ISSN 1927-0887 (Print)
ISSN 1927-0895 (Online)

Journal of Food Research

Vol. 8, No. 6 December 2019

Canadian Center of Science and Education®

JOURNAL OF FOOD RESEARCH

An International Peer-reviewed and Open Access Journal of Food Research

Journal of Food Research is an international, double-blind peer-reviewed, open-access journal. JFR is published by the Canadian Center of Science and Education in both print and online versions. JFR aims to promote excellence through dissemination of high-quality research findings, specialist knowledge, and discussion of professional issues that reflect the diversity of this field.

The scopes of the journal include:

- History and Culture of Food
- Novel Foods and Sensory Analysis
- Food Quality and Safety
- Food Chemistry and Toxicology
- Food Microbiology
- Food Nutrition and Health
- Nutraceuticals and Functional Food
- Food Engineering and Physical Properties
- Food Laws and Regulations
- Food Economics and Marketing
- Concise Reviews and Hypotheses in Food Science

The journal is included in:

- AGRICOLA
- BASE
- Chemical Abstracts Service (CAS)
- Global Health
- InfoBase Index
- JournalFinder
- JournalTOCs
- SHERPA/RoMEO
- Ulrich's
- WorldCat

Open-Access Policy

We follow the Gold Open Access way in journal publishing. This means that our journals provide immediate open access for readers to all articles on the publisher's website. The readers, therefore, are allowed to read, download, copy, distribute, print, search, link to the full texts or use them for any other lawful purpose. The operations of the journals are alternatively financed by publication fees paid by authors or by their institutions or funding agencies.

Copyright Policy

Copyrights for articles are retained by the authors, with first publication rights granted to the journal/publisher. Authors have rights to reuse, republish, archive, and distribute their own articles after publication. The journal/publisher is not responsible for subsequent uses of the work.

Submission Policy

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the authorities responsible where the work was carried out. However, we accept submissions that have previously appeared on preprint servers (for example: arXiv, bioRxiv, Nature Precedings, Philica, Social Science Research Network, and Vixra); have previously been presented at conferences; or have previously appeared in other "non-journal" venues (for example: blogs or posters). Authors are responsible for updating the archived preprint with the journal reference (including DOI) and a link to the published articles on the appropriate journal website upon publication.

The publisher and journals have a zero-tolerance plagiarism policy. We check the issue using the plagiarism prevention tool and a reviewer check. All submissions will be checked by iThenticate before being sent to reviewers.

This is an 'Open Access' journal published under a creative commons license. You are free to copy, distribute, display, and perform the work as long as you clearly attribute the work to the authors.

JFR accepts both Online and Email submission. The online system makes readers to submit and track the status of their manuscripts conveniently. For any questions, please contact jfr@ccsenet.org.

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

EDITORIAL TEAM

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

CONTENTS

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Effect of Extraction Conditions for Recovery of Chlorogenic Acid and Flavonoids from Wet Cider Apple Pomace under Subcritical Water

Salis Ibrahim[1], Regina Santos[2] & Steve Bowra[3]

[1]University for Development Studies, PO Box TL 1350, Tamale, Ghana

[2]University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

[3]Department of Research and Development, Phytatec (UK) Ltd., Plas Gogerddan, Aberystwyth, SY23 3EB, UK

Correspondence: Salis Ibrahim, University for Development Studies, PO Box TL 1350, Tamale, Ghana. Tel: +233-244-222-176. E-mail: isalis@uds.edu.gh

Received: August 23, 2019 Accepted: September 6, 2019 Online Published: September 18, 2019

doi:10.5539/jfr.v8n6p1 URL: https://doi.org/10.5539/jfr.v8n6p1

Abstract

Industrial wet cider apple pomace was subjected to subcritical water mediated hydrolysis in a batch reactor with varying experimental conditions such as solid-to- solvent ratio (1-8 % w/v), temperature (100- 200 ^{o}C) and a residency time (10-30 min) to understand the effects of the experimental conditions on overall recovery of polyphenolic compounds. Chlorogenic acid and some flavonoids were identified and quantified by high performance liquid chromatography (HPLC-DAD). Higher yields of chlorogenic acid and the flavonoids were obtained between 100- 150 ^{o}C for residence time of 20 minutes. Solid-to solvent ratio and temperature played a significant role in the recovery of the polyphenolic compounds ($p< 0.05$). The results demonstrated that, residence time at a fixed extraction condition was less significant ($p> 0.05$). However, it was significant for yield of degradation compounds such as protocatechuic aldehyde, 5- HMF and furfural. Subcritical water selectively influenced the chemical structure of the polyphenolic compounds.

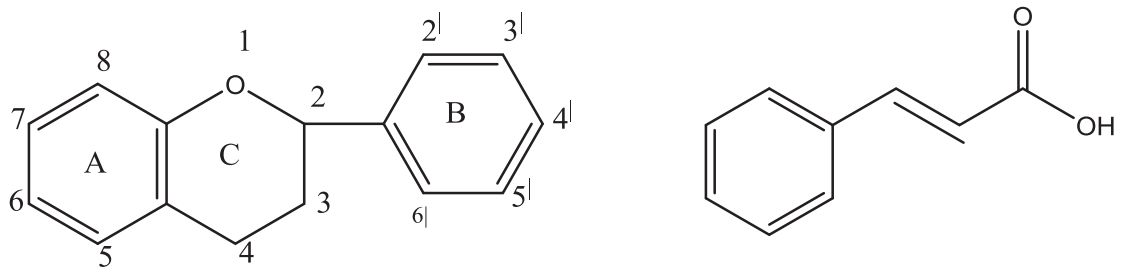
Keywords: cider apple pomace, chlorogenic acid, flavonoids, subcritical water extraction

1. Introduction

Apples (*Malus domestica*) are extensively cultivated in many moderate temperate regions of the world. The fruits are enjoyed for their taste and flavour but also acknowledged as a good source of antioxidants, vitamins, and dietary fibre (Brendan, 2010). The global annual production in 2010 was approximately 70×10^6 tonnes covering a 4.8×10^6 hectares (FOA, 2011) thus making apples the 4th most cultivated fruit globally next to banana, oranges and grapes.

Apples can be categorised into, culinary, desert and cider apple varieties. Cider apples are distinct from the desert and culinary apples, due to the high content of tannin and their fibroid nature. There are over seventy cider apple varieties recorded in the United Kingdom and about twenty three reported in France (Johansen, 2000). Of the total production of apples, at least 71% of the fruit is eaten fresh while 20% is processed into apple juice and cider after fermentation, the balance is used to produce apple purees, spirits and other by-products of apple (Joshi, 1997, Joshi & Attri, 1991, Kaushal et al., 2002). Apple pomace is the residue generated after juice extraction and is composed of 20- 35% by weight of the original production feedstock. The amount of the pomace generated and its composition will depend on the variety of the apple and the techniques used in extracting the juice (Mahawar et al., 2012). Approximately 9×10^6 tonnes of apple pomace are produced worldwide per the estimation of the amount of apples processed. The use and the amount of pomace vary according to country. China is the largest producer of apple pomace in the world with estimated annual yield of over 3 million tons since 2009 (Yue et al., 2012). In India, approximately 1 million tonnes of apple pomace every year is produced, of which 10,000 tonnes are exploited (Shalini and Gubta, 2010). At least, 80,000 tonnes of pomace are generated in Brazil each year and primarily utilised as animal feed (Vendruscolo et al., 2008). In northern Spain, 20,000 tonnes per annum of the pomace is generated and accounts for a significant proportion of the global cider apples production (Diñeiro García et al., 2009). Apple pomace is a heterogeneous biomass residue consisting of peels, discarded apples, seeds, core, stems and exhausted apple tissue and a potential source of carbohydrate, fibre, polyphenolic compounds and pectin which can serve as valuable feedstock for food, feed, pharmaceutical or

cosmetic purposes(Cetkovic et al., 2008, Kennedy et al., 1999, Lu & Foo, 1997, Lu & Foo, 2000). Apples represent an important source of flavonoids in diets within Europe and the United States. Approximately 22% of polyphenolics consumed in the United states are derived from apples (Vinson et al., 2001). The major phenolic compounds found in apples include; chlorogenic acids, epicatechins procyanidins, phloridzin and the quercetin conjugate (Bhushan et al., 2008). It has been revealed that different polyphenolic compounds have potential to impede proliferation of cancer cells, control immune and inflammatory response, and defend against oxidation of lipids (Hollman et al., 1997, Liu, 2003). Polyphenolic compounds are grouped into classes according to the number of carbon atoms in the molecule and also their chemical structure and are classified as phenolic acids and flavonoids which represent non-nutrient natural compounds derived from plants. Flavonoids by far constitute the most diverse group of polyphenolic compounds in plants. Ordinarily they are found as glycoside derivatives and over 4000 flavonoids have been documented in plants (Harborne & Williams, 2000). The list keeps growing because they can be many patterns of substitution of the ''hydroxyl, methoxy or glycosyl'' groups of the primary structure, to form more complex ones (Véronique, 2005). Flavonoids are soluble in water and can often be extracted directly with water. The backbone molecule of flavonoids contains 15 carbon atoms and have $C_6 - C_3 - C_6$ structure skeleton with ring A and B been phenolic in nature which are linked together by 3 grouped carbons as shown in figure 1.



Flavonoid general structure Hydroxycinammic acid structure

Figure 1. general structure for Flavonoids and Phenolic acid

The variations in the chromate ring C as a result of hydroxylation pattern allow flavonoids to be classified further into six (6) sub-groups; flavonols, flavanols, flavones, flavanones anthocyanin, isoflavones (Scalbert & Williamson, 2000, Tsao & McCallum, 2009). Chalcones are also classified as a member of the flavonoids, although they lacked the heterocyclic ring C (Tsao & McCallum, 2009). Phenolic acids constitute non – flavonoids polyphenolic compounds which are further grouped into two main types, as benzoic acid and cinnamic acid derivatives (Manach et al., 2004). Free phenolic acids are obtained from fruits and vegetables but are often in a bound form in grains and seeds and can be freed by acid or alkaline hydrolysis or treatment with enzymes (Adom & Liu, 2002, Chandrasekara and Shahidi, 2010, Kim et al., 2006). Hydroxycinnamic acids are derived from cinnamic acids and comprised primarily of p-coumaric, caffeic, ferulic and sinapic acids glycosylated derivatives or esters of quinic, shikimic or tartaric acid. Chlorogenic acid which is found in many fruits and in particular coffee is an ester of caffeic and quinic acids. Caffeic acid is the most abundant phenolic acid representing over 75% of the total of hydrocinnamates in most fruits (D'Archivio et al., 2007, Manach et al., 2004).

Innovative methods have been explored to recover the polyphenolic compounds from natural sources to reduce the extraction time, the amount of solvent used, and improve yields and quality of extracts (Wang & Weller, 2006). The traditional solvent extraction methods are often time consuming and require large volumes of solvents (Luque de Castro & Garcia-Ayuso, 1998). Moreover, the procedure is laborious and produces low selectivity and extraction yield (Ibáñez et al., 2008). However, simply substituting the solvent type usually results in low yields of recovery because of poor solute-solvent affinities and moreover the cost could be higher. Therefore, advanced techniques of extraction with reduced recovery time and organic solvents consumption and increase pollution prevention are required. Subcritical water extractions are perceived as efficient routes to recover polyphenolic compounds from plant matrices. Water is environmentally friendly, non-toxic, easily accessible and inexpensive solvent for the extraction of phytochemicals from plant materials (Çam & Aaby, 2010). In addition it has been shown that, subcritical water mediated extraction is efficient and can provide higher extraction yields from solid samples (Luque de Castro et al., 1999; Ibrahim et al., 2018). Subcritical water refers to liquid water at a temperature between the atmospheric boiling point and the critical temperature (374^{o}C)

of water. The most essential factor to consider in subcritical water procedure is the variation of it dielectric constant. Water at room temperature has a dielectric constant of approximately 80 and decreases to 27 at 250^{o}C, which is similar to that of ethanol. Therefore by modulating temperature and pressure water can perform like organic solvents solubilising low polarity analytes (Nieto et al., 2010). Moreover the enhancement of extraction efficiency of subcritical water can be attributed to an improvement in the solubility and mass transfer effects as a result of the modification of physico-chemical properties of water at elevated temperature, which also leads to disruption of surface equilibria (Ong et al., 2006). Therefore, the increase in temperature can break the solute-matrix association as a result of van der Waal forces, hydrogen bonding, and dipole attractions of the solute molecules and other active sites in the matrix. Subcritical water has been shown to demonstrate selectivity towards separate groups of compounds at selected temperatures depending on their polarities. Polar compounds are extracted at lower subcritical temperatures whereas less polar ones are recovered at higher temperatures (Ibañez et al., 2003). Water under subcritical conditions has high concentrations of H^+ and OH^- ions which can catalyse acid-base reactions (Arai et al., 2013). Therefore, other water mediated reactions such as hydrolysis and dehydration can occur at the high temperature (Brunner, 2009). The modified physico-chemical properties at the elevated temperature contribute to the disruption of solute-matrix interactions thereby improving solubility and mass transfer effects leading to elution of compounds that are strongly bound to cell wall. The eluted compounds can undergo transformation at the high temperatures to generate other compounds of different chemical properties and structures (Plaza et al., 2010).

The current research assessed the utility of subcritical water in the recovery of chlorogenic acid and selected flavonoids from wet industrial apple pomace with no co-solvents or modifiers added. Different levels of the wet apple pomace were employed to assess the recovery of the phenolic acid and flavonoids under subcritical water conditions. The impact of extraction parameters such as solid-to-solvent ratio, temperature and residence time in a batch reactor under subcritical water mediated operation were investigated to study the relationship between experimental factors and all responses. The composition and structure of the polyphenolics at different temperature levels were determined.

2. Material and Methods

2.1 Chemicals/Reagents

Phenolic standards comprising, Chlorogenic acid (≥ 95%), ± Catechin hydrate (≥ 90%) (-) Epicatechin (≥ 90), Phloridzin dihydrate (≥ 99%), Procyanidin B2 (≥ 90%), Quercetin -3-β-D-glucoside (≥ 90%), Quercetin-3-D-galactoside (≥ 97%), Phloretin, Chromasolv for HPLC water were purchased from Sigma-Aldrich (UK). Acetonenitrile, methanol and glacial acetic acid were obtained from Fisher Scientific (UK).

2.2 Apple Pomace Sample

Apple pomace sample was supplied by Universal Beverages Limited (UBL) a subsidiary company of Bulmers, UK. The pomace residue was derived from 7 cider apple varieties comprising Dabinett, Brown Snout, Harry Master Jersey, Chisel Jersey, Vilberie, Michelin, and Yarlinton Mill. The apple pomace residue was very heterogeneous, made-up of peels, seeds, apple flesh and therefore was thoroughly mixed to ensure replicate samples were representative of the population of samples. Portions of the apple pomace were collected for dry weight determination and the rest frozen and ~~then~~ later homogenized for 30 seconds using Moulinex domestic blending machine to minimize variability in batch-to-batch analysis.

Dry weight of the apple pomace was determined using AOCS (American Oil Chemist Society Standard) standard protocol using a laboratory oven (STATUS international, UK) at 103 ± 3°C.

2.3 Experimental Design

Design of experiments for the subcritical water extraction were done according to (Ibrahim et al., 2018) which were composed of screening experiments and the actual design by response surface methodology (RSM). Screening experiments were conducted to identify effects of dependent variables such as temperature, solid to solvent ratio and residency time on overall recovery of the polyphenolic compounds. The results of the screening experiments were used, with the aid of Stat-Ease Design Expert software 7.0, to construct experiments using the central composite rotatable design (CCRD. Twenty experimental points were generated in all, comprising of 14 trials and 6 replicates runs around the centre point. The extraction programme is shown in Table 1.

Table 1. Design of experment by Central Composite Rotatable Design (CCRD) using 3 factors under subcritical water extraction

Standard	Run	Factor 1	Factor 2	Factor 3
		A: Solid/Solvent ratio %	B: Temp ℃	C: Residence time min
20	10	4.5	150.0	20.0
19	20	4.5	150.0	20.0
18	4	4.5	150.0	20.0
17	15	4.5	150.0	20.0
16	17	4.5	150.0	20.0
15	9	4.5	150.0	20.0
14	11	4.5	150.0	37.0
13	12	4.5	150.0	3.0
12	8	4.5	234.1	20.0
11	2	4.5	66.0	20.0
10	19	9.5	150.0	20.0
9	3	0.5	150.0	20.0
8	16	8.0	200.0	30.0
7	14	1.0	200.0	30.0
6	1	8.0	100.0	30.0
5	18	1.0	100.0	30.0
4	7	8.0	200.0	10.0
3	6	1.0	200.0	10.0
2	5	8.0	100.0	10.0
1	13	1.0	100.0	10.0

2.4 Subcritical Water Extraction of Polyphenolics

Polyphenolic compounds from the apple pomace were extracted under subcritical water mediated hydrolysis conditions according to extraction programme in table 1, using the Parr instrument model (5521), a stainless-steel reactor vessel (300 mL) of diameter (2.5 in) with heating jacket and a magnetic stirring at 1240 rpm and integrated cooling system. A back pressure regulatory valve was employed to control internal pressure of vessel. Nitrogen gas was used to purge the reactor and to pressurize the reaction vessel to 50 bar. Crude extracts after subcritical water extraction were collected from the reaction vessel and centrifuged using Beckman J2-20 centrifuge. Supernatants were collected and analysed for phenolic acids and flavonoids and other degradation products.

2.5 Separation, Identification and Quantification of Polyphenolic Compounds by High Performance Liquid Chromatography (HPLC)

Reverse phase high performance liquid chromatographic (HPLC) procedure previously described by (Schieber et al., 2001) was used to characterise and quantify the phenolic compounds within the subcritical water extracts. The polyphenolics were resolved based on their respective affinities between the mobile and the stationary phase. The separation of phenolic compounds in extracts were performed using the Agilent 1100 series HPLC system coupled with a DAD-UV detector (Agilent Technologies- Germany), supplied with a solution Chemstation software for both online and offline analysis. A Prodigy 5µm ODS3 100A, C18 (250 x 4.6 mm I.D) column from Phenomenex (Torrance, CA, USA) was the stationary phase with a guard column operated at 40℃. The mobile phase consisted of 2% (v/v) of the glacial acetic acid in water as eluent A. Eluent B was made with 0.5% of acetic acid in 50:50 (v/v) of water and acetonitrile. Eluent C was (100%) acetonitrile. The gradient solvent systems programmed for the separation with a flow rate of 1ml/min were as follows: beginning with 10% of B and increasing the gradient to 55% B in 50 minutes. Further increased from 55% B to 100% B was done in 10 minutes and finally decreased from 100% B to initial 10% B in 5 minutes. Eluent C was used in reconditioning the column under isocratic flow by pumping 100% C for 10 minutes, and 10% B also for 10 minutes. Volume of all samples injected at a time was 10µl and phenolic compounds were monitored at 280nm (flavanols), 320nm (hydrocinnamic acid) and 370nm (flavonols). Retention times and spectra data were collected and identifications of the phenolic compounds were done using their respective retention times and spectra data of respective standards. Quantification of the phenolic compounds were done making use of their chromatographic peak areas at maximum absorbance.

2.5.1 Preparation of Polyphenolic Standards

A standard of chlorogenic acid at concentration of 1mg/ml was prepared by weighing 0.05g of the solid into a 50ml volumetric flask and making it up to the mark with HPLC water. 5ml of the 1mg/ml of the stock solution was pipetted into a 50ml volumetric flask and adding HPLC grade water to the mark to yield a solution of concentration 0.1mg/ml. The solution was transferred into a 50ml tube covered with aluminium foil to prevent oxidation from light. 10, 100, 200, 300, 500 µl of the 0.1mg/ml of the chlorogenic acid solution were pipetted into separate HPLC vials and adjusting total volume to 1ml using HPLC water with 1000µl micropipettes. The solutions were thoroughly mixed to give final concentrations of 1, 10, 20, 30 and 50µg/ml. 1ml of the 0.1mg/ml (100µg/ml) was also pipetted into the vial to obtain six different concentrations of samples for the calibration curve. The autosampler of the HPLC was loaded with the standard and were analysed by the method described above. Other phenolic standards were prepared similarly to the chlorogenic acid. Calibration curves were obtained by plotting areas under peaks against concentrations.

2.5.2 Typical Calculation of Concentration of Chlorogenic Acid

The calibration curve for standard chlorogenic acid is shown in Figure 2 below;

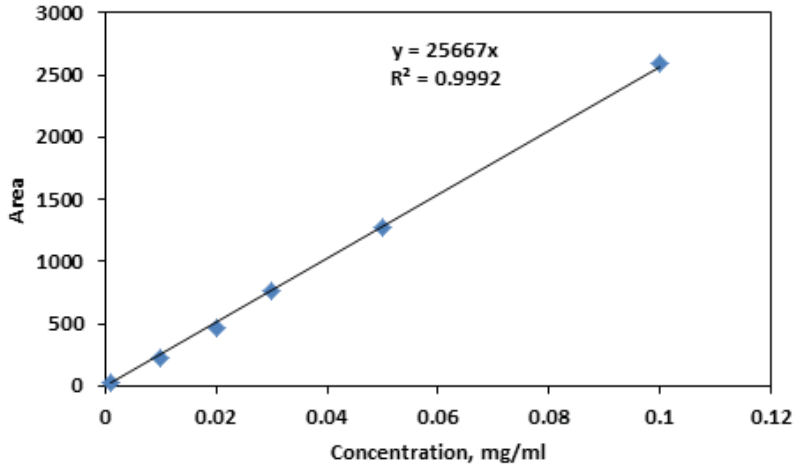


Figure 2. Calibration curve of standard chlorogenic acid

The regression equation for standard chromogenic acid is

$$y = 26612x$$

Where y represent peak area, and x is concentration of standard.

$$Amount\ of\ sample\ (interpolated) = \frac{y}{26612}$$

The purity of the standard used was taken into consideration and corrected. Purity of Chlorogenic acid used ≥95%, therefore the multiplier was;

$$M = \frac{1}{0.95} = 1.0526$$

And amount of Chlorogenic acid in sample $= \left[\left(\frac{y}{26612}\right) \times 1.0526\right]\ mg/ml$

Conversion of mg/ml to $mg/g\ DW$ of apple pomace used was

$$Amount\left(\frac{mg}{g}DW\right) = \frac{(Amount\ \left(\frac{mg}{ml}\right) \times (Volume\ of\ extract, ml)}{Dry\ weight\ of\ apple\ pomace\ used, g}$$

Similar procedure was used to construct calibration curves for other phenolic standards and their concentrations derived from the graphs.

3. Results and Discussion

The dry matter content of the homogenised apple pomace was 26.2 ± 0.1g/100g fresh weight. Dry matter content of apple pomace reported in the literature was 26.4g/100g fresh weight (Vasil'ev et al., 1976).

3.1 Identification of Polyphenolic Compounds under Subcritical Water Extracts

The extracts from the various design parameter combinations from the subcritical water extraction were analysed using HPLC-DAD. The main phenolic compounds identified were, chlorogenic acid, phloridzin, quercetin-3-galactoside, quercetin-3-glucoside, procyanidins B2., and phloretin glycosides. Figures 3- 5 represent chromatograms at temperatures, 100°C, 150°C and 200°C. Table 2 show the retention times of standard polyphenolic compounds.

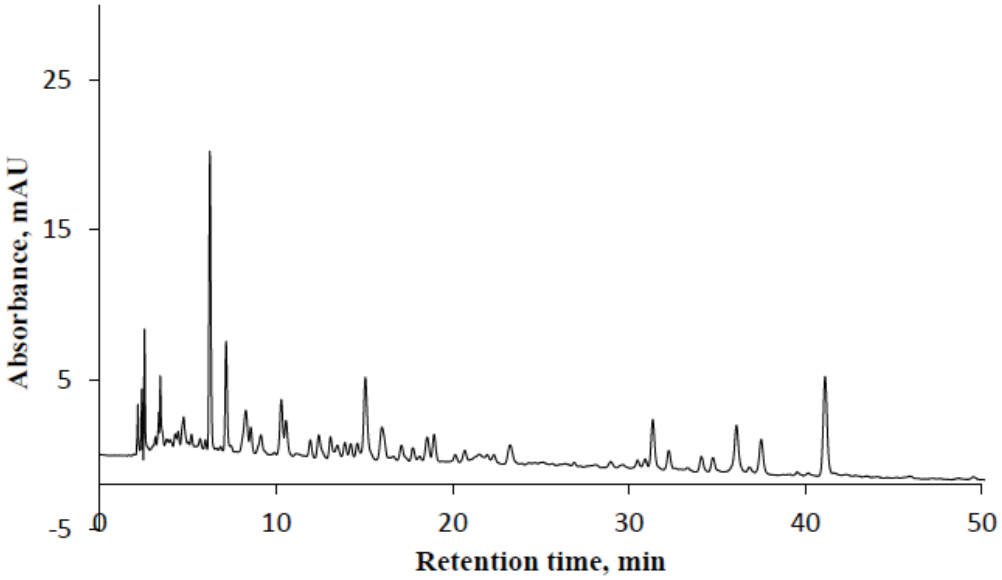


Figure 3. Chromatogram of subcritical water extract of wet cider apple pomace at 100°C

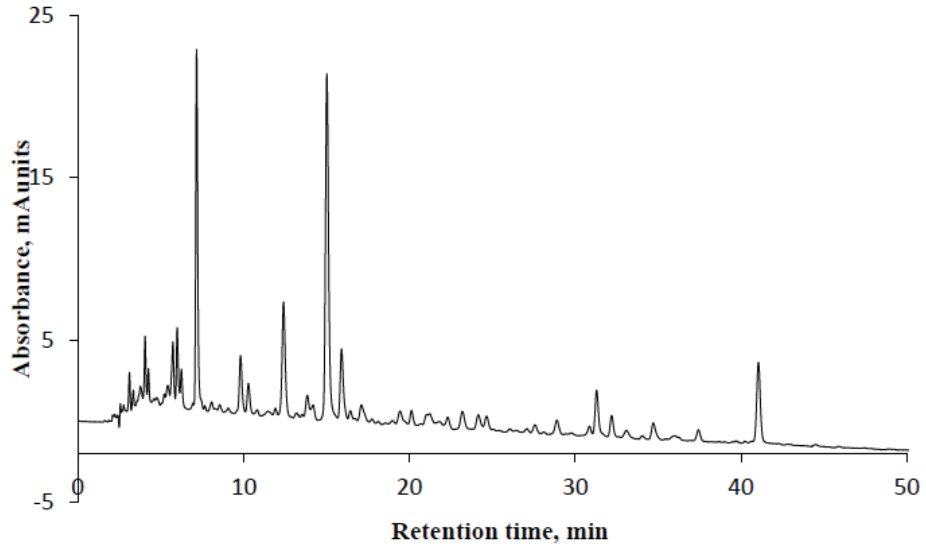


Figure 4. Chromatogram of subcritical water extract of wet cider apple pomace at 150°C

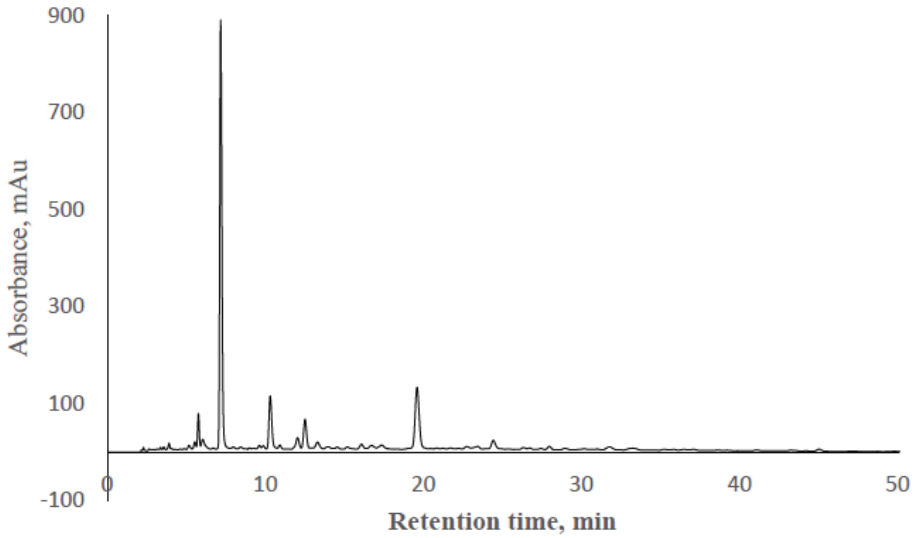


Figure 5. Chromatogram of subcritical water extract of wet cider apple pomace at 200°C

Table 2. Retention time of standard Phenolic compounds

Phenolic standard	Retention time (min)
Catechin	13.03 ± 0.01
Chlorogenic acid	15.17±0.05
Procyanidin B2	16.13±0.07
Caffeic acid	17.5± 0.08
Epicatechin	18.6 ±0.07
P-Coumaric acid	25.3 ± 0.08
Ferulic acid	29.3±0.20
Quercetin- 3- galactoside	31.47± 0.11
Quercetin -3-glucoside	32.4±0.01
Phloridzin	41.5 ± 0.23
Phloretin	50.32±0.12
5-HMF	7.3±0.05
Furfural	10.4±0.06
Protocatechuic aldehyde	12.4± 0.06

Monomeric procyanidins like catechin and epicatechin were barely detected and the reason could be that the UV-DAD was not very good enough for their detection. Protocatechuic aldehyde was detected from 100°C,150°C and 200°C. The HPLC –DAD characterisation of the polyphenolic compounds of subcritical conditions at 100°C reveals among others similar compounds under the aqueous acetone extraction (Ibrahim et al., 2019). These polyphenolic compounds with exception of protocatechuic aldehyde were the main polyphenolic compounds recovered from apple pomace using pressurised ethanol/water as solvents. (Wijngaard & Brunton, 2009). Phenolic compounds like anthocyanidins were not detected although the apple pomace sample used was a blend of varieties of cider apples including the red ones which were thought to contain high levels of the flavonoid. Anthocyanidins are highly unstable and may even oxidize before the start of the analysis (Delgado-Vargas et al., 2000). However, there were other peaks typically of phenolic nature by their UV- spectra but standards were not available to identify them. Other compounds like 5-hydroxymethylfurral (5-HMF), furfural and Protocatechuic aldehyde are not usually reported in solvent extracts.

3.2 Determination of Concentration of Phenolic Compounds

The concentration of phenolic compounds identified were derived from the calibration curves of their pure standards and were recorded as mean values ± standard error and expressed in mg/kg of dry weight of apple pomace as shown in Table 3 and Table 4.

Table 3. Mean concentration of Chlorogenic acid, Phloridzin, Quercetin-3-galactoside; Quercetin-3-glucoside (mg/kg) dry weight under subcritical water extraction of wet apple pomace

Std Order	Sample	CGA	PHL	Q-3-gal	Q-3-glu
6	8-100-30	35.05 ± 6.3	38.74 ± 3.6	16.54 ± 2.5	10.84 ± 1.9
11	4.5-66-20	6.91±0.0	50.24±5.0	22.36 ± 6.9	ND
9	0.5-150-20	ND	ND	ND	ND
18	4.5-150-20	60.37±1.8	57.61 ± 2.4	12.712 ± 0.7	21.43 ± 0.2
2	8-100-10	24.67 ± 1.3	31.43 ± 1.32	16.66 ± 0.5	12.91± 0.4
3	1-200-10	ND	ND	ND	ND
4	8-200-10	ND	ND	ND	ND
12	4.5-234-20	ND	ND	ND	ND
15	4.5-150-20	60.14±0.4	48.30 ± 7.0	14.45 ± 0.5	18.68 ±
20	4.5-150-20	59.18 ± 2.8	43.48 ± 1.7	11.29 ± 0.2	19.46 ±
14	4.5-150-37	54.90 ± 1.7	44.80 ± 2.3	10.59 ± 2.4	20.88 ± 2.6
13	4.5-150-3	59.4 ± 2.4	50.49 ± 2.8	25.07 ± 1.1	22.97 ±1.7
1	1-100-10	58.39±0.6	60.73 ±	76.16 ± 0.7	ND
7	1-200-30	ND	ND	ND	ND
17	4.5-150-20	60.56 ± 1.5	45.29 ± 2.6	15.30 ± 0.9	18.32 ± 4.9
8	8-200-30	ND	ND	ND	ND
16	4.5-150-20	34.49±1.2	22.37±4.3	25.33 ± 1.67	28.24 ± 4.7
5	1-100-30	122.21±1.9	250.20 ± 22.2	97.00± 0.7	ND
10	9.5-150-20	40.21±0.4	31.53 ± 1.2	5.83 ± 0.7	8.87 ± 2.9
19	4.5-150-20	78.33±2.7	89.21 ± 1.02	19.24 ± 1.4	23.59 ± 4.9

Note: Sample 8-100-30 reads; 8% solid/solvent @ 100^{o}C for 30 minutes;

CGA- Chlorogenic acid; PHL- Phloridzin; Q-3-gal- Quercetin-3-galactoside; Q-3-glu-Quercetin-3-glucoside, ND –not detected.

Table 4. Mean concentration of Procyanidin B2, Protocatechuic aldehyde, 5-HMF and Furfural (mg/kg) dry weight under subcritical water extract of wet apple pomace

Std order	Sample	Pr-B2	PCA	Furfural	5-HMF
6	8-100-30	57.67 ± 5	ND	16.09 ± 1.7	4.19 ± 0
11	4.5-66-20	ND	ND	ND	ND
9	0.5-150-20	ND	ND	ND	150.89 ± 8.1
18	4.5-150-20	ND	39.6 ± 0.3	628.75±71	194.05 ± 33.0
2	8-100-10	48.55 ± 0.1	ND	4.40 ± 0.23	2.02 ± 1.4
3	1-200-10	ND	674.2 ± 0.1	17980.39± 406	3754.93 ± 67.7
4	8-200-10	ND	337.8 ± 0.5	6653.63± 103	633.27 ± 2.7
12	4.5-234-20	ND	1133.2 ± 0.6	15787.68± 265	1543.86 ± 13.5
15	4.5-150-20	ND	40.5 ± 0.2	590.89 ± 42.5	168.32 ± 6.1
20	4.5-150-20	ND	38.6 ± 1.4	631.12 ± 14.5	175.12 ± 6.0
14	4.5-150-37	ND	62.2 ± 0.0	1042.49± 74.1	342.83 ± 1.8
13	4.5-150-3	ND	18.4 ± 0.1	177.61 ± 20.3	33.36 ± 1.8
1	1-100-10	ND	ND	46.27±0.4	6.48 ± 0.2
7	1-200-30	ND	966.4 ± 0.1	40330.39± 786	5767.29±170.5
17	4.5-150-20	ND	39.7 ± 0.1	588.45 ± 1.57	162.24 ± 3.1
8	8-200-30	ND	357.4 ± 23.9	9038.32 ± 158.6	795.93 ± 1.8
16	4.5-150-20	ND	41.8 ± 0.1	753.07 ± 148	201.94 ±46.3
5	1-100-30	ND	ND	97.78 ± 9.3	4.34 ± 0.5
10	9-150-20	ND	44.5 ± 1.1	652.88 ± 1.03	212.95 ± 5.0
19	4.5-150-20	ND	41.6 ± 0.0	594.97± 11.9	185.97 ± 7.0

Note: 1-100-30 reads 1% solid-to-solvent ratio at 100^{o}C for 30 minutes residence time; Pr-B2= Procyanidin B2; PCA= Protocatechuic aldehyde ND= not detected;

3.3 Model Analysis of Chlorogenic Acid and other Flavonoids in the Subcritical Water Extracts

Chlorogenic acid, phloridzin, quercetin-3-galactoside, quercetin-3-glucoside, procyanidin B2 and degradation compounds (protocatechuic aldehyde, 5-HMF and furfural) were evaluated for effects of solid-to solvent ratio, temperature and residence time on overall recovery of the dependent variables using concentrations of each compound in table 3 and table 4. Individual concentrations were each entered into design expert 7.0 software for model selection. The model selection criteria were that, selected model should be significant and exhibit an insignificant lack of fit, in addition to satisfactory levels of adequacy. ANOVA results at 95% confidence interval for all proposed models were significant ($p<0.05$) with satisfactory level of adequacies ($R^2 >0.9$) and predicted R^2 largely agreed with adjusted R^2. Coefficient of variations and variance of inflation factors were at acceptable levels. Transformed reduced quadratic models were appropriate and revealed the effect of each dependent variable on extraction conditions. The summary on the levels of significance of design factors and their interaction are shown in Table 5.

Table 5. Summary of the levels of significance of design factors and interaction terms under subcritical water extraction

Response	Significant level ($p < 0.05$)								
	A	B	C	AB	AC	BC	A^2	B^2	C^2
CGA	✓	✓		✓				✓	
PHL	✓	✓		✓			✓	✓	
Q-3-gal.	✓	✓		✓			✓		
Q-3-glu.	✓	✓		✓			✓		
Pr-B2	✓	✓		✓			✓		
PCA	✓	✓	✓		✓	✓	✓		
5-HMF	✓	✓	✓	✓		✓	✓		
Furfural	✓	✓	✓	✓		✓	✓		

Note: CGA-chlorogenic acid; PHL-phloridzin; Q-gal- quercetin-3-galactoside; Q-glu- quercetin-3- glucoside; Pr-B2-procyanidin B2; A-solid-to-solvent ratio; B-temperature, and C- residence time in reactor, ✓- significant.

Solid-to-solvent ratio (A), temperature (B) and their interaction (AB) had significant effects on the yield of all responses. Effect of residence time was not significant on recovery of the phenolic compounds. This observation was consistent with earlier published report of flavonoids extraction under subcritical water extraction (Plaza et al., 2013). Residence time influenced the yield of protocatechuic aldehyde, 5-HMF and furfural as well it's (residence time) interaction with temperature. Higher temperatures and extended time favoured the yield of the decomposition or degradation products.

3.3.1 Effects of Extraction Conditions on Recovery of Chlorogenic Acid

Yield of chlorogenic acid was significantly affected by loading ratio (solid/solvent) and temperature as well as their interaction ($P<0.05$). Only temperature showed significant effect in the quadratic term ($p<0.05$). Residence time had no significant influence on the recovery of chlorogenic acid ($p > 0.05$). Maximum concentration of chlorogenic acid (122.2 mg/kg) was extracted around 100°C. Chlorogenic acid had been reported to degrade at temperature 112°C during pressurised liquid extraction of antioxidants from apple pomace using a mixture of ethanol and water as solvent (Wijngaard & Brunton, 2009). A phenolic compound which share similar spectra characteristics with chlorogenic acid was observed at temperature at 150°C with retention time 16.06 minutes. Chlorogenic acid which is an ester of caffeic acid and quinic acid, has several isomers and classified based on the number and position of the acyl groups attached to them. The isomers include; 5-O- caffeeoylquinic acid (common one), 4-O- caffeeoylquinic acid and others with two caffeic acid molecules referred to as iso chlorogenic acid comprising 3,4, dicaffeeoyliquinic acid and 3,5, dicaffeeoyliquinic acid (Clifford, 2000). On the other hand, Winjgaard and Brunton reported, an increased in chlorogenic acid concentration at temperatures higher than 160°C and attributed the release from a possible noncovalent relationship of chlorogenic acid with melanoidins. Similarly Plaza and co-workers predicted maximum concentration of 5-caffeoquinic acid (chlorogenic acid) from apple pomace to elute between 175-200°C (Plaza et al., 2013). Chlorogenic acid or any of its isomers were not detected at temperature 200°C in this investigation. The transformed polynomial model for chlorogenic acid as in (1);

$$\sqrt{Chlorogenic\ acid} = -14.66360 - 1.11557A + 0.38727B + 0.19207C + 5.57786 \times 10^{-3}AB$$

$$-1.09174 \times 10^{-3}BC - 1.55493 \times 10^{-3}B^2 \quad (1)$$

was used to navigate the design space and no prediction of any higher concentrations for chlorogenic acid at higher temperatures was observed. Response surface plots for recovery of chlorogenic acid showing the effects of solid/solvent ratio, temperature at a fixed residence time of 20 minutes is shown in Figure 6.

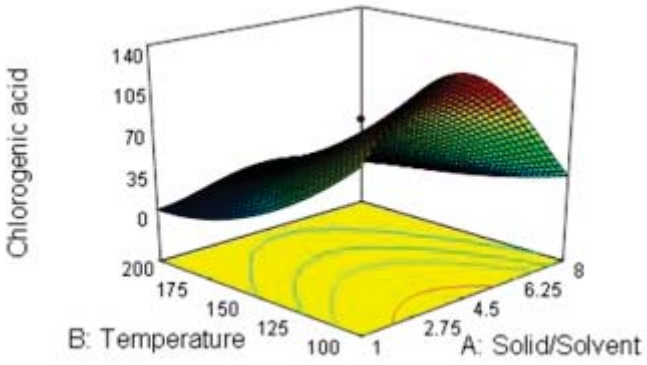


Figure 6. Response surface plot for the effects of temperature (°C)and solid/solvent ratio % (w/v) on the concentration of Chlorogenic acid (mg/kg) der weight under subcritical water extraction of apple pomace for a residence time 20 minutes

3.3.2 Effects of Extraction Conditions on Recovery of Phloridzin

Phloridzin is a major phenolic glycoside found in apple trees and has a characteristic bitter taste that contribute to the original flavour of cider (Whiting & Coggins, 1975). Maximum concentration of the dihydrochalcone glycosides in extracts was 250.2mg/kg at 100°C, a condition consistent with similar extractions using ethanol/water as solvents for extraction (Wijngaard & Brunton, 2009). However, it began to degrade at around 120°C. Phloridzin was not detected at temperature 200°C. The transformed polynomial model is shown in equation (2).

$$\sqrt{Phloridzin} = +8.95591 - 3.88579A + 0.22440B + 0.014146AB + 0.11739A^2 - 1.32240 \times 10^{-3}B^2 \quad (2)$$

The response surface plots show highest amount of phloridzin at 100℃ and stay relatively constant until around 120℃ and gradually decreases to zero at 200℃. The model could be used to navigate the design space. Similar to Chlorogenic acid, Plaza et al., 2013 reported a deviation of response surface plots for phloridzin under pressurised hot water extraction of apple peels (Plaza et al., 2013), predicting higher yield at the highest temperatures(170-200°C). Response surface plots showing the effects of temperature, solid- to- solvent ratio at fixed residence time of 20 minutes on the yield of phloridzin is shown in Figure 7.

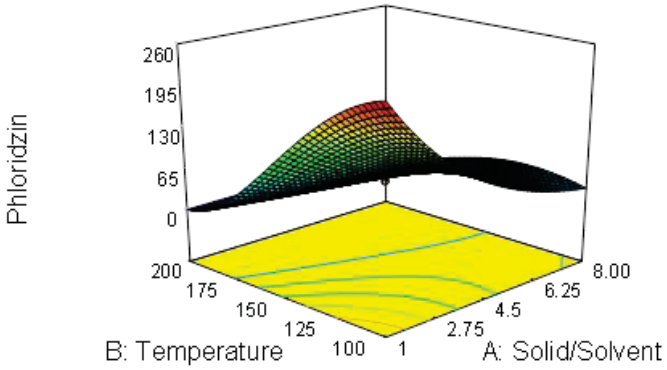


Figure 7. Response surface plot for the effects of temperature (°C) and solid/solvent ratio % (w/v) on the amount of Phloridzin (mg/kg) dry weight under subcritical water extraction of apple pomace for residence time of 20 minutes

3.3.3 Effects of Extraction Conditions on Recovery Procyanidin B2

Structural and molecular weight variation within proanthocyanidins makes their evaluation very challenging. Moreover, their complexation with other non-soluble polymers, under estimate their quantification due to incomplete extraction (Pérez-Jiménez et al., 2009). About 50-93% of apple procyanidins may be retained within cell wall material during processing of apple juice (Le Bourvellec & Renard, 2012). Concentrations of procyanidin B2 under subcritical water extraction varied from 0.00 – 57.67± 5.30mg/kg dw and the yield of procyanidin B2 was both solid-to solvent ratio and temperature dependent as well as their interaction. Residence time had no influence on overall amount of procyanidin B2 under the subcritical water extraction within selected range. Procyanidin B2 was not detected at 150^{o}C and beyond suggesting that the amount originally accumulated at 100^{o}C would have been degrading. Therefore, it was not advisable to pursue predictive modelling based on the selected range. Although the design expert suggested predictive model and this could not be relied upon due to limited data because contour plots may be misleading. Optimal temperature conditions of 80-140^{o}C were reported for extracting procyanidins from grape pomace using ethanol /water mixture as solvents (Monrad et al., 2010).

3.3.4 Effects of Extraction Conditions on Recovery of Quercetin Glycosides

The two quercetin glycosides have differed in the way they responded to changes in temperature and solid- to-solvent ratio. Quercetin-3-galactoside was influenced slightly by temperature whereas quercetin-3-glucoside was controlled by solid-to-solvent ratio both in the quadratic terms. The interaction between solid-to solvent ratio and temperature was positive for quercetin-3-galactoside and negative for quercetin-3-gluctoside. The difference in the behaviour could be attributed to the structural configuration of galactose and glucose moieties attached to the quercetin aglycone. Although glucose and galactose can both occur in either open or cyclic structures, the five (5) hydroxyl groups in glucose can be arranged in a specific way on the six- carbon backbone whereas galactose has a carbonyl towards the end of chain. These configurations contribute to the different boiling points of the sugars despite identical molecular weights as they are isomers (180.156g/mol for glucose and 180.16g/mol for galactose). Boiling points of glucose and galactose are 146- 150^{o}C and 167^{o}C respectively. Boiling points are reflection of the strength of the forces between molecules and indication of ease of separation of components which are bound together. Both quercetin glycosides have positive linear temperature coefficients which suggested their concentrations increase in extracts initially when temperature was raised up to 100^{o}C. However further increase in temperature negatively affected the yields of the glycosides shown in the polynomial model equations (3) and (4).

$$Quercetin-3-galactoside = +95.97583 - 31.76127A + 0.45032B + 0.085086AB + 1.63824A^2 - 4.32336 \times 10^{-3} B^2 \quad (3)$$

$$quercetin-3-glucoside = -15.02656 + 16.48077A + 0.016964B - 0.016964AB - 1.45421A^2 \quad (4)$$

The coefficients values of interaction between solid-to solvent and temperature for both glycosides indicated that, it was not advantageous to the yield of quercetin-3-galactoside when both factors are increased simultaneously, whereas it positively influences the amount of the quercetin-3-glucoside as shown in the response surface plots in Figures 8 and 9.

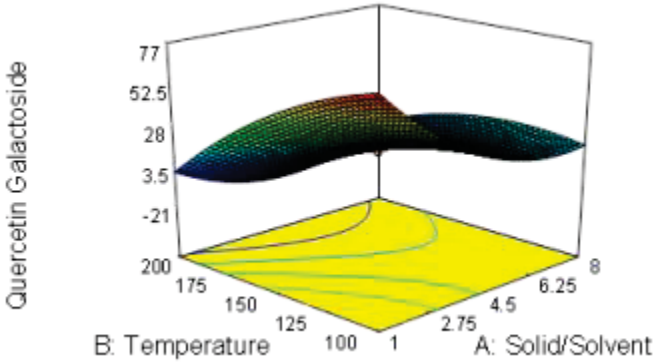


Figure 8. Response surface plot for the effects of temperature (^{o}C) and solid/solvent ratio %(w/v) on the amount of Quercetin-3- galactoside (mg/kg) dry weight for 20 minutes residence time under subcritical water extraction of apple pomace

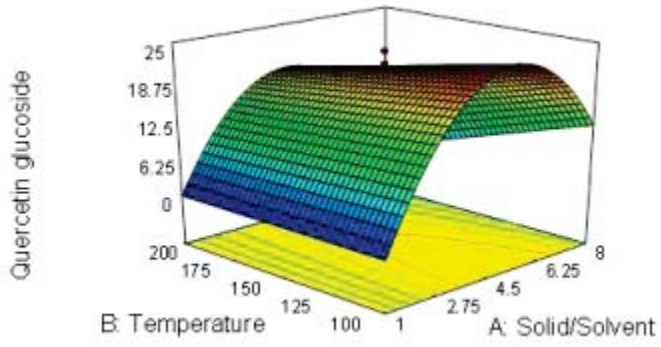


Figure 9. Response surface plot for the effects of temperature (^{o}C)and solid/solvent ratio %(w/v) on the amount of Quercetin-3- glucoside (mg/kg) dry weight for 20 minutes residence time under subcritical water extraction of apple pomace

3.3.5 Effects of Extraction Conditions on Recovery Protocatechuic Aldehyde

3, 4 – dihydroxybenzaldehyde otherwise known as protocatechuic aldehyde is not found freely in apples. Protocatechuic aldehyde which demonstrates strong effect by suppressing replication of hepatitis B-virus both in vitro and in vivo studies is a stable product of thermal degradation of chlorogenic acid (Moon & Shibamoto, 2010; Zhou et al., 2007). The phenolic aldehyde was also the main stable compound from caffeic acid degradation under subcritical water conditions within 160-240℃ (Khuwijitjaru et al., 2014). Water soluble protocatechuic aldehyde in the extracts ranged from 0 – 966.4 mg/kg within the limits of design parameters. Protocatechuic aldehyde was not detected at 100℃ but noticeable at 150℃ , and was stable in the extract even up to 234℃. Yield of protocatechuic aldehyde increases from 150℃ to 200℃ with increasing residence time up to 30 minutes. However, it decreases as solid- to- solvent ratio approaches 8% meaning decreasing the loading and increasing both temperature and residence time improves the yield of protocatechuic aldehyde as in equation (5).

$$\sqrt{PCA} = -25.29499 - 2.30741A + 0.27115B - 0.052261C - 0.014120AB - 0.016414AC + 1.41183 \times 10^{-3}BC + 0.44974 \times 10^{-3}A^2 \quad (5)$$

Response surface plots depicting this behaviour of temperature, solid to solvent ratio and residence time are shown Fig 10 below;

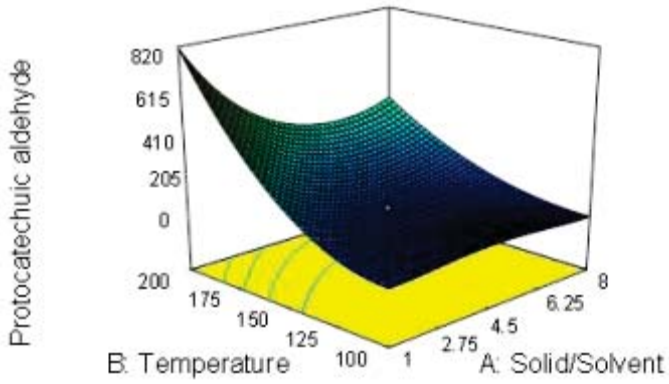


Figure 10. Response surface plot for the effects of temperature (^{o}C) solid/solvent ratio %(w/v) on theamount of Protocatechuic aldehyde (mg/kg) dry weight for 20 minutes residence time under subcritical water extraction of apple pomace

3.3.6 Effects of Extraction Conditions for 5-HMF and Furfural

5-HMF and furfural constitutes furan derivatives and are by-products of hexose and xylose degradation respectively (Ulbricht et al., 1984). The subcritical water extracts of the apple pomace contained varying

concentration of monomeric sugars which degraded at higher temperatures and extended periods under the batch operations to produce the two maillard agents. 5-HMF and furfural varied significantly with temperature and solid to solvent ratio as shown in the model equations (6) and (7).

$$\sqrt{5HMF} = -57.06203 - 1.78796A + 0.63427B - 0.26542C - 0.059059AB - 0.037606AC + 4.38730 \times 10^{-3}BC + 0.92923A^2 \quad (6)$$

$$\sqrt{Furfural} = -77.74299 - 22.99902A + 1.22185B - 2.21599C - 0.098922AB + 0.021109BC + 3.49756A^2 \quad (7)$$

High temperature favoured the formation of the furanic compounds which are also influenced by pH (Purlis, 2010). Increase in temperature under subcritical conditions decreased the pH of water which enhances the dehydration reaction to 5-HMF (Aida et al., 2007). 5- HMF and furfural were not detected at temperatures 66°C which is consistent with results reported earlier (Çam & Aaby, 2010, Schieber et al., 2001). Lower concentrations of 2.02 mg/kg and 4.4 mg/kg of 5-HMF and furfural were recorded at 100°C respectively. Concentrations of 5767.3 mg/kg and 40330.4 mg/kg of HMF and furfural respectively were achieved at 200°C with corresponding increase in browning of extract suggesting caramelisation reactions taking place. The response surface plots showing the effects of solid-to solvent ratio and temperature on maillard agents are shown in Figure 11 and 12.

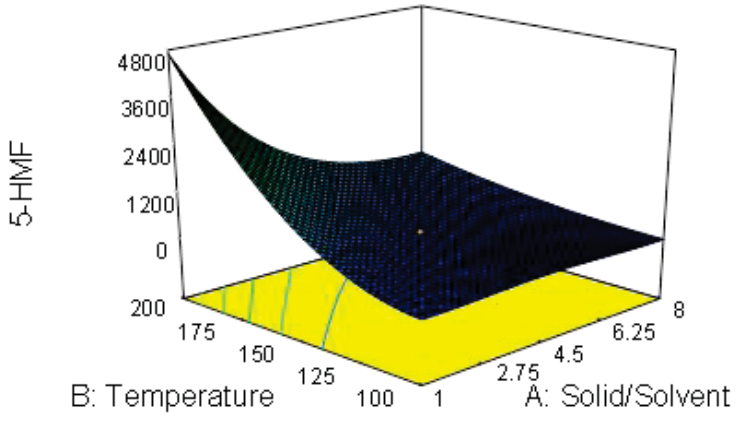


Figure 11. Response surface plot of effects of temperature (°C) and solid-to solvent ratio %(w/v) on 5-HMF (mg/kg)dry weight for 20 minutes residence time under the subcritical water extraction of apple pomace

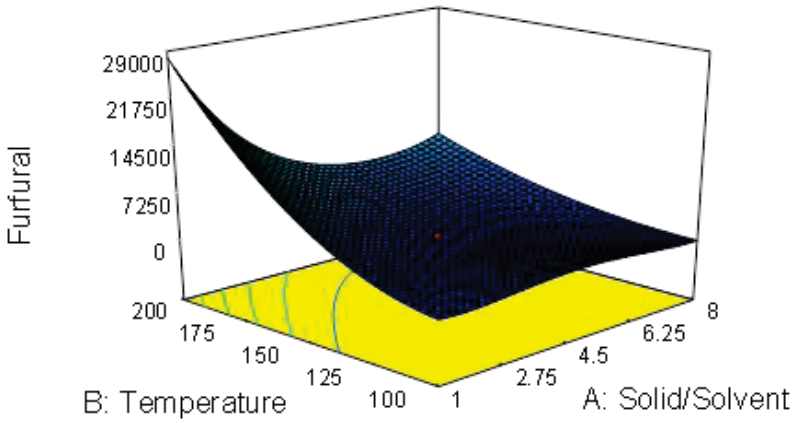


Figure 12. Response surface plot of effects of temperature (°C)and solid-to solvent ratio %(w/v) on furfural (mg/kg) dry weight for 20 minutes extraction time under the subcritical water extraction of apple pomace

4. Conclusion

Recovery of chlorogenic acid and flavonoids were strongly influenced by solid to solvent ratio and temperature under the subcritical water mediated extraction of the cider apple pomace. Residence time during extraction at lower subcritical water temperature had little significance on overall recovery of the polyphenolic compounds. However, its effect at higher temperature was significant and resulted in the formation of degradation products like protocatechuic aldehyde and furan derivatives. Higher yields of chlorogenic acid and the flavonoids were obtained between 100- 150 ^{o}C for residence time of 20 minutes. Solid-to solvent ratio and temperature played a significant role in the recovery of the polyphenolic compounds ($p< 0.05$). The subcritical water condition selectively affected the chemical structure of the phenolic acid and the flavonoids particularly quercetin-3-glucoside and quercetin-3- galactoside.

Acknowledgement

We greatly acknowledge Universal Beverages Limited (UBL), UK for supplying the cider apple pomace sample and Ghana Education Trust Fund (GETFund) for supporting the research financially.

References

Adom, K. K., & Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry, 50*, 6182-6187. https://doi.org/10.1021/jf0205099

Aida, T. M., Sato, Y., Watanabe, M., Tajima, K., Nonaka, T., & Hattori, H. (2007). Dehydration of -glucose in high temperature water at pressures up to 80MPa. *The Journal of Supercritical Fluids,* 381-388. https://doi.org/10.1016/j.supflu.2006.07.027

Arai, Y., Sako, T., & Takebayashi, Y. (2013). Supercritical fluids: molecular interactions, physical properties and new applications. *Springer Science & Business Media*.

Brendan, L. (2010). *Apples*. Washington DC, United State International Trade Commission.

Çam, M., & Aaby, K. (2010). Optimization of Extraction of Apple Pomace Phenolics with Water by Response Surface Methodology. *Journal of Agricultural and Food Chemistry, 58*(16), 9103-9111. https://doi.org/10.1021/jf1015494

Cetkovic, G., Canadanović-Brunet, J., Djilas, S., Savatović, S, Mandić, A., & Tumbas, V. (2008). Assessment of polyphenolic content and in vitro antiradical characteristics of apple pomace. *Food Chemistry, 109*(2), 340-347. https://doi.org/10.1016/j.foodchem.2007.12.046

Chandrasekara, A., & Shahidi, F. (2010). Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *Journal of Agricultural and Food Chemistry, 58*, 6706-6714. https://doi.org/10.1021/jf100868b

Clifford, M. N. (2000). Chlorogenic acids and other cinnamates–nature, occurrence, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture, 80*(7), 1033-1043. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<1033::AID-JSFA595>3.0.CO;2-T

D'Archivio, M., Filesi, C., Di Benedetto, R., et al. (2007). Polyphenols, dietary sources and bioavailability. *Annali dell'Istituto Superiore Di Sanita, 43*, 348-361.

Delgado-Vargas, F., Jimenez, A. R., & Pardes-Lopez, O. (2000). Natural pigments: carotenoids, anthocyanins and betalainsecharacteristics, biosynthesis, processing and stability. *Critical Reviews in Food Science and Nutrition, 40*, 173-289. https://doi.org/10.1080/10408690091189257

Diñeiro, G. Y., Valles, B. S., & Picinelli, L. A. (2009). Phenolic and antioxidant composition of by-products from the cider industry: Apple pomace. *Food Chemistry, 117*(4), 731-738. https://doi.org/10.1016/j.foodchem.2009.04.049

FOA (2011). Food and Agricultural Organization of the United Nations. Retrieved from http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567 FOASTAT [Accessed 2011]

Foo, L.Y. and Lu, Y. (1999). Isolation and identification of procyanidins in apple pomace. *Food Chemistry, 64*(4), 511-518. https://doi.org/10.1016/S0308-8146(98)00150-2

Harborne, J., & Williams, C. (2000). Advances in flavonoid research since 1992. *Phytochemistry, 55*, 481-504. https://doi.org/10.1016/S0031-9422(00)00235-1

Hollman, P. C., van Trijp, J., Buysman, M. N., van der Gaag, M. S., Mengelers, M. J., de Vries, J. H., & Katan, M. B. (1997). Relative bioavailability of the various antioxidant flavonoid quercetin from various foods in

man. *FEBS Letters, 418*(1-2), 152-156. https://doi.org/10.1016/S0014-5793(97)01367-7

Ibáñez, E., Mendiola, J. A., Rodríguez-Meizoso, Javier, S. F., Guillermo, R., & Alejandro, C. (2008). Antioxidants in plant foods and microalgae extracted using compressed fluids. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 7*(8), 3301-3309.

Ibrahim, S., Santos, R., & Bowra, S. (2019). Optimisation of Organic Solvent Mediated Solubilisation of Apple Pomace Polyphenolic Compounds Using Response Surface Methodologies. *International Journal of Chemistry, 11*(2), 1-21. https://doi.org/10.5539/ijc.v11n2p1

Ibrahim, S., Santos, R., & Bowra, S. (2018). Optimization of Subcritical Water Mediated Extraction of Apple Pomace Polyphenolics and their Antioxidant Activity. *J Chromatogr Sep Tech, 9*, 410. https://doi.org/10.4172/2157-7064.1000410

Johansen, K., (2000). Cider Production in England and France - and Denmark? Danbrew Ltd. A/S. Retrieved from https://www.yumpu.com/en/document/read/10581991/cider-production-in-england-and-france-and-denmark

Joshi, V. K. (1997). Fruit wines, 2nd edition Dr. YS Parmar University of Horticulture and Forestry, *Solan, India*.

Joshi, V.K., & Attri, B.L. (1991). Importance and scope of fruit based fermented beverages in India. *Beverage and Food World, 17*(4), 9-10.

Joshi, V. K., Kaushal, N. K., & Thakur, N. S. (1996) Apple pomace sauce development, quality of fresh and stored products. *Journal of. Food Science and Technology, 33*, 414-419.

Joshi, V. K., & Sandhu, D. K. (1996). Preparation and evaluation of animal feed using solid state fermentation of apple. *Bioresource Technology, 56*, 251-255. https://doi.org/10.1016/0960-8524(96)00040-5

Kaushal, N., Joshi, V., & Sharma, R. (2002). Effect of stage of apple pomace collection and the treatment on the physical-chemical and sensory qualities of pomace papad (fruit cloth). *Journal of Food Science and Technology, 39*, 388-393.

Kennedy, M., List, D., Lu, Y., Newman, R. H., Sims, I. M, Bain, P. J. S., Hamilton, B., & Fenton, G. (1999). Apple pomace and products derived from apple pomace: Uses, composition and analysis. *Berlin: Heidelberg: Springer.* https://doi.org/10.1007/978-3-662-03887-1_4

Khuwijitjaru, P., Suaylam, B., & Adachi, S. (2014). Degradation of Caffeic Acid in Subcritical Water and Online HPLC-DPPH Assay of Degradation Products. *Journal of Agricultural and Food Chemistry, 62*(8), 1945-1949. https://doi.org/10.1021/jf404850a

Kim, K.-H., Tsao, R., Yang, R., & Cui, S. W. (2006). Phenolic acid profiles and antioxidant activities of wheat bran Extracts and the effect of hydrolysis conditions. *Food Chemistry, 95*(3), 466-473. https://doi.org/10.1016/j.foodchem.2005.01.032

Le Bourvellec, C., & Renard, C. (2012) Interactions between polyphenols and macromolecules: quantification methods and mechanisms. *Critical Reviews in Food Science and Nutrition, 52*(3), 213-248. https://doi.org/10.1080/10408398.2010.499808

Liu, R. H. (2003). Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. *The American journal of clinical nutrition, 78*(3), 517-520. https://doi.org/10.1093/ajcn/78.3.517S

Lu, Y., & Foo, L. Y. (1997). Identification and quantification of major polyphenols in apple pomace. *Food Chemistry, 59*(2), 187-194. https://doi.org/10.1016/S0308-8146(96)00287-7

Lu, Y., & Foo, L. Y. (2000). Antioxidant and radical scavenging activities of polyphenols from apple pomace and vegetables. *Food Chemistry, 68*(1), 81-85. https://doi.org/10.1016/S0308-8146(99)00167-3

Luque de Castro, M. D., & Garcia-Ayuso, L. E. (1998). Soxhlet extraction of solid materials: An outdated technique with a promising innovative future. *Analytica Chimica Acta, 369*, 1-10. https://doi.org/10.1016/S0003-2670(98)00233-5

Luque de Castro, M. D., Jiménez-Carmona, M. M., & Fernández-Pérez, V. (1999). Towards more rational techniques for the isolation of valuable essential oils from plants. *TrAC Trends in Analytical Chemistry, 18*(11), 708-716. https://doi.org/10.1016/S0165-9936(99)00177-6

Mahawar, M., Singh, A., & Jalgaonkar, K. (2012). Utility of apple pomace as a substrate for various products: A review. *Food and Bioproducts Processing, 90*, 597-605. https://doi.org/10.1016/j.fbp.2012.04.007

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition, 79*, 727-747. https://doi.org/10.1093/ajcn/79.5.727

Monrad, J. K., Howard, L. R., King, J. W., Srinivas, K., & Mauromoustakos, A. (2010). Subcritical Solvent Extraction of Procyanidins from Dried Red Grape Pomace. *Journal of Agricultural and Food Chemistry, 58*(7), 4014-4021. https://doi.org/10.1021/jf9028283

Moon, J. K., & Shibamoto, T. (2010). Formation of volatile chemicals from thermal degradation of less volatile coffee *Journal of Agricultural and Food Chemistry, 58*, 5465. https://doi.org/10.1021/jf1005148

Nieto, A., Borrull, F., Pocurull, E., & Marcé, M. (2010). Pressurized liquid extraction: a useful technique to extract pharmaceuticals and personal-care products from sewage sludge. *TrAC Trends in Analytical Chemistry, 29*(7), 752-764. https://doi.org/10.1016/j.trac.2010.03.014

Ong, E. S., Cheong, J. S. H., & Goh, D. (2006). Pressurized hot water extraction of bioactive or marker compounds in botanicals and medicinal plant materials: Review. *Chromatography. A, 1112*, 92-102. https://doi.org/10.1016/j.chroma.2005.12.052

Pérez-Jiménez, J., Arranz, S., & Saura-Calixto, F. (2009). Proanthocyanidin content in foods is largely underestimated in the literature data: An approach to quantification of the missing proanthocyanidins. *Food Research International, 42*(10), 1381-1388. https://doi.org/10.1016/j.foodres.2009.07.002

Plaza, M., Abrahamsson, V., & Turner, C. (2013). Extraction and Neoformation of Antioxidant Compounds by Pressurized Hot Water Extraction from Apple Byproducts. *Journal of Agricultural and Food Chemistry, 61*, 5500-5510. https://doi.org/10.1021/jf400584f

Plaza, M., Amigo-Benavent, M., Del Castillo, M. D., Ibáñez, E., & Herroro, M. (2010). Facts about the formation of new antioxidants in natural samples after subcritical water extraction. *Food Research International, 43*(10), 2341-2348. https://doi.org/10.1016/j.foodres.2010.07.036

Purlis, E. (2010). Browning development in bakery products – A review. *Journal of Food Engineering, 99*(3), 239-249. https://doi.org/10.1016/j.jfoodeng.2010.03.008

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *The Journal of Nutrition, 130*(8), 2073S-2085S. https://doi.org/10.1093/jn/130.8.2073S

Schieber, A., Keller, P., & Carle, R. (2001). Determination of phenolic acids and flavonoids of apple and pear by high- performance liquid chromatography. *Chromatography. A, 910,* 265-273. https://doi.org/10.1016/S0021-9673(00)01217-6

Shalini, R., & Gubta, D. K. (2010). Utilization of pomace from apple processing industries: a review. *Food Science Technol, 47*(4), 365-371. https://doi.org/10.1007/s13197-010-0061-x

Tsao, R., & McCallum, J. (2009). *Chemistry of Flavonoids*. In; Eds.; Blackwell Publishing: USA: Ames, IA.

Ulbricht, R., J., Northup, S., J., & Thomas, J., A. (1984). A Review of 5-Hydroxymethylfurfural (HMF) in Parenteral Solutions. *Toxicological Sciences, 4*(5), 843-853. https://doi.org/10.1016/0272-0590(84)90106-4

Vasil'ev, Y. I., Morozov, A. N., Zaiko, G. M., & Moiseeva, V. G. (1976). The utilization of waste from vegetable and fruit canning plants. *Konservnaya-i-Ovoshchesushil naya-Promyshlennost, 3*, 32-34.

Vendruscolo, F., Albuquerque, P. M., Streit, F., & Esposito, E. (2008) Apple Pomace: A Versatile Substrate for Biotechnological Applications. *Critical. Reviews.in Biotechnology, 28*(1), 1-12. https://doi.org/10.1080/07388550801913840

Véronique, C. (2005). Polyphenols in foods are more complex than often thought. *Am J Clin Nutr, 81*(1), 2235-2295. https://doi.org/10.1093/ajcn/81.1.223S

Vinson, J. A., Su, X., Zubik, L., & Bose, P. (2001). Phenol antioxidant quantity and quality in foods: fruits. *Journal of Agricultural and Food Chemistry, 49*, 5315-5321. https://doi.org/10.1021/jf0009293

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology, 17*, 300-312. https://doi.org/10.1016/j.tifs.2005.12.004

Whiting, G. C., & Coggins, R. A. (1975). Estimation of the monomeric phenolics of ciders. *Journal of the Science of Food and Agriculture, 26*(12), 1833-1838. https://doi.org/10.1002/jsfa.2740261205

Wijngaard, H. H., & Brunton, N. (2009). The optimization of extraction of antioxidants from apple pomace by pressurized liquids. *Journal of Agricultural and Food Chemistry, 57*(22), 10625-10631. https://doi.org/10.1021/jf902498y

Wijngaard, H. H., & Brunton, N. (2010). The optimisation of solid–liquid extraction of antioxidants from apple pomace by response surface methodology. *Journal of Food Engineering, 96*(1), 134-140. https://doi.org/10.1016/j.jfoodeng.2009.07.010

Yue, T., Bai, X., Zhang, H., & Yuan, Y. (2012). Fractionation and anti-inflammatory effects of polyphenol – enriched extracts from apple pomace. *Bangladesh Journal of Pharmacology, 7,* 28-32. https://doi.org/10.3329/bjp.v7i1.10194

Zhou, Z., Zhang, Y., Ding, X., Chen, S., Yang, J., Wang, X., Jia, G., Chen, H., Bo, X., & Wang, S. (2007). Protocatechuic aldehyde inhibits hepatitis B virus replication both in vitro and in vivo. *Antiviral Research, 74*(1)*,* 59-64. https://doi.org/10.1016/j.antiviral.2006.12.005

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Production Methods and Physicochemical Characteristics of Cassava Inoculum and Attiéké from Southern Côte d'Ivoire

Justine Bomo Assanvo[1], Georges N'zi Agbo[1], Pierre Coulin[2], Christoph Heuberger[2] & Zakaria Farah[2]

[1]Laboratory of Biochemistry and Food Science, Félix Houphouët Boigny University, Abidjan, Côte d'Ivoire

[2]Institute of Food Science and Nutrition/Laboratory of Food Chemistry and Technology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland

Correspondence: Justine Bomo Assanvo, Laboratory of Biochemistry and Food Science, UFR Biosciences, Félix Houphouët Boigny University, Abidjan, 22 BP 582 Abidjan 22, Côte d'Ivoire. Tel: 225-0778-6755. E-mail: justinebomo2015@gmail.com

Received: August 22, 2019 Accepted: September 6, 2019 Online Published: September 18, 2019

doi:10.5539/jfr.v8n6p18 URL: https://doi.org/10.5539/jfr.v8n6p18

Abstract

Attiéké is a food from Côte d'Ivoire exported today in several countries. For evaluating production processes, determinant factors and quality attributes of attiéké, a production survey and a physicochemical study were carried out. The survey included 170 producers from the departments of Abidjan, Dabou and Jacqueville, major production areas. Traditional attiéké (Adjoukrou, Ebrié, Alladjan) and a commercial type Garba were investigated for physicochemical analyses. The cassava variety (98% of producers) had no effect on traditional cassava inoculum but had an impact on attiéké quality. The step of fermentation is necessary. The difference between studied attiéké related to microflora of inoculum whose identification may provide adequate explanations on the product. Respect for the various steps of manufacturing process is also essential. Physicochemicals confirmed observed differences between attiéké types. Organoleptic characteristics are criteria of differentiation between attiéké. The quality of preference is well-made grains without fibers. The results obtained also highlighted the risks faced by regular consumers of Garba due to its high cyanide content (12 mg/100g MS) compare to other attiéké (4.41 mg/100g MS).

Keywords: cassava, inoculum, production, attiéké, physicochemicals, organoleptic characteristics, Côte d'Ivoire

1. Introduction

Cassava, *Manihot esculanta* Crantz is one of the most important food crops in Côte d'Ivoire. With an estimated production of 4.54 million tonnes in 2016 (FAO, 2018), and a consumption of 100-110 kg / year per inhabitant living in urban areas, cassava plays a crucial role in food security of both rural and urban populations as well as job and incomes for the involved actors. According to FAO data, between 45 and 50% of Ivorian cassava production go to urban markets. Mainly consumed in the forms of placali (fermented dough) and attiéké (steamed semolina), cassava occupies a dominant place in culinary habits of Ivorians (Anonymous 1, 2016).

Several varieties of cassava exist and can be classified into three broad groups according to the root content in cyanogenic glucosides. These products are found in high doses in bitter varieties, justifying their transformation before consumption (Assanvo et al., 2017). Thus, toxic cassava varieties are used in manufacturing many fermented products, because of a better technological transformation aptitude. Among these traditional foods, attiéké remains the most consumed food (Assanvo, Agbo, Behi, Coulin, & Farah, 2006)

Cyanide detoxification occurs when plant tissue is disrupted and glycosides, known as lydene and lotaustraline, after disruption of the root cell structure, come into contact with B-glycosidases found in distinct intracellular compartments in intact tissue, being cleaved and producing glucose and α-hydroxynitriles. The latter, when catalyzed by hydrrixinitrile lyase, transforms into HCN and corresponding ketones in a process called cyanogenis (Cagnon, Cereda, & Pantarotto, 2002).

Originally, Attiéké was prepared and consumed exclusively in a restricted ethno-cultural setting in the Ivorian lagoon complex where the Adjoukrou, Ebrié, Alladjan, Avikam, Aizi and Neo ethnic groups live. Of these, Adjoukrou, Ebrié and Alladan remain the largest producers and consumers. However, the product has overflowed its original environment and is now consumed throughout the country and even beyond its borders

because of its "ready-to-eat" presentation (Assanvo et al., 2006). The attiéké, semolina or couscous of steamed cassava is a food produced from fermented cassava paste.

The multiple manufacturing methods of attiéké vary according ethnic groups and are based on a principle of traditional and non-standardized fermentation. In addition, attiéké has shifted to market production, a result of ever-increasing demand in large urban centers (Diop, 1992). The lack of control of production factors (cassava inoculums, temperatures and time) by new producers justifies most of the constraints related to production, including manufacturing defects and low yield (Assanvo, 2008).

The aim of this study was to highlight manufacturing processes, insufficiencies in commercial activity, physicochemical quality of the various ingredients, risks incurred by consumers and quality attributes sought by traditional producers of attiéké.

2. Method

2.1 Sampling for Surveys

The study was conducted from June to December 1999 in the departments of Abidjan, Dabou and Jacqueville (Lagoon Region), areas of regular attiéké production (Figure 1).

A pre-survey was conducted in Adiopodoumé in April 1999 to collect information for completing the questionnaire. The village of Adiopodoumé is located at 17 km from Adjamé, on the outskirts of Yopougon where lives a large proportion of Ebrié. A complementary survey was carried out in 2006 and 2017 for an update of data.

The investigation focused on factors determining the quality of finished products. They were raw material, inoculum, stages of attiéké production, different products obtained and their organoleptic characteristics. The selling prices were also sought. The sampling method was 3 cluster sampling with, at the primary level, the departments surveyed, at the secondary level the villages (or production sites) and at the tertiary level the producers surveyed (Table 1). The chosen primary units (departments surveyed) corresponded to a production area of an attiéké type: Abidjan (type Ebrié in majority and type Garba), Dabou (Adjoukrou type exclusively) and Jacqueville (Alladjan type only).

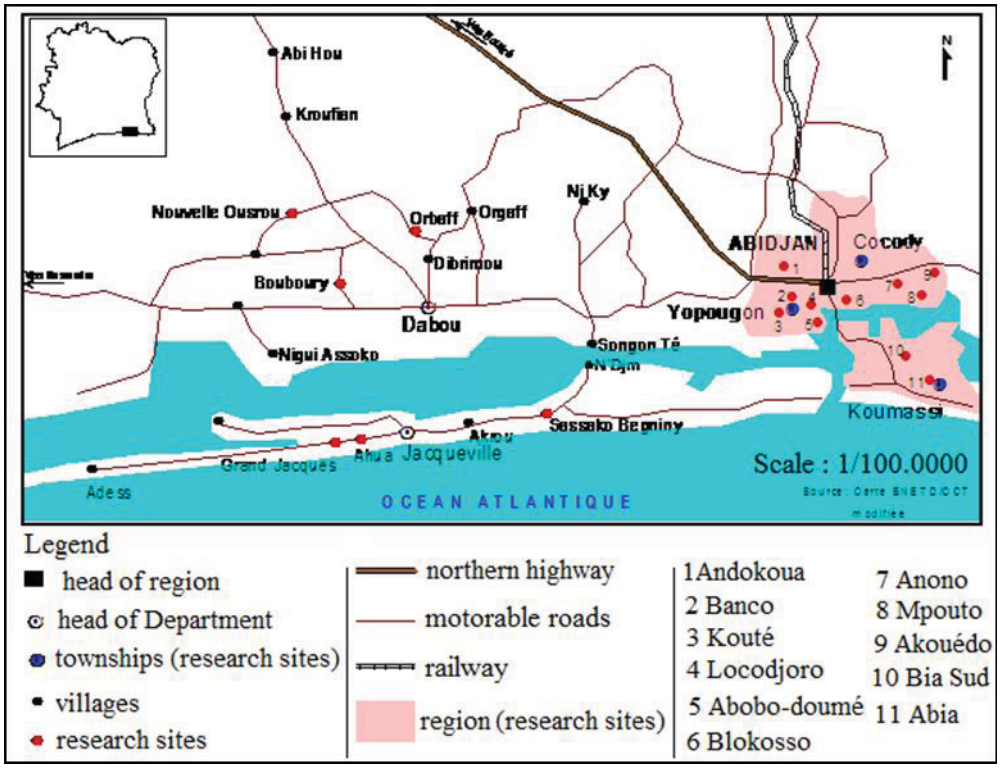


Figure 1. Map of study site presenting the attiéké production areas included in the survey

Scale: 1/1000.000; source: Map BNETD / CCT modified

Table 1. Sample size used for the attiéké production survey

Department of Lagoon region	Number of villages	Number of producers surveyed	Type of attiéké studied
Abidjan	11	110	Ebrié, Garba
Dabou	3	30	Adjoukrou
Jacqueville	3	30	Alladjan
Total	17	170	

In each department, 3 production villages were randomly selected. However in Abidjan zone, 11 villages were investigated. In each village, 10 producers randomly selected were interviewed. 170 traditional producers were interviewed in 17 production sites.

2.2 Sampling of Attiéké

Of 10 respondents in each village, three producers were randomly selected for collecting 100 g of attiéké. A total of 54 samples was collected, including 9 samples of each type of attiéké and Garba. At the laboratory, samples were dried and used automatically for determination of particle sizes. 54 other attiéké samples were used for physicochemical analyzes. For each producer, a total of 36 samples (9 inoculums/ethnic group/each attiéké) were also collected, directly transported and stored -18 ° C at the laboratory for physicochemical analyzes.

2.3 Sampling for Calculation of Quantities Required for Attiéké Production

At each sampling and each stage of manufacturing process, weighings were performed for calculating the amount required. This was performed from 9 yield values of IAC (Improved African Cassava) variety transformation.

2.4 Physicochemical Analyzes

2.4.1 Determination of Particle Size

50 g of attiéké were lyophilized (freeze dryer Christ Alpha 1-2) and reduced (MFC grinder, IKA ® LABORTECH,) in flour and passed through an air draft sieve (Alpine AG 910) during a rotation of 10 minutes. The characteristic variable of separation was the diameter equivalent to that of the largest sphere passing geometrically through the meshes of the sieve considered (Melcion, 2000).

2.4.2 Determination of Dry Matter of Cassava Inoculums and Attiéké Studied

Ten (10) g of sample were weighed (AG 24 Delta Range scale) and oven dried (Memmert) at 103 ± 2 °C for 24 hours. Dry matter was determined at constant weight AOAC (1984). Experiments were repeated three times.

2.4.3 Determination of pH and Total Titratable Acidity

10 g of attiéké or inoculum were suspended in distilled water (90 ml) and homogenized. The pH was measured using a Calimatic 761 pH meter, Knick, the further solution, obtained after adding distilled water (100 ml) and 2% phenolphthalein (8 drops), was titrated with NaOH 0.1 M (Amoa-Awua, Appoh, & Jakobsen, 1996). The assays were repeated 3 times.

2.4.4 Enzymatic Determination of Acid Levels

The determination of lactic acid and acetic acid was carried out by the enzymatic method of Böhringer Mannheim (acetic acid kit No. 0148261, D-/L-lactic acid kit No. 1112821, R-Biopharm GmbH, D-64293 Darmstadt).

2.4.5 Determination of Starch

Briefly the suspension obtained from 100 mg of attiéké flour and 25 ml of ethanol 40% (in distilled water) was mixed using a magnetic stirrer for 20 minutes at room temperature and centrifuged (Mistral 4L centrifuge) for 5 minutes at 2000 g. The pellet in distilled water (25 ml) was boiled for 20 minutes in a water bath and hydrolysed by adding 1 ml of termamyl ® (Novo Nordisk Ferment). A control sample of standard starch was glucose.

The absorbance of the resulting NADPH was measured at 340 nm with spectrophotometer (WTW photolab S12). The amount of glucose released is stoichiometrically equal to the amount of NADPH formed. The tests were repeated 3 times.

2.4.6 Determination of Total and Reducing Sugars

Attiéké flour (1 g) was treated with ethanol (80% v/v) and defecated with lead acetate solutions (10% v/v) and oxalic acid (10% v/v) according to the method of Agbo, Uebrsax and Hosfield (1985). Each extract obtained (1

ml) was treated with phenol sulfuric (5% v/v) for total sugars (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), or DNS (3, 5 Dinitro-Salicylic acid) for reducing sugars (Bernfeld, 1951).

The absorbance of the various solutions was measured at λ = 490 nm for total sugars and λ = 540 nm for reducing sugars using a spectrophotometer (WTW photolab S12). Standards were glucose for total sugar and glucose/fructose for reducing sugars. Each sample was assayed in triplicate.

2.4.7 Determination of Cyanide

This assay was performed on 100 mg of attiéké flour using picrate method (Bradbury, Egan, & Bradbury, 1999). A kit (all reagents) has been produced: kit B2 (cassava semolina) and Absorbances (A) was measured at 510 nm with spectrophotometer (WTW photolab S12) following immersion of picrate paper in distilled water (5 ml) for 30 minutes.

A control (value = 0) and a standard (expected value = 50 ppm) were included. Total cyanide content (ppm) was calculated using the formula: Cyanide (ppm) = 396 A. The assays were performed in triplicate for each sample.

2.4.8 Protein Determination by the Kjeldahl Method

The protein assay was performed using Kjedahl method associated to a Buchi. The determination of protein was made on 1 g to 0.01 mg of attiéké fine powder. The mineralization was carried out using a Büchi 435 digester (Büchi Laboratoriums, Technik AG). The distillation and determination of nitrogen were carried out using a distillation unit Büchi 339 (Büchi Laboratoriums, Technik AG,). The protein content was obtained directly at this unit, using a conversion factor of 6.38. A triple determination was performed on each sample (Commission du Manuel Suisse des Denrées Alimentaires, 1994).

2.4.9 Determination of Percentage of Mineral Substances (ash)

3 g of attiéké flour was burned in a muffle furnace at 450ºC and incinerated until complete mineralization. The residue was determined by the gravimetric method (AOAC, 1990) in triplicate.

2.4.10 Determination of Fat

Fat determination was performed using Soxhlet method (AOAC, 1995). The fat contained in 8 g of attiéké flour was extracted with 180 ml of petroleum benzine or petroleum ether at 100 °C for 4 hours. After evaporation, fat amount weighed in triplicate.

2.4.11 Determination of Total Dietary Fiber (fat)

The total dietary fiber assay was performed using the modified enzymatic-gravimetric method (Commission du Manuel Suisse des Denrées Alimentaires, 1994).

2.4.12 Calculation of Digestible Carbohydrates

The percentage of digestible carbohydrates in samples was obtained by difference between dry matter and sum of protein (% P), fat (% MG), ash (% C) and fiber.

$$\% \text{ Digestible carbohydrate} = MS - \Sigma (\% MF + \% P + \% C + F) \quad (1)$$

2.4.13 Calculation of Production Yield

At each stage of manufacturing process of each type of attiéké, the quantities of the various stages (from cassava to attiéké) were weighed. The final yield of the transformation (%) of cassava roots in attiéké was determined.

2.5 Statistic Analysis

The results of production surveys were recorded in a database and frequencies were calculated. The analysis were performed using SAS software (version 8.0) based on biochemical parameters. A one-way model of variance analysis was used. The factor is the type of attiéké.

The dependent variables (responses) considered were biochemical parameters. Using the Student-Newman-Keul multiple comparison tests with the relative risk assessed, a ranking of averages was performed.

The significant threshold is α = 0.05.

The results of particle size distribution were interpreted by applying the logarithmic form of the Rosin, Rammler and Sperling equation.

3. Results

3.1 Attiéké Production

3.1.1 Raw Materials

The main raw material of attiéké, is roots of sweet and bitter cassava depending on the regions of production. Previously, attiéké was produced from only bitter varieties or the mix. Today, mixture is predominant, bitter variety (IAC) becoming insufficient. Most producers (about 98%) state bitter varieties give a better quality attiéké. The cassava roots used were fresh (0-1 week) depending on cassava varieties.

3.1.2 Production Techniques of Attiéké

3.1.2.1 Principle of Manufacture and Desired Characteristics

According to producers surveyed (98%), cassava variety has an impact on attiéké quality, however other parameters such as fermentation, granulation, drying and steaming, remain decisive stages for obtaining a good attiéké.

3.1.2.2 Preparation of Cassava Inoculum

In the study area, two types of inoculum are used (Figure 2):

Figure 2. Two different types of inoculum obtained after 2-3 days of cassava root fermentation

A) Inoculm of peeled and boiled cassava root (Assanvo, 2008)

B) Inoculum based on unpeeled and braised cassava root (Assanvo, 2008)

The peeled and boiled cassava root inoculum is increasingly used.

The braised cassava root inoculum is endangered in all regions visited due to preparing constraints according to producers (82.35%).

The traditional inoculum called "Lidjrou" in Adjoukrou language, "Magnan" in Ebrié and "Bédé Fon" in Alladjan is obtained from a spontaneous fermentation. This inoculum is made from one or many varieties (sweet or bitter) of cassava. About 2-3 kg of fresh cassava roots were peeled, washed and boiled in water (100 ° C ± 2) for 5-10 minutes. The cassava was cooled at room temperature (28-35 °C) and packaged in a bag or set of fillets already used for previous fermentations. The whole is put in a basket or cardboard and kept at room temperature (28-35 °C) for 1-3 days. For braised inoculum, non peeled cassava is cooked on embers.

Cassava variety does not matter much for obtaining a good inoculum. It should only be well fermented, very soft to the touch, yellowish (covered with mushrooms), releasing a pleasant smell when ready. 2-3 days post fermentation, cassava is cleaned and crushed. The inoculum still represents 5-10% of fresh cassava roots used for preparing attiéké and Garba.

3.1.2.3 Peeling and Washing

The fresh roots are peeled, crushed and washed several times for eliminating all visible impurities and preserving the color of finished product. For attiéké Garba, all these treatments are useless and one washing can be done.

3.1.2.4 Red Palm Oil

Oil is mixed with inoculum just before grinding. Its amount usually did not exceed two tablespoons: 0.1% for Adjoukrou and Alladjan and about 0.1-0.15% for Ebrié. For Garba, about 1-2% is added. Producers used discolored by heating or refined (rich in vitamins A and E) palm oil. 98% of producers state the small amount of added oil has an impact on the color of attiéké and prevents grains from stick.

3.1.2.5 Grinding of Crushed Cassava, Inoculum and Palm Oil

The mixture palm oil-inoculum is added to cleaned fresh cassava roots before grinding or to the resulting paste. The smaller the grinder's mesh (diameter = 1.5-2 mm), the more it gives a fine paste. For attiéké Garba, the sieve meshes are larger (diameter = 3 mm), leading to a coarser texture of the finished product.

3.1.2.6 Wetting with Water of the Dough

Water (about 10-20% of paste) is added to the dough for well homogenization. The quantity of water is greater for attiéké Ebrié (10-20%) than Adjoukrou and Alladjan types (7-10%). However, the dough should not be too liquid or compact.

3.1.2.7 Fermentation of Dough

The fermentation time (12-15 hours) of dough varies according producer who feels quality to the touch. All producers unanimously state the inoculum makes dough suitable for processing into grains. The fermentation technique does not differ according types of attiéké. For Attiéké Garba, the time and quality of fermentation were variable. This time (≤ 6 hours) largely depends on the delivery time and quantity ordered.

3.1.2.8 Pressing of Fermented Dough

All the producers surveyed used a mechanical screw press for pressing fermented dough in a nylon bag to eliminate excess water and facilitate granulation. Producers knew the appropriate humidity rate checked to the touch. Depending on the daily amounts of cassava roots used, the pressing time was 1-3 hours. This stage was also dependent on cassava variety. To save time, some producers combined fermentation (about 2-8 hours post fermentation) and pressing. This process is widely used for attiéké Garba.

3.1.2.9 Sieving the Dough after Pressing

After pressing, the fermented flour is sieved successively through large meshes (4-5 mm) and small mesh (1.5-2 mm) for removing impurities, and obtaining semolina (crackling) or cleaning and fining flour ready for granulation.

3.1.2.10 Granulation of Flour

Grains were obtained using either a plastic bowl (510 liters) for Ebrié or a wooden bowl (Adjoukrou and Alladjan). Granulation occurs as a result of rotational movements and is stopped when grains are round, well-formed and solid (Figure 3). Producers can decide to favor a size: large, medium, small and fine grains.

The choice of grain size is related to the type of attiéké. Hand granulation does not exist in manufacturing process of Garba type.

3.1.2.11 The Drying of Uncooked Grains

Drying is used to hardening grains and reducing water amount for maintaining certain moisture in the finished product after cooking. Grains are sun dried on large plastic sheets (Ebrié) or raffia trays (Adjoukrou and Alladjan) for at least 1 hour depending on solar radiation. The quality of drying is appreciated by the naked eye. In rainy weather, grains are exposed in the kitchen at 30-40ºC due to fire.

Ebrié from Abidjan used various drying racks (table or stool) for hygiene measures. There is no drying stage for attiéké Garba, resulting in pasty and tacky characteristics.

3.1.2.12 The Winnowing of Uncooked Dried Grains

This step is made in raffia trays (Adjoukrous and Alladjans) or plastic bowls (Ebriés). It requires the wind, for eliminating fibers and small size grains. The winnowing step is not applied for attiéké Garba containing too fibers.

Figure 3. Uncooked semolina of cassava roots before drying (Assanvo, 2008)

3.1.2.14 The Cooking

Steaming is done using traditional couscous cooker about 20-30 minutes. The cooked grains are cohesive with a slightly translucent appearance (Figure 4).

A characteristic pleasant slightly fermented smell emerges. However, cooking with wood fire revealed a small aroma of charcoal. The color (cream, light yellow or beige or off-white, Figure 5) depended on cassava variety and oil (case of attiéké Garba).

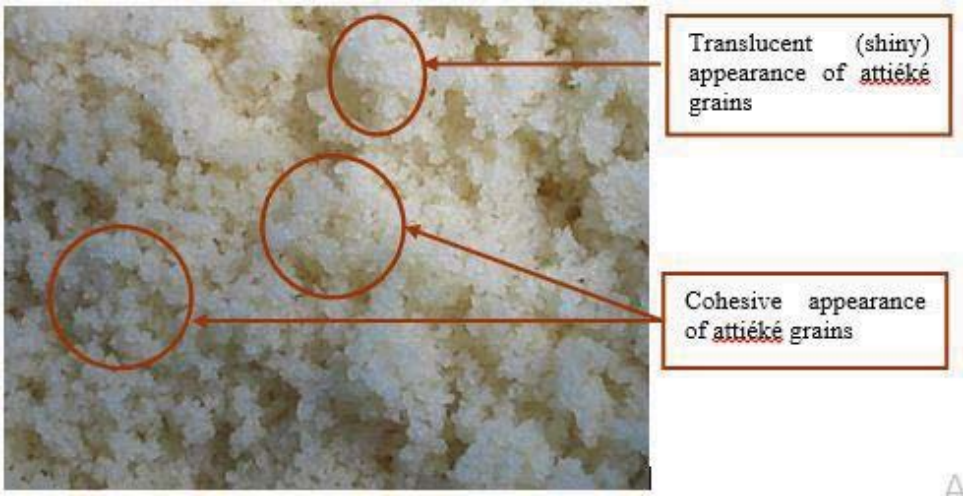


Figure 4. Translucent and cohesive aspects of attiéké (Assanvo, 2008)

Translucent (shiny) appearance of the grains

Cohesive appearance of attiéké grains

Figure 5. Colors of freshly prepared Ebrié attiéké (Assanvo, 2008)

a) Off-white color

b) Yellow-cream color

3.2 Characterization of Different Types of Attiéké

Each type of attiéké is linked to a process specific to each ethnic group. Thus, the Adjoukrou produce attiéké Adjoukrou, the Alladjan, attiéké Alladjan and the Ebrié, attiéké Ebrié. The Ebrié put more emphasis on production of attiéké Garba than the other ethnic groups. The manufacturing processes of studied attiéké are presented respectively in Figures 6a, 6b, 6c and 6d. Table 2 summarizes differences between the quality of traditional attiéké (Adjoukrou, Alladjan and Ebrié) and commercial attiéké Garba (Figure 7).

3.2.5 Quality Descriptors of Attiéké Types and their Importance

A list of quality descriptors of attiéké has been established among producers. The descriptors are ranked in order of importance of percentages as attributed by the 170 producers interviewed. These results revealed the importance of taste (94%), odor (64%), shelf life (56%) and color (40%), (Figure 8).

Color (40%) and translucent appearance (30%) also appear as descriptors that highly matter in the appreciation of producers as attributes rounded grains, well-formed grains (30%) and quantity of fibers (20%).

Table 2. Differences in assessment parameters of traditional attiéké (quality 1) and Garba (quality 2)

Rating parameters	Attiéké (quality 1)	Garba (quality 2)
Age of inoculum:	2-3 days	Indefinite, may vary depending on demand (1-4 days)
Quantity of inoculum in relation to the amount of fresh cassava roots	On average 7-10% depending on producing ethnic group (attiéké type)	On average 2-5% compensated by a large amount of oil
Quantity of discolored palm oil after heating	Small (0.1%)	Large (1-2%)
Grains	Well-formed	Poorly formed or absence
Grains drying	Mandatory	Eliminated
Presence of fibers (winnowing)	Very little	Much
Humidity	Normal (varying on average from 44-49% depending on the type of attiéké)	Abnormal (varying on average from 49-61% and can reach 80% sometimes)
Conservation	1 week at least	2-3 days
Consumption	Family and commercial	Only commercial
Cost	Expensive (1-2 fold more expensive than the Garba)	Cheaper
Quality (taste, appearance, odor)	Very good to good	Good to bad

Other descriptors (provides a well-being, stickiness, size, moisture, elasticity) were secondary for the 170 producers (Figure 8). Descriptors such as firmness, attiéké well-cooked and flavor had no importance.

3.2.6 Frequency of Production, Average Daily Quantities and Prices

The production of attiéké per week is done at frequency of 2-3 times depending on the ethnic group (Table 3). "Agbodjama" is prepared once or twice (few times) a week.

The average amount produced/day was around 30 kg for attiéké and 60 kg for Garba. However, this quantity may vary up or down depending on the amount and variety of cassava, or available labor. Attiéké Garba is cheaper than the other three types of attiéké. Commercial attiéké Ebrié is cheaper compared to Adjoukrou and Alladjan types. However, attiéké "Agbodjama" is the most expensive. Table 3 gives prices/kg by type of attiéké.

Attiéké Ebrié is most often packaged in plastic bags of 100 CFA F ball unlike the Adjoukrou and Alladjan types. The prices of balls of Attiéké Adjoukrou and Alladjan ranged from 150 CFA F to 250 CFA F. The smallest share of attiéké Garba sold for immediate consumption at the retailer costs 50 CFA F. This rate does not exist for the usual attiéké.

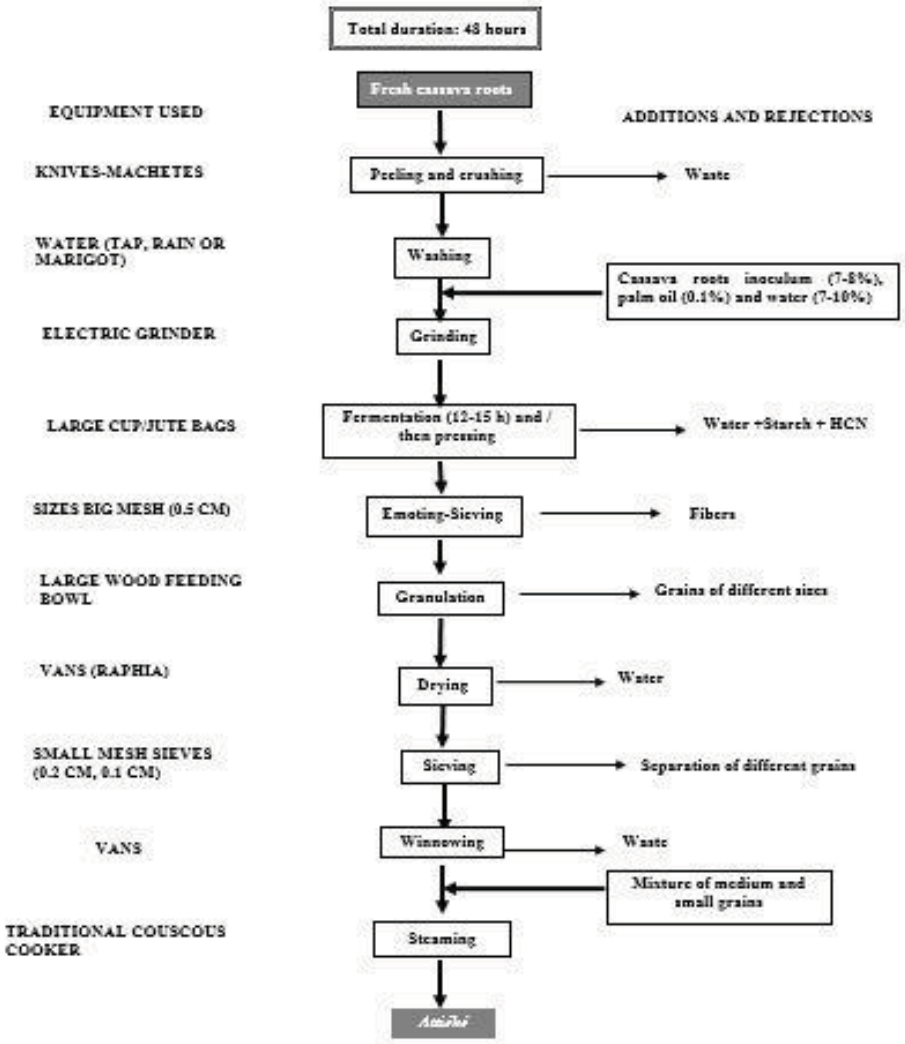


Figure 6 (a). Diagram of the traditional manufacturing process of attiéké Adjoukrou

Table 3. Frequencies of production, daily quantities and prices of different types of attiéké according to the producing ethnic Groups

Types of attiéké	Frequency of Production by Producer/6 days	Quantity (Kg/day)	Price/Kg (CFA F)
Adjoukrou	2-3	40-50	350-400
Alladjan	2-3	30-40	300-350
Ebrié Agbodjama	1	20-25	450-500
Usual Ebrié	2	40-50	250-300
Garba	5	50-60	150-200

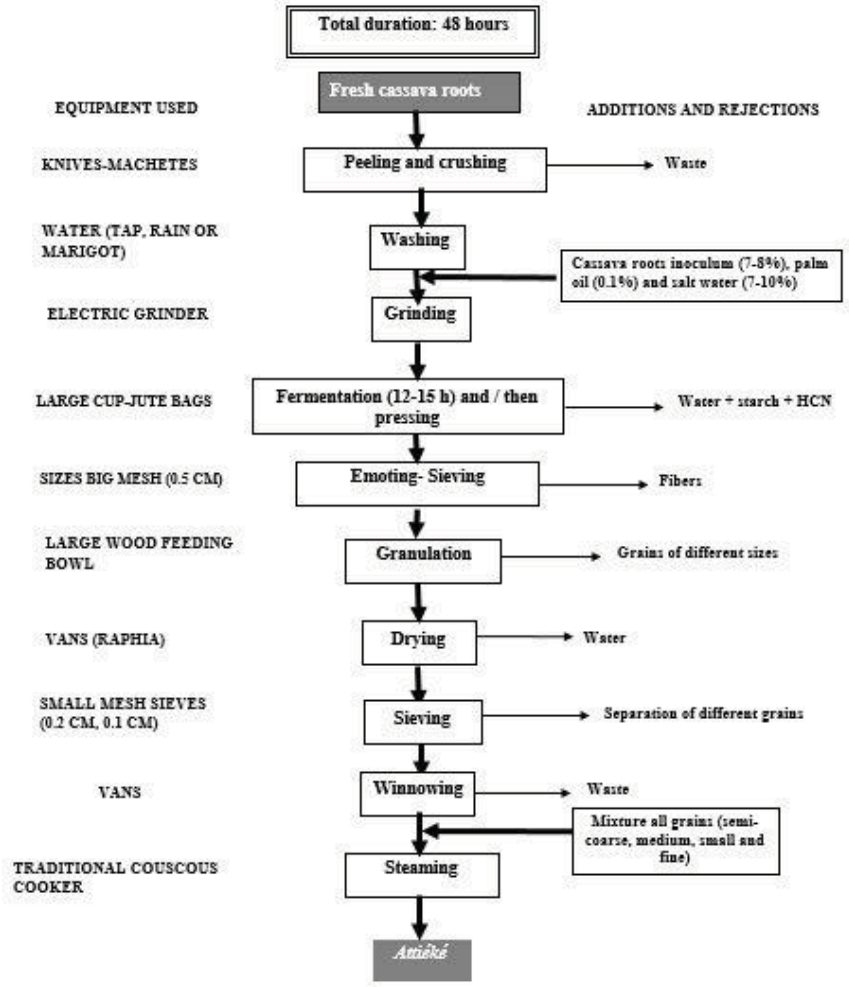


Figure 6 (b). Diagram of the traditional process of manufacturing attiéké Alladjan

3.3 Physicochemical Parameters of Inoculum and Attiéké

3.3.1 Granulometry of Attiéké

The grains size of studied attiéké can be classified in 5 categories: extra fine (Ø <0.80 mm), fine (0.80 mm ≤ Ø <1.00 mm), small (1.00 mm ≤ Ø <1.50 mm), medium (1.50 mm ≤ Ø <2.00 mm) and large (2 mm ≤ Ø <3 mm). On average, 20% of dried attiéké grains had a diameter < 1 mm, 70% had a diameter between 1 and 2 mm and 10% diameter ≥2 mm but < 3 mm.

These size ranges may vary slightly depending on the variety of cassava used, fermentation and ability to make grains easily. Garba usually had grains with diameter < 0.8 mm.

3.3.2 Physicochemical Characteristics of Inoculums from Boiled Cassava Variety IAC

There was no significant difference between cassava inoculums regardless of types of attiéké (Table 5). Only the inoculum of Garba had low rates for titratable acidity, lactic acid and acetic acid. Its pH was higher than the other inoculums (Table 4). Fresh cassava was not acid (pH = 6.01), (Table 4).

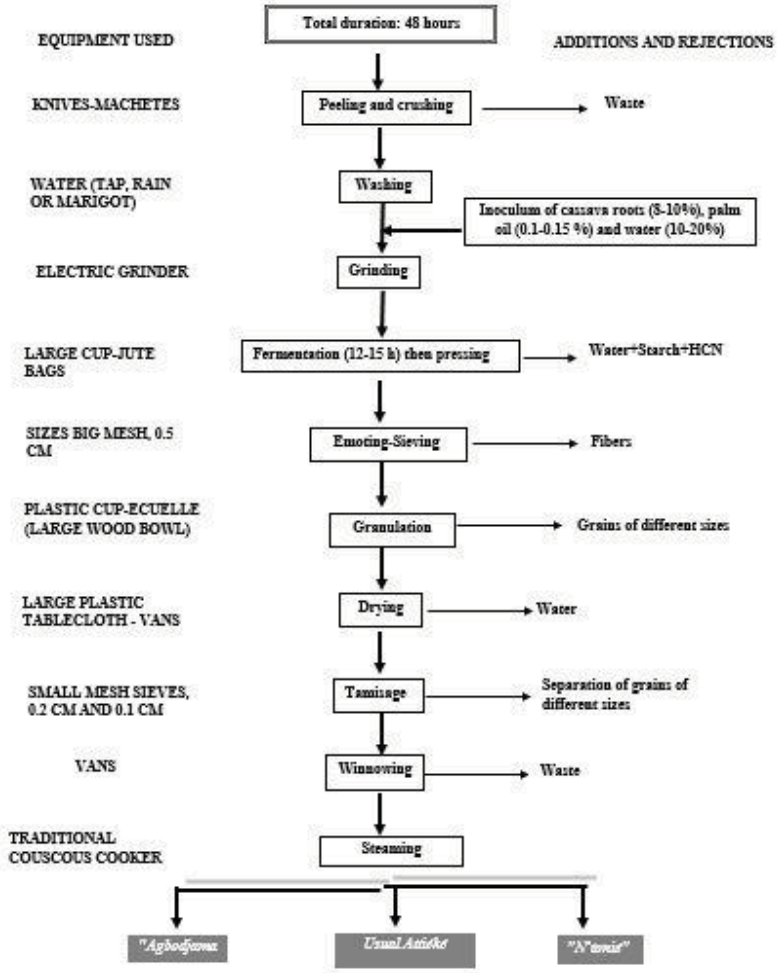


Figure 6 (c). Diagram of the traditional manufacturing process of attiéké Ebrié

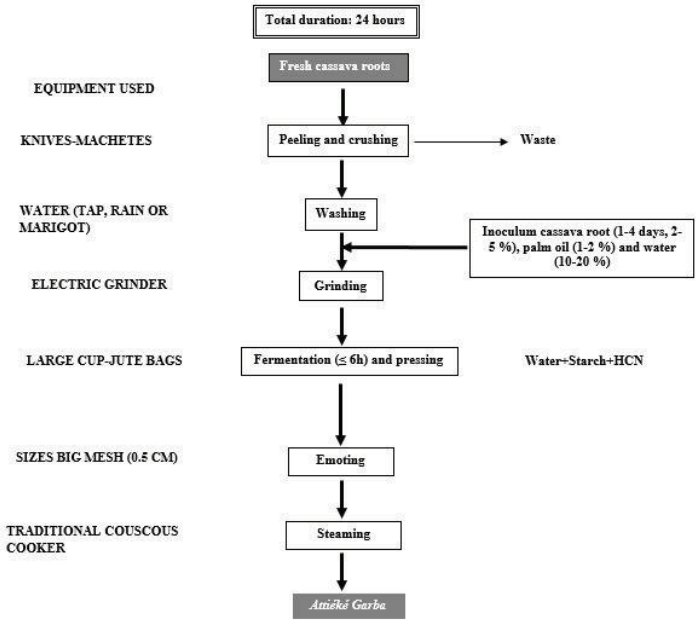


Figure 6 (d). Diagram of the traditional manufacturing process of attiéké Garba

Figure 7. Attiéké Garba packaged in a plastic bag and on a plate (Assanvo, 2008)

Table 4. Average of physicochemical characteristics of samples of boiled or fresh cassava inoculum.

Physicochemical Characteristics studied	Fresh cassava IAC Variety	Ebrié	Adjoukrou	Alladjan	Garba
	Mean ± standard deviation				
pH	6.01±0.11 a	4.9±0.03 b	5.2±0.08 b	5.3±0.04 b	5.48±0.01 c
Titratable acidity (mEq/100g)	0.1±0.01 a	0.36±0.12 b	0.33±0.05 b	0.30±0.06 b	0.25±0.07 c
Lactic acid	0.09±0.05 a	0.27±0.07 b	0.25±0.09 b	0.23±0.08 b	0.18±0.01 c
Acetic acid	0.02±0.01 a	0.08±0.03 b	0.07±0.05 b	0.08±0.02 b	0.05±0.01 c

N=36

The averages followed by the same letters in same line are not significantly different at 5%

IAC = Improved African Cassava Variety

3.3.3 Physicochemical and Biochemical Characteristics of Studied Attiéké

On average, the pH of studied attiéké was acid and varied from 4.56 (usual attiéké Ebrié) to 4.70 (attiéké Garba). This gave attiéké its acid character without being sour. The pH of attiéké Garba is the least acid. Attiéké "Agbodjama," Adjoukrou and Alladjan had less acid pH than attiéké Ebrié and "N'tonié" which had the same pH (Table 5).

Attiéké Garba had a significant different pH ($p < 0.05$) from other attiéké.

The acidity of attiéké Garba is lower than the other studied attiéké (Table 5). Analysis of variance showed a significant difference between Garba and other types of attiéké.

The moisture content of Garba was significantly different ($p < 0.05$) from Agbodjama (43.52%), usual attiéké Ebrié (48.45%), "N'tonié" (44.55%), attiéké Adjoukrou (46.71%) and attiéké Alladjan (47.41%).

The lactic acid rate was low in all types of attiéké but was higher in usual attiéké Ebrié (1.13±0.17%). In contrast, its content in attiéké Garba remained the lowest (0.58 ± 0.16%).

The level of acetic acid was very low regardless of attiéké types (Table 5).

All biochemical parameters sought in the four studied attiéké are present in varying amounts (Table 6). At statistical level, there is a significant difference ($P < 0.05$) at least between two types of attiéké (Table 6).

The starch content was high and varied on average from 95 g/100g DM (attiéké Ebrié Agbodjama) to 78.89 g/100g DM (attiéké Garba). Analysis of variance indicated a significant difference between starch content of studied attiéké. The starch content of attiéké Garba was different ($p < 0.05$) and the lowest (Table 6).

Total sugar (1.17-1.58 g/100g DM) and reducing sugar contents of studied attiéké (0.15-0.43 g/100g DM) were very low. Attiéké Agbodjama had the highest rate (2.05±1.03 g/100g DM) while the lowest total sugar content was recorded for attiéké Garba (1.14±0.17 g/100g DM. The ANOVA indicated a significant difference ($p < 0.05$) between the 4 types of attiéké, meaning at least two attiéké were different from the others.

Table 5. Mean rate of physicochemical characteristics of 54 attiéké samples

Physicochemical characteristics studied	Agbodjama Ebrié	Usual Ebrié	N'tonié Ebrié	Adjoukrou	Alladjan	Garba
	Mean ± standard deviation					
Dry matter (%)	56.48±2.14 a	51.55±2.71 b	55.45±0.35 a	53.29±0.98 b	52.60±1.20 b	47.41±7.84 c
Humidity (%)	43.52±2.14 a	48.45±2.71 b	44.55±0.35 a	46.71±0.98 ab	47.41±1.20 b	52.59±7.84 c
pH	4.65±0.03 a	4.56±0.13 ab	4.58±0.01 ab	4.67±0.11 a	4.63±0.15 a	4.70±0.18 c
Titratable acidity (mEq/100g)	0.85±0.04 b	1.20±0.10 a	1.02±0.02 a	0.83±0.09 c	0.90±0.12 b	0.68±0.20 d
Lactic acid	0.79±0.11 a	1.13±0.17 b	0.97±0.12 b	0.77±0.23 c	0.84±0.09 a	0.58±0.16 d
Acetic acid	0.06±002 a	0.10±0.04 b	0.07±0.01 a	0.09±0.03 b	0.07±0.012 a	0.04±0.05 a

N=54, The averages followed by the same letters in same line are not significantly different at 5%

Attiéké N'tonié = attiéké Ebrié possessing small round grains of the same size

Attiéké Agodjama = attiéké Ebrié possessing round grains compared to fish eggs with substantially the same size

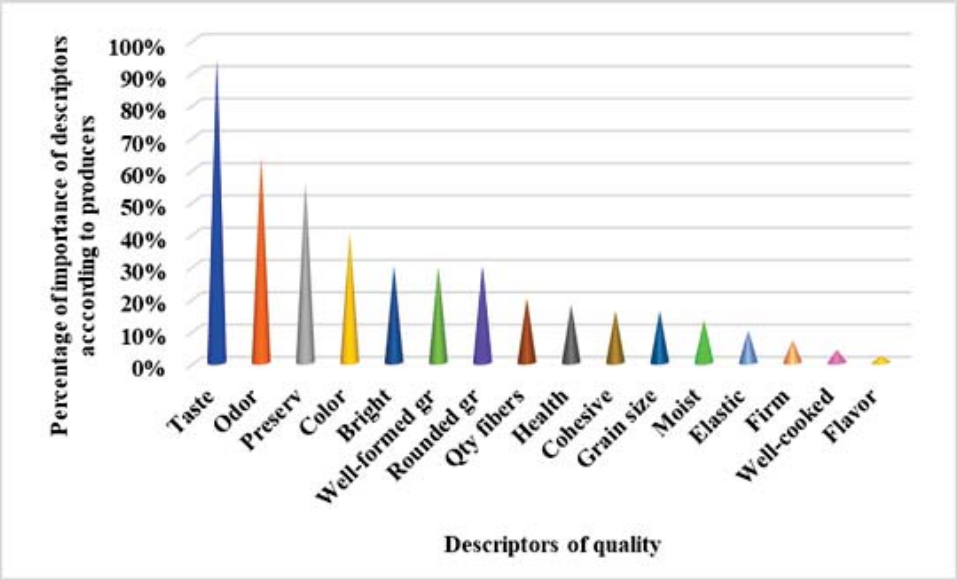


Figure 8. Importance percentages of the quality descriptors of attiéké defined by producers

Preserv: good conservation; bright aspect: translucent character; Gr well formed: well-formed grains; Rounded gr: rounded grains; Qty fibers: amount of fibers; Health: provides a well-being; cohesive: cohesion between grains; Well cooked: well-cooked attiéké

The very low reducing sugar rates ranged from 0.43±0.18 g/100g DM (attiéké Ebrié Agbodjama) to 0.15±0.66 g/100g DM (attiéké Garba). Usual attiéké Ebrié, Adjoukrou and Alladjan were not significantly different (α = 0.05%), (Table 6). Attiéké Garba had the highest contents of fiber (2.53±1.05 g/100g DM), fat (1.22±0.38 g/100g DM) and cyanide (12±2.02 g/100g DM), (Table 6). There was a significant difference between the types of attiéké studied, at least two attiéké were different from the others (Table 6).

The fat content was very low and varied from 0.057 to 1.22% of dry matter. The ash content of attiéké from cassava variety IAC was between 1.62 (attiéké Garba) and 1.90% (attiéké Adjoukrou).

Table 6. Mean rate of biochemical characteristics of 54 attiéké samples

Physicochemical characteristics studied	Agbodjama Ebrié	Usual Ebrié	N'tonié Ebrié	Adjoukrou	Alladjan	Garba
	Mean ± standard deviation					
Starch (mg/100g)	95.35±0.64 a	91.74±0.85 a	83.12±1.12 b	93.51±1.65 a	90.94±0.97 a	78.89±2.16 b
Total sugars	2.05± 1.03 a	1.66 ± 0.55 b	1.32 ± 0.67 c	1.55 ± 0.37 b	1.44 ± 0.25 b	1.14 ± 0.17 d
Reducing sugars	0.43 ± 0.18 a	0.33 ± 0.14 b	0.27 ± 0.08 bc	0.35 ± 0.17 b	0.30 ± 1.02 b	0.15 ± 0.10 c
fibers	1.15 ± 0.57 a	1.43 ± 0.63 b	1.10 ± 0.71 a	1.56 ± 0.88 c	1.48 ± 0.92 b	2.53 ± 1.05 d
Protein (mg/100g)	4.3 ± 2.06 a	3.95 ± 1.12 a	4.00 ± 2.25 a	4.2 ± 2.07 a	3.89 ± 1.23 a	3.02 ± 1.33 b
Fat (mg/100g)	0.075 ± 0.02 a	0.078 ± 0.04 a	0.074 ± 0.05 a	0.065 ± 0.08 a	0.057 ± 0.035 b	1.22 ± 0.38 c
Ash (mg/100g)	1.78 ± 1.21 a	1.80 ± 1.18 a	1.76 ± 0.84 a	1.90 ± 0.68 b	1.84 ± 1.07 c	1.62 ± 1.05 d
Cyanide (mg/100g)	4.95 ± 1.01 a	5.18 ± 1.51 a	4.62 ± 0.78 b	3.88 ± 1.03 c	3.52 ± 1.15 c	12 ± 2.02 d

N = 54, The averages followed by the same letters in one line are not significantly different at 5%

Attiéké Ntonié =attiéké Ebrié possessing small round grains of the same size

Attiéké Agodjama = attiéké Ebrié possessing round grains compared to fish eggs with substantially the same size

The protein contents ranged between 3.02±1.33 g/100g DM (attiéké Garba) and 4.3 ± 2.06 g/100g DM (attiéké Ebrié Agbodjama). There was a significant difference between attiéké Garba and other types of attiéké that had similar level of protein.

3.3.4 Production Yield of the 4 Types of Attiéké

The final yield of attiéké production showed that cassava variety IAC had the highest value for attiéké Garba (38.07%), and the lowest (31.93%) for attiéké Alladjan. However, the yield of attiéké Alladjan was close to that of attiéké Adjoukrou (32.42%) and usual attiéké Ebrié (33.83%).

The fiber content is higher in attiéké Garba compared to other types of attiéké and ranges from 1.10±0.71g/100g DM (″N'tonié″) to 2.53±1.05 g/100g DM for the Garba.

4. Discussion

4.1 Influence of Traditional Attiéké Production Technologies on Its Quality

4.1.1 Technological Process of Attiéké and Constraints Related to Food Chain (from Cassava to Consumer)

The results on the impact of cassava variety and inoculum on the quality of traditional attiéké confirmed those of Kouadio, Kouakou, Angbo and Mosso (1991) focused on traditional preparing methods of attiéké in Southern Côte d'Ivoire. The traditional manufacturing methods of inoculum and attiéké, preserving the finished product are similar to a few nuances. Nowadays, great similarities were observed because of cultural mix and growing demand. The consequence of this increasing demand is reduction of the time of manufacturing process. This time rose to 12-15 hours while it was 1-2 days according to Kouadio et al. (1991). Also, the proportion of inoculum varies depending on cassava variety used (quantity and quality). Appreciation of the end and quality of fermentation has remained the same, using the touch (Kouadio et al., 1991). The semolina obtained following modification of dough texture during fermentation, will facilitate shaping of small granules with capacity to absorb a large amount of water (Assanvo et al., 2006). According to Moorthy and Mathew (1998) and McFeeters (2008), lactic acid bacteria, main agents of fermentation of cassava paste, contribute to the texture, flavor, and production of aromatic compounds. Grinding facilitates fermentation, suppressing cellular structures, homogenizing the environment and promoting development of microorganisms (Mescle & Zucca, 1996). For beneficial impact of this step on attiéké quality (Anonymous 2, 2005), ensuring best conditions is important for this process.

The adjustments in manufacturing attiéké Garba may lead to serious consequences on consumers' health. Risks are associated with cyanide residues in the finished product due to reduction in fermentation time of bitter cassava (Assanvo, Agbo, & Farah, 2019).

In the case of attiéké Garba, some steps such as granulation and drying are removed and cooking time is reduced. However, these steps help eliminating cyanide. Zacarias, Esteban, Rodrigues, & de Souza Nascimento (2017) confirm fears and possible exposure to cyanide may be predicted. Heuberger (2005) support these results suggesting cyanide compounds less than 10 mg/kg may be considered safe for consumption. According to Obilie, Tano-Debrah and Amoa-Awua (2004), attiéké produced in Southwestern Ghana ("akyeke") is not at risk of toxicity because all steps of the manufacturing process are followed. Cyanide in "akyeke" is very low (1.4-2.8

mg CN equivalent/kg dry matter) and the cassava varieties used are sweet (69.3-110 mg CN equivalent/kg dry matter).

Cassava variety also has impact on the color of attiéké as different colors were observed for the different types studied. According to Sotomey Ategbo, Mitchikpe, Gutierrez and Nago (2001), colors of attiéké most often depend on cassava varieties, moisture of rolled dough and amount of palm oil. New cooked attiéké has a color ranging from yellowish to whitish or dark, cream color is preferred in Côte d'Ivoire. The color darkens more 3-5 days post preparation with quality deterioration. Bavaro et al. (2017), reported molds are responsible for dark color following spontaneous fermentation. Also the presence of oil could causes a slight oxidation.

Food packaging should allow food reaching consumers under optimal conditions (Anonymous 3, 2006). Packaging helps maximizing the life of products, carrying this important information on the label (Anonymous 3, 2006). At the moment, the types of packaging used by producers is not a guaranty of safety, no scientific study determines their quality.

4.1.2 Importance of Quality Descriptors of Attiéké According Producers

Quality attributes are of paramount importance in the assessment of attiéké by producers (Assanvo, Agbo, Brunnchweiler Beez, Monsan, & Farah, 2018). The descriptor taste is capital in the choice of a good attiéké. Slightly acid taste is sought by producers. Attiéké can have a sweet taste regardless of acidity or even a neutral flavor due to sugars responsible for the flavor of fermented foods (Bourdichon et al., 2012). Lactic acid and acetic acid (Coulin, Farah, Assanvo, Spillmann, & Puhan, 2006) have a very high perception threshold and have an impact on organoleptic qualities due to their high concentrations (Bourdichon et al., 2012). Organic acids derived from glycolysis are important precursors of aromas. Odor is the second attribute in the choice of producers followed by the "long life" descriptor (56%). Both descriptors (odor and taste) are benchmarks for producers in assessing the quality of finished products. In addition, Maille (2003) showed that unpleasant odors had a negative impact on sale outlets.

Producers also appreciated good attiéké when well cooked. Indeed, the conservation of attiéké is very often linked to the well-cooked character. Cooking is a very important step, and has an impact on storage, hygienic and toxic qualities of attiéké.

Color and bright appearance appear as important descriptors in producer's appreciation. Attiéké must have a shine due to translucent nature. Color plays an important role in assessing the quality and first impressions of a food in terms of maturity, impurities, appropriate or defective technological treatment, poor storage conditions, an early microbial deterioration (Nout, Hounhouigan, & Boekel, 2003). Ebrié prefer the off-white color, Adjoukrou and Alladjan the light yellow (or cream) color.

The other descriptors such as fiber, well-being, grain size, moisture, firmness, and flavour, appear secondary but had some importance. Attiéké Garba differs from usual attiéké by presence of fibers. The term "provides a well-being" puts more emphasis on hedonic characters (pleasure felt) of food.

The moisture of attiéké must not be beyond the desired limit since it should neither be perceived as wet or dry. Traditional producers indicated importance of feeling firmness and graininess of attiéké grains. In conclusion, all quality attributes are important for producers.

4.1.3 Consequence of Attiéké Quality on Price and Frequency of Production

The attiéké quality influences its selling price and production frequency. The more the manufacturing processes are shortened and poorly executed, the less expensive is attiéké and the more the quantity produced/day/week is high. The production of Garba is related to growing demand and relative high cost of other types of attiéké (Assanvo et al., 2019). Three main reasons support its consumption: very low cost, large quantity served and especially energy supply.

Attiéké agbodjama and usual attiéké were respectively two-fold or one and half-fold as expensive as attiéké Garba. "Agbodjama" is the most expensive attiéké because its manufacturing requires too time and great efforts for granulation and winnowing. The prices of attiéké on markets of Abidjan vary often according to seasons. In rainy season, these prices increase related to drying difficulties.

4.2 Physicochemical Characteristics of Attiéké Types

4.2.1 Influence of Fermentation on the Quality of Different Types of Attiéké

The use of IAC variety for producing attiéké in the three regions surveyed allows assessing the impact of inoculum on organoleptic quality of attiéké. The physicochemical analysis confirmed the statements of producers. Whatever the attiéké, lactic and acetic acids were present and affected the acidulous taste and aroma. The rates of

these acids in traditional attiéké and Garba were low but indicated that the fermentation is heterolactic due to actions of traditional cassava inoculum.

The starch content of attiéké agbodjama is higher than all other attiéké produced because the producers take the time to enlarge, round and harden the grains. However, high amount of starch is due to incomplete release and lost during fermentation (Hatew et al., 2015).

Total and reducing sugar rates were low but highest in attiéké from well fermented doughs. Bad fermentation may explain their lowest rate in Garba.

4.2.2 Influence of Manufacturing Process on Production Yield

Several factors influence the performance of production. Losses occur throughout the manufacturing process and vary depending on the step. Among those having the greatest impact were peeling, crushing, grinding, pressing and winnowing (Nago, 1995). Losses recorded were up to 66.17% for attiéké Ebrié, 67.58% for attiéké Adjoukrou, 68.07% for attiéké Alladjan and 61.93% for attiéké Garba. In terms of yield, attiéké Garba (around 34%) appears more profitable since many losses are minimized as possible.

Yield may be related to useful material (cassava variety). The higher the dry matter in a variety, the more variety gives a lot of attiéké. According to Njukwe, Hanna, Kirscht, & Araki (2013), the main criteria for choosing a variety is obviously its productivity in dry matter or starch. The age of harvest also can influence the yield of transformation.

5. Conclusion

Surveys of production showed that cassava inoculum is being prepared similarly in all three regions visited. For producers, cassava variety does not matter for a good inoculum which has a decisive impact on fermentation of fresh paste. It lightenes the dough for a better ability to form grains (semolina) and imposes desired taste and smell of attiéké. Making a good attiéké is correlated with some attributes of quality. A good attiéké has a slightly acid and sweet taste, a very characteristic smell and aroma of fermented cassava found pleasant, at least one week lifespan, a bright color, a spongy character expressing a cohesive and elastic texture. The firm and granular texture is appreciated during the appetizer. Attiéké Garba does not meet these characteristics and consumers may be at risk due to cyanide. Future investigations are needed for reducing cyanide in this product.

The control of external factors as immediate working environment, temperature, humidity, seasons can improve the quality of attiéké.

Acknowledgments

The authors thank the Laboratory of Biochemistry and Food Sciences (University FHB, Côte d'Ivoire), the Institute of Food Science of the Federal Polytechnic School of Zurich (Switzerland), the Swiss Center for Scientific Research in Côte d'Ivoire and the International Foundation for Science (IFS) for their technical and financial support.

References

Agbo, N. G., Uebrsax, M., & Hosfield, G. (1985). An efficient extraction technique of sugars from dry edible be vulgaris and estimation an HPLC. *Université Nationale de Côte d'Ivoire Annales Série c. (sciences)*, *tome XXI*, 10.

Amoa-Awua, W. K. A., Appoh, F. E., & Jakobsen, M. (1996). Lactic acid fermentation of cassava dough into agbelima. *International Journal of Food Microbiology*, *31*, 87-98. https://doi.org/10.1016/0168-1605(96)00967-1

Anonymous 1 (2016). *Manioc- production*. Retrieved from http://kko-international.com/manioc/

Anonymous 2 (2005). *Intérêt de la fermentation*. Mansion N., Rapport INRA, mission communication. Catherine.Foucaud@versailles.inra.fr

Anonymous 3 (2006). *The BASICS 6*. Retrieved from http://www.eufic.org/index/fr/

AOAC: Association of Official Analytical Chemists (1984). *Official Methods of Analysis* (14th Ed.). Washington, DC (USA).

AOAC: Association of Official Analytical Chemists (1990). *Official Methods of Analysis* (15th Ed.). Washington DC (USA). Available on: https://law.resource.org/pub/us/cfr/ibr/002/aoac.methods.1.1990.pdf

AOAC: Association of Official Analytical Chemists (1995). *Official methods of analysis* (16th Ed.). Washington DC, USA.

Assanvo B. J., Agbo N. G., & Farah, Z. (2019). Surveys on the Consumption of Attiéké (Traditional and a Commercial, Garba) in Côte d'Ivoire. *Journal of Food Research, 8*(4), 23-36. https://doi.org/10.5539/jfr.v8n4p23

Assanvo, J. B. (2008). *Evaluation of traditional Ivorian attiéké qualities: Survey on production and consumption, physicochemical, microbiological and sensory characterization of four cassava varieties (IAC, Bonoua, Olekanga and TMS 4 (2) 1425)* (Unpublished doctoral dissertation), University of Cocody, Abidjan, Côte d'Ivoire.

Assanvo, J. B., Agbo, G. N., Brunnchweiler Beez, J., Monsan, V., & Farah, Z. (2018). Sensory profiling and hedonic evaluation of Attiéké from local and improved cassava varieties. *Food and Nutrition Sciences, 9*, 1472-1497. https://doi.org/10.4236/fns.2018.912107

Assanvo, J. B., Agbo, G. N., Coulin, P., Monsan, V., Heuberger, C., Kati-Coulibaly, S., & Farah, Z. (2017). Influence of microbiological and chemical quality of traditional starter made from cassava on attiéké produced from four cassava varieties. *Food Control*, *78C*, 286-296. http://dx.doi.org/10.1016/j.foodcont.2017.02.066

Assanvo, J. B., Agbo, N. G., Behi, Y. E. N., Coulin P., & Farah, Z. (2006). Microflora of traditional starter made from cassava for "attiéké" production. *Food Control, 17*, 37-41. https://doi.org/10.1016/j.foodcont.2004.08.006

Bavaro, S. L., Susca, A., Frisvad, J. C., Tufariello, M., Chytiri, A., Perrone, G., … Bleve, G. (2017). Isolation, Characterization, and Selection of Molds Associated to Fermented Black Table Olives. *Frontiers in Microbiology, 8*, 1356. https://doi.org/10.3389/fmicb.2017.01356

Bernfeld, P. (1951). Enzymes of starch degradation and synthesis. *Advances in Enzymology and Related Subjects of Biochemistry*, *12*, 379-428. https://doi.org/10.1002/9780470122570.ch7

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L., Hammes, W. P., … Hansen, E. B. (2012). Food fermentations: Microorganisms with technological beneficial use. *International Journal of Food Microbiology, 154*(2012), 87-97. https://doi.org/10.1016/j.ijfoodmicro.2011.12.030

Bradbury, M. G., Egan, S. V., & Bradbury, J. H. (1999). Picrate paper kits for determination of total cyanogens in cassava roots and all forms of cyanogens in cassava products. *Journal of the Science of Food and Agriculture*, *79*, 593-601. https://doi.org/10.1002/(SICI)1097-0010(19990315)79:4<593::AID-JSFA222>3.0.CO;2-2

Cagnon, J. R., Cereda, M. P., & Pantarotto, S. (2002). Glicosídeos cianogênicos da cassava: biossíntese, distribuição, destoxificação e métodos de dosagem. In: M. P. Cereda, coordenador. *Tuberosas amiláceas latino-americanas* (Vol. 2, p. 83-99). São Paulo: Fundação Cargill.

Commission du Manuel Suisse des Denrées Alimentaires (1994). *Manuel suisse des denrées alimentaires: méthodes pour l'analyse et l'appréciation des denrées alimentaires et des objets usuels* (5ème éd.). Service fédéral de l'hygiène publique; Suisse. Conseil fédéral, Berne: EDMZ.

Coulin, P., Farah, Z., Assanvo, J., Spillmann, H., & Puhan, Z. (2006). Characterisation of microflora of attiéké, a fermented cassava product, during traditional small-scale preparation. *International Journal of Food Microbiology*, *106*, 131-136. https://doi.org/10.1016/j.ijfoodmicro.2005.06.012

Diop, A. (1992). *L'attiéké dans la région d'Abidjan: Analyse économique de la filière traditionnelle à travers quelques types d'organisation (Adjoukrou, Ebrié, Attié…)*, (Unpublished doctoral dissertation), Université Nationale de Côte d'Ivoire, Centre Ivoirien de Recherches Economiques et Sociales (CIRES), Abidjan, Côte d'Ivoire.

Dubois, M., Gilles, K., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350-356. https://doi.org/10.1021/ac60111a017

FAO (2018). Retrieved from http://www.fao.org/cote-divoire/actualites/detail-events/fr/c/1112318/

Hatew, B., Podesta, S. C.,Van Laar, H., Pellikaan, W. F., Ellis, J. L., Dijkstra, J., & Bannink, A. (2015). Effects of dietary starch content and rate of fermentation on methane production in lactating dairy cows. *Journal of Dairy Science, 98*(1), 486-499. https://doi.org/10.3168/jds.2014-8427

Heuberger, C. (2005). *Cyanide content of cassava and fermented products with focus on attiéké and attiéké "garba"*, (Doctoral dissertation ETH Nº 16247), Swiss Federal Institute of Technology, Zurcih.

https://doi.org/10.3929/ethz-a-005182128

Kouadio, N. A., Kouakou, K. E., Angbo S. F., & Mosso, K. (1991). Etude comparative des méthodes traditionnelles de préparation de l'attiéké dans le sud de la Côte d'Ivoire. *Cahier Scientifique et Technique*, *108*, 703-706. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=FR19950104789

Maille, V. (2003). L'influence des odeurs sur le consommateur: la tendance à la recherche des sensations et au comportement exploratoire comme variables modératrices. *Revue française du marketing, 194*(4/5), 49-61. Retrieved from http://archives.marketing-trends-congress.com/2002/Materiali/Paper/Fr/MAILLE.pdf

McFeeters, R. (2008). Fermentation microorganisms and flavor changes in fermented foods. *Journal of Food Science*, *69*, 35-37. https://doi.org/10.1111/j.1365-2621.2004.tb17876.x

Melcion, J.-P. (2000). La granulométrie de l'aliment: Principe, mesure et obtention. *INRA Production Animale, 13*(2), 81-97. https://www6.inra.fr/productions-animales/content/download/4045/41840/version/1/file/Prod_Anim_2000_13_2_01.pdf

Mescle, J. F., & Zucca, J. (1996). Les facteurs du développement In Coordonnateurs: C. M. Bourgeois, J. F. Mescle, & J. Zucca. *Microbiologie Alimentaire, tome 1: Aspect microbiologique de la sécurité et de la qualité des aliments* (2ème édition). Techniques et Documentation. Lavoisier, Paris.

Moorthy, S., & Mathew, G. 1998. Cassava fermentation and associated changes in physicochemical and functional properties. *Critical Reviews in Food Science and Nutrition, 38*, 73-121. https://doi.org/10.1080/10408699891274174

Nago, C. M. (1995). La préparation artisanale du gari au Benin: Aspects technologiques et physico-chimiques (Artisanal gari production in Benin. Technological and physico-chemical aspects) In Agbor Egbe T., Brauman A., Griffon D., Trèche S. (Ed), *Transformation Alimentaire du Manioc*, (pp. 475-493). ORSTOM.

Njukwe, E., Hanna, R., Kirscht, H., & Araki, S. (2013). Farmers perception and criteria for cassava variety preference in Cameroon. *African Study Monographs, 34* (4), 221-234. Retrieved from http://jambo.africa.kyoto-u.ac.jp/kiroku/asm_normal/abstracts/pdf/33-1/34-4/njukwe4.pdf

Nout, R., Hounhouigan, J. D., & Boekel, T. V. (2003). *Food, Processing, Conservation and Quality*. Backhuys Publishers and CTA, Wageningen, The Netherlands, 268 p.

Obilie, E. M., Tano-Debrah, K., & Amoa-Awua, W. K. (2004). Souring and reduction of cyanogenic glycosides during the processing of cassava into akyeke in Ghana. *International Journal of Food Microbiology, 93*, 115-121. https://doi.org/10.1016/j.ijfoodmicro.2003.11.006

Sotomey M., Ategbo E.-A., Mitchikpe E., Gutierrez M.-L., & Nago, M. C. (2001). L'attiéké au Bénin. Innovations et diffusion de produits alimentaires en Afrique. CERNA, CNEARC, CIRAD, France.

Zacarias, C. H., Esteban, C., Rodrigues, G. L., & de Souza Nascimento, E. (2017). Occupational exposure to hydrogen cyanide during large-scale cassava processing, in Alagoas State, Brazil. *Cadernos de Saùde Pùblica, 33*(7), 1-14. https://doi.org/10.1590/0102-311X00073416

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Fatty Acid Composition of Oil from Groundnuts and Oyster Nuts Grown in Uganda

Juliet Hatoho Musalima[1], Patrick Ogwok[1] & Diriisa Mugampoza[1]

[1]Department of Food Technology, Faculty of Science, Kyambogo University, Kampala, Uganda

Correspondence: Juliet Hatoho Musalima, Department of Food Technology, Faculty of Science, Kyambogo University, Kampala, Uganda. Tel: 256-701-617-002. E-mail: julietmusalima@gmail.com

Received: August 29, 2019 Accepted: September 27, 2019 Online Published: October 11, 2019

doi:10.5539/jfr.v8n6p37 URL: https://doi.org/10.5539/jfr.v8n6p37

Abstract

This study determined the fatty acid (FA) composition of oil from groundnuts and oyster nuts in Uganda. The FA composition was determined by Gas Chromatography/Mass Spectrometry with FID. Oil from groundnuts contained 39.71 to 55.89% oleic, 20.21 to 35.59% linoleic and 11.91 to 17.16% palmitic acids. Oil from Serenut cultivars contained cis 11-eicosenoic acid (C20.1), cis 11, 14 eicosadienoic acid (C20.2) and cis 11, 14, 17 eicosatrienoic acid (C20.3ω3) which were not detected in traditional cultivars. Oyster nut oil was high in linoleic acid at 41.02 to 44.86% and palmitic acid at 33.58 to 38.11% while oleic acid was low with amounts <10%. The polyunsaturated to saturated FA ratios of oil from groundnuts and oyster nuts were >0.45, the minimum recommended by FAO. The atherogenic (AI) and thrombogenic indices (TI) of <1 and the hypocholesterolemic to hypercholesterolemic index (h/H) of >4 in groundnut oil were favorable for cardiovascular health. Indices for oyster nut oil were ≤1for AI and >1for TI. The h/H was low compared to that of groundnut oil. Results showed little distinction between the FA compositions of oil from traditional and improved groundnuts suggesting that breeding may not have significant effect on major FAs. Oyster nut oil contained saturated and unsaturated FA in a ratio of 1:1. The lipid health indices for groundnut oil were within recommendations while those of oyster nuts were less desirable. Oyster nut oil should therefore be consumed with moderation.

Keywords: fatty acids, groundnut, oil, oyster nut, Uganda

1. Introduction

Nuts have been recognised as a high fat food group (Hollis & Mattes, 2007). Most nuts contain low levels of saturated and high amounts of unsaturated fatty acids (Kris-Etherton et al., 1999). Fatty acids (FA) in nuts have structural and metabolic roles in the body (Arbex et al., 2015). Groundnuts (*Arachis hypogea* L.) are the second most widely grown and consumed legumes in Uganda after common beans (*Phaseolus vulgaris*). Their production is in Northern, Eastern and Southern parts of Uganda with Eastern region being the highest producer (Okello, Biruma, & Deom, 2010). In North and Eastern Uganda, groundnuts are valued as cash crops and a valuable source of oil and protein (Mugisha, Lwasa, & Mausch, 2014; Okello et al., 2010). Groundnuts contain approximately 40 to 50% oil (Ntare, Diallo, Ndjeunga & Waliyar, 2008). Despite being an excellent source of oil, the highest proportion of groundnuts produced in Uganda is consumed as snacks, stew and paste with low value added to the crop (Okello et al., 2010; Mugisha, Lwasa, & Mausch, 2014). Vegetable oils are important sources of polyunsaturated fatty acids (PUFA), alpha linolenic and linoleic acids (Yehuda, 2003). Utilization of the oil depends on FA composition and fat soluble vitamins (Shad, Pervez, Zafar, Nawaz, & Khan, 2012; Strayer, Belcher, Fine, & Mcbrayer, 2006). Groundnut oil is considered healthier than saturated oil due to its high monounsaturated FA content and is resistant to rancidity because of the anti oxidant vitamins (Asibuo et al., 2008; Shad et al., 2012).

The groundnut research program at the National Arid and Semi-Arid Resources Research Institute (NaSARRI) in Serere, Uganda is focused on developing groundnut varieties with improved agronomic characteristics. Breeding at NaSSARI resulted in large scale production and commercialization of improved groundnut (Serenut) cultivars. These cultivars are high yielding, high quality, resistant to diseases, and quick maturing (Okello et al., 2010; Okello, Deom, Puppala, Monyo, & Bravo-Ureta, 2016).

Today's consumers are concerned about the nutritional value and safety of foods they consume. It is therefore

important to establish any nutritional and safety differences between new varieties and traditional ones. Even though modification was intended for another purpose, it may result in some unintended positive or negative changes in the product. Little information however, is available on the nutritional profile of these cultivars in comparison with their traditional counterparts.

Oyster nut, *Telfairia pedata*, is a perennial climbing vine and a member of the *Cucurbitaceae* family (Jumbe et al., 2016). It is common in Tanzania, Kenya and some parts of Uganda. Oyster nuts contain 55 to 60% oil (Ajayi et al., 2004; Hopkins & Chisholm, 1964). Oyster nuts are eaten raw or cooked are especially mentioned as source of food for women during the lactating period. The nuts have been reported to have other non-food uses ascribed to the properties of their oil. Ajayi et al. (2004) asserted that oyster nuts may stay in good condition for up to eight years despite their high concentration of oil. Oyster nut oil however, may have limited application due to lack of information regarding its chemical composition.

Despite the indiscriminate utilisation of groundnuts and their products, there is scant information on the FA composition of oil from the Serenut and traditional cultivars as well as oyster nuts in Uganda. To establish whether breeding improves the nutritional properties of the oil, this study determined the FA composition of oil from Serenut and traditional groundnut cultivars. Oyster nut oil was characterised to establish its constituent fatty acids. Determining these parameters could add to the nutritional data base for crops grown in Uganda and allow for consumption from an informed perspective.

2. Materials and Methods

2.1 Sample Collection and Preparation

Fourteen improved cultivars (Serenuts 1 to 14) and six traditional groundnut cultivars (Acholi white, *Igola*, *Egoromoit, Rudu* white, *Rudu* red and Red beauty) were studied. Serenut 1 to 14 were collected from National Arid and Semi-Arid Research Resources Institute (NaSARRI) in Serere. Traditional cultivars were purchased from markets in Soroti, Arapai, and Achorimongin markets in Teso sub-region, Eastern Uganda. Groundnuts were shelled, sorted, hulled and finely crushed to obtain groundnut flour. Oyster nuts were obtained from Kamuli district in Eastern, Dokolo district in Northern, and Luwero district in central Uganda and transported to the laboratory. Nuts were cleaned and sorted according to gender, the flat nuts were classified as female and the creased nuts as male. A total of 18 samples comprising of 9 male and 9 female were peeled to remove the fibrous shell. Splitting of the inner shell was done using a knife to release the oil-bearing cotyledon. Oyster nut cotyledons were pounded using a mortar and pestle to obtain a paste. Groundnut flours and oyster nut paste were stored in the refrigerator (4^{o}C) prior to oil extraction. All assays were done in triplicate.

2.2 Oil Extraction

Oil extraction was done according to Bligh and Dyer (1959). Groundnut flour and oyster nut paste were separately weighed (10 g) into a 250 ml flat bottomed flask then chloroform was added (100 ml). Flask contents were mixed using an ultraturax homogenizer (IKA T18, Bergkirchen, Germany) for 2 min. The mixture was transferred into a 40 ml dionex vial and centrifuged at 2000 rpm for 5 min. The chloroform layer was filtered through a filter paper (Macherey-nagel, 125 mm) containing anhydrous sodium sulphate. Twenty milliliters of the filtrate was concentrated under a stream of nitrogen at 40 ^{0}C.

2.3 Fatty Acid Composition

2.3.1 Preparation of Fatty Acid Methyl Esters

Fatty acid methyl esters (FAME) were prepared according to AOAC (2000) method number 969.33. Fat was weighed (0.5 g) in a 40 ml glass vial and diethyl ether added (2 ml). The mixture was then vortexed until oil was dissolved. Methanolic potassium hydroxide was added (0.5 ml) and the mixture allowed to react for 15 min. during which the solution became cloudy due to soap formation.

2.3.2 Extraction of the Fatty Acids from the Soap Solution

Distilled water was added (2 ml) to the FAME solution followed by 10 ml of hexane. The mixture was vortexed to allow phase separation. The organic layer was transferred to a clean test tube then distilled water was added (2 ml). The mixture was allowed to stand to allow phase separation. This was repeated until the water used to wash the organic layer showed no color change with phenolphthalein. The organic layer was transferred (1 ml) to a 1.5 ml GC vial and injected (1 µl) onto a 30 m x 0.32 mm x 0.5 µm solgel wax column with polyethylene-glycol as the stationary phase and helium gas at 20 psi as the mobile phase. The column was mounted in a GC/FID (Varian chrompack CP-3800, USA). The injector temperature was 260°C. The temperature of the column was kept at 50°C for 5 min. after injection and thereafter increased to 180 °C at a rate of 20°C/min., followed by an increase of

2°C/min to 200°C, held for 11 min. and then finally ramped to 250°C at 2°C/min. and held for 2.5 min. Fatty acids were identified by analyzing a reference standard mixture (Supelco 47885-U, Sigma Aldrich, Belgium) under the same conditions as the test portion. The retention distances of the standard were compared with those of the test portion. The esters appeared in order of increasing number of carbon atoms and of increasing level of unsaturation for the same number of carbon atoms. Calculation of the peaks was by normalization which assumes all components of test portion represented on the chromatograms so that the sum of the peaks represents 100% of the constituents.

2.4 Iodine Value

Iodine value was calculated from the fatty acid composition using the method of Hashim et al.(1993).

$$\text{IV} = (\%\ \text{Oleic acid X } 0.8601) + (\%\text{Linoleic acid X } 1.7321) + (\text{Eicosanopentanoic acid X } 0.7854) \quad (1)$$

2.5 Lipid Health Indices

2.5.1 Atherogenic Index and Thrombogenic Indices

Atherogenic index (AI) and thrombogenic index (TI) were calculated according to the following equations by Ulbricht and Southgate (1991).

$$\text{Atherogenic index} = (\text{C14.0x4} + \text{C16.0} + \text{C18.0})/(\sum\text{MUFA} + \sum\omega\text{6PUFA} + \sum\omega\text{3PUFA}) \quad (2)$$

$$\text{Thrombogenic index} = (\text{C14.0} + \text{C16.0} + \text{C18.0})/(0.5\text{xMUFA} + 0.5\text{x}\omega\text{6PUFA} + 3\text{x}\omega\text{3PUFA}) + \frac{\omega 3}{\omega 6} \quad (3)$$

2.5.2 Hypocholesterolemic/Hypercholesterolemic Index

This index indicates potential of a lipid to balance between the good and bad cholesterol. It was calculated from the equation by Santos-Silva, Bessa, and Santos-Silva (2002).

$$\frac{h}{H} = (C18.1\omega 9 + C18.2\omega 6 + C20.4\omega 6 + C18.3\omega 3 + C20.5\omega 3 + C22.6\omega 3)/(C14 + C16) \quad (4)$$

h: Hypocholesterolemic

H: Hypercholesterolemic

2.6 Statistical Analysis

Samples were analysed in triplicate and data presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with a Turkey's least significant difference (LSD) test was used in SPSS version 17.0 (SPSS, Chicago, IL, USA) to evaluate the differences in fatty acids. Differences were considered statistically significant at $p<0.05$.

3. Results and Discussion

The percent FA composition of oil from groundnuts and oyster nuts are presented in Tables 1 and 2, respectively. Oils were found to contain variable levels of FA as discussed below.

3.1 Groundnut Oil

The dominant fatty acids (FA) in groundnut oil were oleic (C18.1), linoleic (C18.2) and palmitic (C16.0) acids with amounts more than 10% (Table 1). Approximately 80% of FA in oil from all cultivars were unsaturated (Table 3). Oleic (C18.1) as the major unsaturated FA varied from 39.71 to 55.89%. This finding is close to the data reported by Khetarpaul, Jood, & Goyal (2007) and Dorni, Paras, Gunendra, & Longvah (2018) who reported 48.90% and 53.77% oleic acid (C18.1), respectively. Linoleic acid (C18.2) ranged from 20.21 to 35.59%. Levels of linoleic acid (C18.2) were in agreement with the findings of Achola et al. (2017) who reported 26.79 to 33.44% while Dorni, Paras, Gunendra, & Longvah (2018) reported 26.96% linoleic acid (C18.2) in groundnut oil. Similarly, Gulluoglu, Bakal, Onat, Sabagh, & Arioglu (2016) reported 2 to 43% linoleic acid (C18.2) in groundnut oil. Palmitic acid (C16.0) concentration varied from 11.91 to 17.16%. Findings were consistent with data by Achola et al. (2017) who reported a range of 14.61 to 18.6% palmitic acid (C16.0) in Serenuts 5 to 10. Shahidi (2005) and Özcan (2010) presented slightly lower ranges of 8 to14% and 7.63 to 11.41% palmitic acid (C16.0), respectively, in groundnut oil. Stearic acid (C18.0) ranged from not detected (nd) to 4.93%. Berry (1982) reported 3.17 to 3.68% stearic acid (C18.0) while Achola et al. (2017) obtained 2.19 to 3.46% stearic acid (C18.0) in Serenuts 5 to 10. Levels of oleic (C18.1), linoleic, (C16.0) and stearic acid (C18.0) varied significantly ($p<0.05$) indicating composition differences among the cultivars. Similarly, behenic acid (C22.0) levels were higher in the traditional cultivars compared to the Serenuts. Oil from red beauty (RB) had the highest amount of behenic acid. Other FAs such as gamma linolenic (C18.3ω6), alpha

linolenic (C18.3ω3), arachidic (C20.0), eicosenoic (C20.1) and eicosadienoic (C20.2) occurred in amounts less than 2% in all cultivars. Levels of these FA were more pronounced in oils from traditional than Serenut cultivars. Arachidic acid (C20.0) is a characteristic FA in groundnuts and their products. The level of arachidic acid (C20.0) should not exceed 4.8% in groundnut oil (Codex, 2001). Dorni, Paras, Gunendra, & Longvah (2018) and Özcan (2010) reported 1.44 to 2.36% and 1.42% arachidic acid (C20.0), respectively, in groundnut oil. The levels of arachidic acid in this study agreed with reports of the aforementioned scholars. Cis 11-eicosenoic acid (C20.1), cis 11, 14 eicosadienoic acid (C20.2) and cis 11, 14, 17 eicosatrienoic acid (C20.3ω3) were detected in oil from Serenut cultivars and not in the other cultivars. The presence of these FA in oil from the Serenut cultivars may be attributed to breeding or growth environment.

Table 1. Fatty acid composition (%) of oil from groundnuts

GNC	C16.0	C18.0	C18.1	C18.2	C18.3ω3	C18.3ω6	C20.0	C20.1	C20.2	C20.3ω3	C22
S1R	15.73±0.11^{e}	2.13±0.10^{a}	50.28±0.61^{fghi}	26.06±0.49^{cd}	1.09±0.07^{i}	0.34±0.03^{cde}	1.02±0.07^{ab}	nd	1.42±0.14^{bcde}	0.52±0.36^{bc}	1.09±0.16^{bcdefg}
S2T	14.84±0.14^{cde}	nd	52.28±0.88^{j}	25.96±0.79^{cd}	1.05±0.04^{i}	0.36±0.02^{cde}	nd	1.24±0.02^{de}	1.05±0.06^{b}	1.40±0.08^{e}	1.48±0.00^{fg}
S3R	14.51±0.27^{cde}	2.89±0.21^{cde}	54.85±0.14^{k}	21.68±0.21^{ab}	0.08±0.01^{bc}	0.28±0.00^{abcde}	nd	1.10±0.03^{c}	1.41±0.28^{bcde}	1.45±0.07^{e}	1.41±0.01^{efg}
S4T	13.62±0.64^{bc}	4.15±0.10^{f}	55.05±0.70^{k}	21.94±0.53^{b}	0.08±0.01^{bc}	0.34±0.02^{cde}	nd	0.68±0.03^{b}	1.57±0.03^{de}	1.60±0.13^{e}	0.85±0.07^{bcd}
S5R	14.84±0.50^{cde}	2.76±0.08^{cd}	48.71±0.65^{fg}	27.16±0.99^{de}	0.05±0.00^{ab}	0.57±0.05^{de}	nd	1.32±0.10^{ef}	1.06±0.03^{bc}	1.66±0.12^{e}	1.59±0.17^{g}
S6T	15.88±0.16^{ef}	3.40±0.18^{e}	50.29±0.13^{ghi}	25.78±0.22^{cd}	0.07±0.01^{abc}	0.51±0.02^{de}	nd	0.70±0.01^{b}	1.08±0.01^{bc}	1.06±0.15^{d}	1.07±0.02^{bcdef}
S7T	14.61±1.26^{cde}	3.15±0.27^{de}	46.31±0.54^{cd}	30.79±0.86^{i}	0.06±0.02^{abc}	0.02±0.00^{ab}	0.12±0.00^{a}	1.20±0.01^{d}	1.48±0.02^{cde}	0.65±0.05^{e}	1.46±0.03^{defg}
S8R	13.78±0.18^{bcd}	3.14±0.05^{de}	48.63±0.52^{ef}	29.25±0.22^{gh}	0.23±0.01^{e}	0.24±0.03^{abcd}	0.06±0.00^{a}	1.15±0.07^{cd}	1.75±0.45^{e}	0.23±0.01^{ab}	1.33±0.04^{cdefg}
S9T	14.56±0.63^{cde}	2.08±0.06^{a}	45.92±0.30^{bcd}	31.91±0.56^{ij}	0.27±0.01^{e}	0.28±0.01^{abcd}	nd	1.33±0.04^{ef}	1.41±0.21^{bcde}	0.26±0.04^{ab}	nd
S10R	15.09±0.05^{de}	2.44±0.22^{ab}	51.85±0.70^{ij}	24.67±0.45^{c}	nd	0.19±0.01^{abcd}	nd	1.36±0.03^{f}	1.26±0.18^{bcd}	1.63±0.10^{e}	1.36±0.02^{defg}
S11T	13.79±0.07^{bcd}	2.36±0.20^{ab}	46.45±1.03^{cd}	34.02±0.39^{k}	0.97±0.01^{h}	0.31±0.15^{abc}	0.92±0.07^{ab}	nd	nd	nd	0.95±0.07^{bcde}
S12R	15.41±0.34^{e}	2.64±0.19^{abc}	52.11±0.09^{j}	27.57±0.26^{ef}	nd	0.37±0.03^{bcde}	1.02±0.04^{ab}	nd	nd	nd	0.98±0.15^{bcdef}
S13T	14.63±0.38^{cde}	2.36±0.15^{ab}	50.30±0.49^{ghi}	30.61±0.12^{hi}	nd	0.97±0.07^{cde}	0.69±0.02^{ab}	nd	nd	nd	0.76±0.58^{b}
S14R	14.86±0.83^{cde}	2.65±0.04^{abc}	49.73±0.39^{fgh}	28.93±0.63^{fg}	nd	0.97±0.07^{f}	1.03±0.06^{b}	nd	nd	nd	1.33±0.27^{cdefg}
AW	17.16±0.11^{f}	4.88±0.12^{h}	39.71±0.65^{a}	35.59±0.39^{l}	nd	0.35±0.31^{cde}	0.55±0.47^{ab}	nd	nd	nd	0.83±0.08^{bc}
IGO	14.83±0.25^{cde}	2.63±0.00^{abc}	50.86±0.45^{hij}	28.87±0.22^{fg}	nd	0.13±0.01^{g}	2.70±0.21^{c}	nd	nd	nd	0.78±0.09^{b}
RR	12.78±0.24^{ab}	4.21±0.48^{ff}	44.74±0.18^{bc}	30.86±0.66^{i}	0.14±0.01^{cd}	nd	nd	nd	nd	nd	3.27±0.12^{h}
RW	12.68±0.17^{ab}	4.80±0.38^{gh}	44.28±0.08^{b}	33.08±0.18^{jk}	0.21±0.02^{de}	nd	nd	nd	nd	nd	3.28±0.05^{h}
EGT	13.75±0.34^{bcd}	4.93±0.23^{h}	55.89±0.12^{k}	20.21±0.18^{a}	0.67±0.02^{g}	nd	nd	nd	nd	nd	3.49±0.09^{hi}
RB	11.91±0.14^{a}	4.74±0.20^{fgh}	46.90±0.49^{de}	30.76±0.11^{hi}	0.59±0.02^{f}	nd	nd	nd	nd	nd	3.86±0.15^{i}

Data are expressed as percentages of total fatty acid methyl esters; Values are means of triplicate determinations, values followed by the same letter within each column are not significantly different (p>0.05) nd: not detected; GNC: Groundnut cultivar; S: Serenut; R: red;T: Tan; AW: Acholi White; IGO: *Igola*; RR: *Rudu* Red; RW: *Rudu* White; EGT: *Egoromoit*; RB: Red Beauty.

3.2 Oyster Nut Oil

Oyster nut oil had high amounts of palmitic acid (C16.0) and linoleic acid (C18.2) but had low amount of oleic acid (C18.0). Palmitic acid (C16.0) ranged from 33.58 to 38.11% (Table 2). This concurs with the concentrations obtained by Hopkins and Chisholm (1964) and Nyagah (2016) who reported 35% and 32.03% palmitic acid (C16.0) in oyster nut oil from South Africa and Kenya, respectively. Minzangi, Mpiana, Samvura, Kaaya, Bertrand, & Kadima, 2015 examined nuts from DR Congo and obtained a lower level of Palmitic acid (C16.0) at 14.06%. Palmitic acid (C16.0) has been positively associated with high serum cholesterol and hence cardiovascular risk (Galli and Calder, 2009; Fattore and Fanelli, 2013; Mancini et al., 2015 and Carta et al., 2017).

The high amount of palmitic acid (C16.0) in this study suggests that oyster nut oil may not favour cardiovascular health. In addition, high levels of palmitic acid (C16.0) may enhance metabolic complications such as insulin resistance and decreased oxidation of FA and glucose in muscles hence their accumulation in tissues (Iggman & Risérus, 2017). However, moderate consumption of oils rich in palmitic acid (C16.0) could provide health benefits as it forms a significant part of cell membrane phospholipids (Calder, 2015). Stearic acid in oil ranged from 9.47 to 13.60%. Hopkins and Chisholm (1964) reported 14% and Nyagah (2016), 10.31% stearic acid (C18.0). Contrary to our findings, Minzangi et al. (2015) obtained 9.3 % stearic acid (C18.0). In the body, stearic acid is converted to oleic acid. This conversion may help to lower plasma cholesterol (Bonanome & Grundy, 1988; Strayer et al., 2006; Mente et al., 2017). It is, therefore, implied that diet containing more stearic than palmitic acid may improve plasma lipid profile. Saturated FA that were detected in amounts less than1.0% included; butyric acid (C4.0) caproic (C9.0), undecanoic (C11.0), lauric (C12.0), tridecanoic (C13.0), myristic (C14.0), heptadecanoic (C17.0), arachidic (C20.0), heneicosanoic (C20.1), tricosanoic (C23.0) and nervonic acid (C24.0). There were no significant differences (p>0.05) in the levels of different FA based on location or gender. Linoleic acid (C18.2) was the major unsaturated FA detected and it ranged from 41.02 to 44.86%. This result is

close to that obtained by Hopkins & Chisholm (1964) at 44% though slightly lower than that of Nyagah (2016) at 53.66%. The levels of linoleic acid (C18.2) in this study doubled the 22.03% obtained by Minzangi et al. (2015). Linoleic acid (C18.2) has been reported to lower total serum cholesterol, a positive attribute in reducing cardiovascular risk (Ristić-Medić, Vučić, Takić, & Karadžić, Ivana and Glibetić, 2013). Oleic acid (C18.1) ranged from 5.69 to 8.10% (Table 2). While other scholars reported low levels of oleic acid (C18.1) at <10%, Minzangi et al. (2015) presented 41.77%. Levels of Alpha linolenic acid (C18.3ω3) in oil were less than 2%. Literature sources, (Hopkins & Chisholm, 1964; Jumbe et al., 2016) indicate that alpha linolenic acid (C18.3ω3) levels were either too low or undetected.

Table 2. Fatty acids (%) in oyster nut oil

Location	C16.0	C18.0	C18:1	C18:2	18.3ω3	18.3ω6	C20.1	C20.2	C22.1ω9	C23.0	C24.0
Dokolo											
Male	38.11±0.96[a]	13.60±0.21[a]	5.93±0.26[a]	41.02±1.02[a]	nd	nd	nd	nd	nd	0.03±0.00[a]	nd
Female	36.39±3.99[a]	11.49±3.07[a]	8.08±2.05[a]	44.86±2.44[a]	0.03±0.00[a]	0.06±0.01[a]	nd	0.02±0.00[a]	nd	0.13±0.00[a]	nd
Kamuli											
Male	38.72±3.37[a]	10.47±0.56[a]	5.69±2.09[a]	42.88±3.77[a]	nd	nd	nd	nd	nd	0.16±0.00[a]	nd
Female	37.21±3.19[a]	10.29±1.29[a]	7.16±1.70[a]	43.64±3.51[a]	nd	nd	nd	nd	nd	nd	0.03±0.00[a]
Luwero											
Male	35.32±5.01[a]	9.47±2.88[a]	8.10±4.15[a]	44.62±1.22[a]	nd	0.23±0.00[a]	0.22±0.01a	0.01±0.00[a]	0.12±0.00[a]	nd	nd
Female	33.58±3.02[a]	13.56±2.76[a]	6.85±1.58[a]	43.44±2.06[a]	0.01±0.00[a]	0.30±0.01[a]	0.20±0.00a	0.13±0.00[a]	0.14±0.00[a]	nd	nd

Data are expressed as percentages of total fatty acid methyl esters, Values are means of triplicate determinations; values followed by the same letter within each column are not significantly different (p>0.05) nd: not detected.

3.3 Lipid Health Indices for Groundnut Oil

The polyunsaturated to saturated FA ratio (P/S) measures the tendency of the diet to influence the incidence of coronary heart disease (SˇimaT, Bogdanovic´, Poljak, & Petricˇevic´, 2015). This ratio is also important in determining the cholesterolemic effect of dietary lipids. Foods with P/S ratio above 0.45 (FAO, 2010) are considered beneficial due to their potential to lower serum cholesterol (Kostik, Memeti, & Bauer, 2013). The beneficial effect is even more significant when the P/S ratios are >1. The P/S of groundnut oil ranged from 0.99 to 2.15 (Table 3). Nile and Park (2013) and Johnson, Saikia, Mathur, & Agarwal (2009) obtained P/S ratios of 1.8 and 2.28 for groundnut oil. The high P/S ratios in this study suggests that consumption of diet rich in groundnut oil is beneficial for human health. Oils with high P/S ratios have higher nutritional value than ones with less (Kostik et al., 2013). Ramprasath, Jones, Buckley, Woollett, & Heubi (2012) reported that serum cholesterol concentrations are hiked with diets rich in SFA while the opposite effect is provided by diets containing high levels of PUFA. Consumption of diets with a high P/S ratio reduces plasma total and LDL-cholesterol concentrations. Similarly Wen and Chao (1998), suggested that high ratio of P/S is crucial in keeping serum and liver cholesterol low. Experimental data by Iggman and Risérus (2017) suggests that partial replacement of SFA by PUFA hence increasing the P/S ratio may reduce cardiovascular risk. This is in agreement with report from expert consultation of FAO in 2010 on fats and FA in human nutrition (FAO, 2010).

The polyunsaturated to monounsaturated FA (P/M) ratio of oil ranged from 0.39 to 0.91. Polyunsaturated and monounsaturated FAs are linked to reduction of atherosclerosis and cardiovascular disease (Kris-Etherton, 1999; Harlioğlu and Yilmaz, 2011). Diets rich in monounsaturated FA are neutral while polyunsaturated FA lower plasma lipids (Wen and Chao, 1998; Bos et al., 2010). No clear recommendations are available for P/M ratio however, Wen & Chao (1998), experimented with different P/M ratios and reported that high ratios lowered plasma and liver cholesterol and triglycerides. Findings of their experiments suggested that diets containing oil with a P/M ratio of 2.5 reduce the plasma and liver cholesterol of human subjects. Our results were far below the threshold given by the above scholars.

The monounsaturated to saturated FA ratio (M/S) of groundnut oil varied from 1.65 to 3.25 (Table 3). Nile and Park (2013) obtained M/S ratio of 1.06 for groundnut oil. The M/S ratio of this study is slightly higher. Diets with high M/S ratio of >1 decrease plasma HDL-C and triacylglycerides (Yang, Lin, Chang, & Chien, 2017). Sinanoglou, Batrinou, Mantis, Bizelis, & Miniadis-meimaroglou (2013) and Calder (2015) suggested that MUFA are heart protective while SFA may increase risk of cardiovascular disease. The high M/S ratio in this research suggests a healthy balance of FA in groundnuts.

The ratio of omega-6 to omega-3 (ω6/ω3) of groundnut oil varied from not detected (nd) to 228.55. where omega -3 FAs were detected, all the ratios exceeded the optimal range (4:1) suggested by Simopoulos (2010). This is attributed to the very low levels of omega 3 FA in the oil and higher levels of omega-6. Johnson et al.

(2009) obtained ω6/ω3 of 7.5 for groundnut oil while Nile and Park, (2013), reported ω6/ω3 value of 25.0. Literature reports on this ratio are highly variable. Some scholars did not report any ALA in groundnut oi1 while others reported very low amounts hence the high ratios. Omega-3 and omega-6 produce eicosanoids with conflicting effects in the body, omega-3 being anti-inflammatory while omega-6 eicosanoids support formation of thrombus, atheromas and obesity (Simopoulos, 2008, 2016; Alabdulkarim, Bakeet, & Arzoo, 2012) . The oleic to linoleic (O/L) acid ratio of groundnut oil varied from 1.12 to 2.77. Shad et al. (2012) obtained an O/L ratio of 3.53 to 19.79 and suggested that high O/L ratio is stable to oxidation (Shad et al., 2012). Among studied oil, *Egoromoit* exhibited the highest O/L ratio implying that its oil have better oxidative stability than the other oils.

Atherogenic and thrombogenic indices are used to assess nutritional quality of lipids (SˇimaT et al., 2015). The atherogenic Index (AI) of groundnut oil ranged from 0.18 to 0.29 while the thrombogenic index (TI) ranged from 0.31 to 0.58 (Table 3). These ranges were close to the range for some vegetable oils as reported by Turan (2007). Different recommendations have been suggested for AI and TI. According to Hernández-martínez, Gallardo-velázquez, Osorio-, Castañeda-pérez, & Uribe-hernández (2016) levels of <1 are favourable while Ulbricht & Southgate (1991) recommended an upper limit of 0.5 for both AI and TI.. The AI and TI of groundnut oil is comparable to the respective indices; 0.17 to 0.19 and 0.30 to 0.34 reported by Kou, Sabolová, Hor, & Rys, (2018) for oats. Similarly, Attia, Al-harthi, Korish, & Shiboob, (2015; 2017) reported a TI range of 0.38 to 0.78 for eggs. The TI and AI indices of groundnut oil were within recommended levels for good cardiovascular health.

Hypocholesterolemic to hypercholesterolemic index (h/H) indicates potential of a lipid to balance between the good and bad cholesterol. Hernández-martínez et al. (2016) and Osmari, Cecato, Macedo, & Souza (2011) suggested that high h/H of >1 may be beneficial to human health. The Hypocholesterolemic to hypercholesterolemic index for groundnut oil ranged from 4.41 to 6.53. The h/H observed for groundnut oil was close to that (6.14) reported for olive oil (Hashempour-Baltork, Torbati, Azadmard-Damirchi, & Savage, 2018). The high h/H observed in this work implies that consumption of groundnut oil may result in reduced cardiovascular risk.

3.4 Iodine Values for Groundnut Oil

The iodine values (IV) groundnut oil were all in accordance to the recommended range of 86 to 106 except *Egoromoit*, with the lowest IV at 83.08 (Table 3). The highest IV in the Serenuts were observed for Serenut 11T and 13T were 98.88 and 96.27, respectively. Among traditional cultivars, Acholi white and *Rudu* white had the highest IV of 95.79 and 95.38. the iodine values in this study were in agreement with the range reported by Asibuo, (2008) at 85.77 to 98.43 for groundnuts.

Table 3. Total fatty acids (%) and lipid health indices of oil from groundnuts

GNC	SFA	MUFA	PUFA	TUFA	IV	O/L	ω6/ω3	M/S	P/S	P/M	AI	TI	h/H
S1R	20.10±0.16d^{efg}	50.39±0.58^{fg}	29.37±0.42^{cde}	79.76±0.16^{cde}	88.28±0.84^{cd}	1.93	16.97	2.51	1.46	0.58	0.23	0.41	4.87
S2T	16.47±0.13^{b}	53.58±0.88^{i}	29.82±0.85^{de}	83.41±0.13^{gh}	89.94±0.62^{de}	2.01	10.74	3.25	1.81	0.56	0.18	0.31	5.30
S3R	18.94±0.07^{def}	56.05±0.14^{j}	24.93±0.19^{b}	80.99±0.06^{def}	84.73±0.39^{a}	2.53	14.03	2.96	1.32	0.44	0.22	0.40	5.30
S4T	18.68±0.66^{de}	55.82±0.67^{j}	25.53±0.56^{b}	81.35±0.68^{def}	85.36±0.99^{ab}	2.51	13.32	2.99	1.37	0.46	0.22	0.40	5.69
S5R	19.39±0.39^{defg}	50.13±0.67^{fg}	30.50±0.99^{efg}	80.63±0.37^{cdef}	88.93±1.15^{cd}	1.80	16.27	2.59	1.57	0.61	0.22	0.40	5.15
S6T	20.46±0.17^{efgh}	51.09±0.14^{gh}	28.49±0.29^{cd}	79.58±0.21^{bcd}	87.89±0.35^{bcd}	1.95	23.47	2.50	1.39	0.58	0.25	0.46	4.82
S7T	19.48±1.18^{defg}	47.61±0.54^{cd}	33.01±0.91^{i}	80.62±1.25^{cdef}	93.17±1.75^{fgh}	1.50	43.45	2.45	1.70	0.69	0.22	0.43	5.31
S8R	18.45±0.13^{cd}	49.28±0.52^{efg}	31.71±0.61^{fghi}	81.60±0.14^{efg}	92.49±0.25^{fg}	1.66	63.84	2.70	1.72	0.63	0.21	0.41	5.67
S9T	18.77±0.63^{bc}	49.13±0.41^{def}	35.99±0.68^{i}	85.13±0.62^{h}	94.76±0.89^{ghij}	1.44	61.14	2.93	2.15	0.73	0.20	0.39	5.37
S10R	19.63±0.26^{defg}	53.21±0.72^{i}	27.79±0.42^{c}	81.01±0.30^{def}	87.33±0.31^{bc}	2.10	15.14	2.70	1.46	0.52	0.22	0.40	5.09
S11T	17.09±0.81^{c}	46.58±0.84^{bc}	35.46±0.20^{i}	82.03±0.19^{fg}	98.88±0.36^{l}	1.37	15.35	-	-	0.76	0.20	0.37	5.85
S12R	20.03±0.31^{defg}	52.11±0.17^{hi}	27.88±0.27^{c}	79.98±0.31^{cde}	92.57±0.52^{fg}	1.89	35.56	2.60	1.39	0.53	0.23	0.45	5.19
S13T	18.67±0.52^{de}	50.30±0.49^{fg}	31.08±0.04^{ef}gh	81.37±0.50^{def}	96.27±0.50^{k}	1.64	-	2.70	1.67	0.62	0.21	0.42	5.56
S14R	20.26±0.93^{efg}	49.73±0.40^{efg}	30.05±0.66^{def}	79.78±0.89^{cde}	92.88±1.31^{fgh}	1.72	-	2.46	1.48	0.60	0.22	0.44	5.37
AW	24.26±1.41^{i}	39.80±0.75^{a}	36.03±0.77^{i}	75.83±1.51^{a}	95.79±1.21^{ij}	1.11	-	1.65	1.50	0.91	0.29	0.58	4.41
IGO	18.96±0.45^{def}	50.86±0.45^{gh}	30.18±0.03^{def}	81.04±0.45^{def}	93.76±0.77^{fghij}	1.76	-	2.68	1.59	0.59	0.22	0.43	5.46
RR	23.14±0.64^{gh}	45.87±0.34^{b}	32.03±0.68^{hij}	77.80±0.67^{b}	92.02±1.08^{ef}	1.45	228.54	1.98	1.38	0.70	0.22	0.44	5.93
RW	20.76±0.29^{fgh}	45.94±0.24^{b}	34.95±0.20^{i}	80.89±0.43^{def}	95.38±0.36^{hij}	1.34	158.78	2.21	1.68	0.76	0.22	0.44	6.10
EGT	22.18±0.11^{hi}	57.01±0.08^{j}	22.00±0.24^{a}	79.01±0.17^{ab}	83.08±0.23^{a}	2.76	29.98	2.57	0.99	0.39	0.24	0.46	5.54
RB	20.51±0.45^{fgh}	48.38±0.41^{de}	32.71±0.16^{h}	80.17±0.37^{def}	93.72±0.36^{fghi}	1.53	52.32	2.35	1.59	0.68	0.21	0.40	6.53

Data are expressed as percentages of total fatty acid methyl esters; Values are means of triplicate determinations, values followed by the same letter within each column are not significantly different (p>0.05) nd: not detected; GNC: Groundnut cultivar; S: Serenut; R: red; T: Tan; AW: Acholi White; IGO: *Igola*; RR: *Rudu* Red; RW: *Rudu* White; EGT: *Egoromoit*; RB: Red Beauty. S: saturated fatty acid; M: monounsaturated fatty acid; P: polyunsaturated fatty acid; TUFA: total unsaturated fatty acid; O/L: oleic/linoleic ratio; AI: Atherogenic index; TI: thrombogenic index; h/H: hypocholesterolemic to hypercholesterolemic index; IV: Iodine value.

3.5 Lipid Health Indices for Oyster Nut Oil

The ratio of polyunsaturated to saturated fatty acids (P/S) is important in determining the cholesterolemic effect of dietary lipids. High P/S ratios of >1 have a hypo-cholesterolemic effect and higher nutritional value than ones with less (Kostik et al., 2013). The P/S index of oyster nut oil ranged from 0.79 to 1.01 (Table 4). Nutritional guidelines show preference for P/S >0.45 (FAO, 2010). The P/S ratio of oyster nut oil was within recommendations. The MUFA to SFA ratio (M/S) varied from 0.12 to 0.19. Saturated FA have been suggested to increase cardiovascular risk while the converse is reported for MUFA (Grundy, 1997; De Souza et al., 2015; Iggman & Risérus, 2017). Pacheco et al. (2006) studied the effect of different ratios of M/S and indicated that a low ratio resulted in pro-thrombic effect compared to a high ratio.

The ω6/ω3 is an important indicator for decreasing the risk for coronary heart disease (CHD) and hypertension (Simopoulos, 2010). The total omega-3 FA content was <0.5% while omega-6 FA proportions in oil which varied from 39.57 to 47.67% was several folds higher. Accordingly, the ω6/ω3 of >100 was very high compared to the recommended ratio of < 4:1 (Simopoulos, 2002; 2004; Codex, 2005; Rustan and Drevon, 2005 and Patterson, Wall, Fitzgerald, Ross, & Stanton, 2012). Oleic to linoleic acid ratio (O/L) is regarded as a measure of oil stability (Asibuo, 2008). Low O/L hence indicates low oxidative stability (Yanishlieva and Marinova, 2001). On the other hand a high ratio is desirable to enhance oxidative stability and nutritional value (Flagella, Rotunno, Tarantino, Di Caterina, & De Caro, 2002; Mukri et al., 2012). Oyster nut oil had low O/L value of <1 (Table 4). The low O/L ratio in oyster nut oil observed in this study implies that oyster nut oil is susceptible to oxidation.

Atherogenic index (AI) of oyster nut oil ranged from 0.57 to 1.09 while thrombogenic index (TI) ranged from 1.31 to 2.27. Antherogenic index values <1 are desirable for cardiovascular health (Hernández-martínez et al., 2016). The AI for oyster nut oil was close to the recommended level however, the TI exceeded the desirable range of <1. This is attributed to almost balanced levels of SFA of 45.37 to 52.17% compared to the unsaturated FA range of 47.33 to 53.64% in the oil. The AI and TI observed for oyster nut oil were higher than those of bogue obtained from farmed fish reported by S˘imaT et al., (2015). The above indices varied from 0.53 to 0.61 and 0.29 to 0.35 for AI and TI, respectively for bogue. Thrombogenic index shows the tendency to form clots in the blood vessels (Ulbricht & Southgate, 1991). This result implies that despite its vegetable origin, oyster nut oil may have potential to increase cardio vascular risk. The hypocholesterolemic to hypercholesterolemic index (h/H) indicates the influence of fatty acids on cholesterol metabolism (Hashempour-Baltork et al., 2018). The h/H ranged from 0.06 to 1.57. There is no clear criterion for h/H and health however, Osmari et al. (2011) and Hashempour-Baltork et al. (2018), suggested that higher values are beneficial to human health.

3.6 Iodine Values of Oyster Nut Oil

The iodine values (IV) of oil from oyster nuts ranged from 82.76 to 87.59. The highest and lowest values were observed among nuts from Dokolo. The IV of oil from oyster nut is close to the 83.2 reported by (Hopkins & Chisholm, 1964) for oyster nut oil. On the contrary, (Nyagah, 2016) obtained a higher value of 109 for oyster nut oil. Although the IV of oyster nut oil was not included in the codex standard for oils, the value is close to that of safflower and high oleic sun flower which range from 80 to 100 and 78 to 90, respectively. This confirms earlier reports which describe oyster nut oil as a drying oil (Ajayi, Dulloo, Vodouhe, Berjak, & Kioko, 2004).

Table 4. Total fatty acids (%) and lipid health indices of oil from oyster nuts

Source	Gender	SFA	MUFA	PUFA	TUFA	IV	P/S	P/M	M/S	ω6/ω3	O/L	AI	TI	h/H
Dokolo	Male	52.17±1.50[a]	6.23±0.30[a]	41.10±0.98[a]	47.33±1.18[a]	82.76±1.58[a]	0.79	6.6	0.12	-	0.15	1.09	2.19	0.06
Dokolo	Female	48.89±6.66[a]	8.42±2.10[a]	45.22±2.36[a]	53.64±2.18[a]	87.59±4.90[a]	0.94	5.6	0.18	3,938.98	0.18	0.19	1.8	1.48
Kamuli	Male	49.17±2.71[a]	7.26±1.29[a]	43.25±3.99[a]	50.51±2.71[a]	83.28±6.98[a]	0.88	6.17	0.15	-	0.16	1.01	2.01	1.57
Kamuli	Female	49.58±3.19[a]	7.17±1.54[a]	43.64±2.99[a]	50.81±2.28[a]	84.45±7.04[a]	0.89	6.33	0.23	-	0.16	0.94	1.87	1.38
Luwero	Male	45.37±6.05[a]	8.34±4.19[a]	45.25±1.82[a]	53.59±5.58[a]	85.83±3.51[a]	1.01	6.2	0.19	271.65	0.18	0.85	1.77	1.56
Luwero	Female	46.77±3.84[a]	7.12±1.65[a]	44.33±2.63[a]	51.45±2.85[a]	86.91±4.77[a]	0.95	6.47	0.16	262.54	0.16	0.57	1.79	1.57

Values followed by the same letter within each row are not significantly different (p>0.05) nd: not detected; SFA: saturated fatty Acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; TUFA: Total Unsaturated fatty acid; ω6/ω3: Omega-6 to Omega-3 ratio; O/L oleic to linoleic acid ratio; AI: Atherogenic index; TI: Thrombogenic index; h/H: Hypocholesterolemic to hypercholesterolemic index; IV: Iodine value.

4. Conclusion

Breeding may not affect major FA considering the similar levels of oleic acid, linoleic acid, palmitic and stearic acids. Oil from Serenut cultivars contained cis 11-eicosenoic acid (C20.1), cis 11, 14 eicosadienoic acid (C20.2) and cis 11, 14, 17 eicosatrienoic acid (C20.3ω3) but were not detected in that from traditional. This could imply

that breeding enhanced the levels of these fatty acids in improved cultivars. Lipid health indices indicated that oil from groundnut cultivars were favourable for health. Oyster nut oil was abundant in linoleic and palmitic acid. Consequently, the ratio of total saturated FA to unsaturated FA was 1:1. The lipid health indices showed that consumption of oyster nut oil has potential to increase cardiovascular risk; however, the effect of individual SFA needs to be further examined.

Acknowledgements

This work was funded by African Development Bank through the Higher Education in Science and Technology (HEST V) project in partnership with Kyambogo University, Uganda. Authors are very grateful to Dr. David Okello Kalule and Dr. Michael Ugen of the National Semi-Arid Resources Research Institute (NaSARRI) Serere for providing Serenut groundnut cultivars.

References

Achola, E., Tukamuhabwa, P., Adriko, J., Edema, R., Mwale, S. E., Gibson, P., & Okello, D. K. (2017). Composition and variation of fatty acids among groundnut cultivars in Uganda. *African Crop Science Journal*, *25*(3), 291-299.

Ajayi, S. A., Dulloo, M. E., Vodouhe, R. S., Berjak, P., & Kioko, J. I. (2004). Conservation status of Telfairia spp. in sub-Saharan Africa. In *In Regional workshop on Plant Genetic Resources and food security in West and Central Africa.* (pp. 89-95).

Ajayi, S. A., Dulloo, M. E., Vodouhe, R. S., Berjak, P. &, & Kioko, J. I. (2004). Conservation status of Telfairia spp. in sub-Saharan Africa. *Ex Situ Conservation and Genetic Enhancement*, (February 2015), 89-95.

Alabdulkarim, B., Bakeet, Z. A. N., & Arzoo, S. (2012). Role of some functional lipids in preventing diseases and promoting health. *Journal of King Saud University - Science*, *24*(4), 319-329. https://doi.org/10.1016/j.jksus.2012.03.001

AOAC. (2000). AOAC Official Method 963.22 Methyl Esters of Fatty Acids in Oils and Fats, 1-2.

Arbex, A. K., Bizarro, V. R., Santos, J. C. S., Araújo, Lis Marina Mesquita Conceição de Jesus, Ana Luísa Fernandes, M. S. A., Miranda, M., & Salles, Rocha. Denise. Rosso. Tenório. Wanderley & Marcadenti, A. (2015). The Impact of the Essential Fatty Acids (EFA) in Human Health. *Open Journal of Endocrine and Metabolic Diseases*, *5*(7), 98-104. https://doi.org/http://dx.doi.org/10.4236/ojemd.2015.57013

Asibuo, James Yaw, Akromah, Richard, Safo-Kantanka, Osei, Adu-Dapaah, Hans Kofi1, Ohemeng-Dapaah, S. and A. A. (2008). Chemical composition of groundnut, Arachis hypogaea (L)(L) landraces. *African Journal of Biotechnology*, *7*(13), 2203-2208.

Asibuo, J. (2008). Evaluation of nutritional quality of groundnut (Arachis hypogaea L.) from Ghana. *African Journal of Food Agriculture Nutrition and Development*, *8*(2), 133-150.

Attia, Y. A., Al-harthi, M. A., Korish, M. A., & Shiboob, M. M. (2015). hypocholesterolemic , atherogenic , and thrombogenic indices of table eggs in the retail market. *Lipids in Health and Disease*, 1-8. https://doi.org/10.1186/s12944-015-0133-z

Attia, Y. A., Al-harthi, M. A., Korish, M. A., & Shiboob, M. M. (2017). Fatty acid and cholesterol profiles , hypocholesterolemic , atherogenic , and thrombogenic indices of broiler meat in the retail market. *Lipids in Health and Disease*, *16*(40), 1-11. https://doi.org/10.1186/s12944-017-0423-8

Berry, S. K. (1982). Fatty acid composition of 16 groundnut (Arachis hypogaea, L.) cultivars grown under Malaysian conditions. *Pertanika*, *5*(1), 20-24.

Bligh, E. ., & Dyer, W. . (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911-917.

Bonanome, A., & Grundy, S. M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *Journal of Medicine*, *318*(19), 1244-1248.

Bos, M. B., Vries, J. H. M. De, Feskens, E. J. M., Dijk, S. J. Van, Hoelen, D. W. M., Siebelink, E., … Groot, L. C. P. G. M. De. (2010). Effect of a high monounsaturated fatty acids diet and a Mediterranean diet on serum lipids and insulin sensitivity in adults with mild abdominal obesity *. *Nutrition, Metabolism and Cardiovascular Diseases*, *20*(8), 591-598. https://doi.org/10.1016/j.numecd.2009.05.008

Calder, P. C. (2015). Functional Roles of Fatty Acids and Their Effects on Human Health Effects of Saturated Fatty Acids on. *Journal of Parenteral and Enteral Nutrition*, *39*(1), 18S-32S.

https://doi.org/10.1177/0148607115595980

Carta, G., Murru, E., Banni, S., & Manca, C. (2017). Palmitic Acid : Physiological Role , Metabolism and Nutritional Implications. *Frontiers in Physiology*, *8*(902), 1-14. https://doi.org/10.3389/fphys.2017.00902

Codex. (2005). Codex Alimentarius, FAO/ WHO, Rome, Italy. *Codex-Stan 210, Codex Standard for Named Vegetable Oils*, *8*(2).

de Souza, R. J., Mente, A., Maroleanu, A., Cozma, A. I., Ha, V., Kishibe, T., … Anand, S. S. (2015). Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *British Medical Journal*, *351*, h3978. https://doi.org/10.1136/bmj.h3978

Dorni, C., Paras, S., Gunendra, S., & Longvah, T. (2018). Fatty acid Profile of edible oils and fats consumed in India. *Food Chemistry*, *238*, 9-15.

FAO. (2010). *Fats and fatty acids in human nutrition, Report of an expert consultation 10-14 November 2008, FAO Food and Nutrition paper 91*. Rome.

Fattore, E., & Fanelli, R. (2013). Palm oil and palmitic acid : A review on cardiovascular effects and carcinogenicity Palm oil and palmitic acid : a review on cardiovascular effects and carcinogenicity. *International Journal of Food Sciences and Nutrition*, *64*(5), 648-659. https://doi.org/10.3109/09637486.2013.768213

Flagella, Z., Rotunno, T., Tarantino, E., Di Caterina, R., & De Caro, A. (2002). Changes in seed yield and oil fatty acid composition of high oleic sunflower (Helianthus annuus L.) hybrids in relation to the sowing date and the water regime. *European Journal of Agronomy*, *17*(3), 221-230.

Galli, C., & Calder, P. C. (2009). Effects of fat and fatty acid intake on inflammatory and immune responses: A critical review. *Annals of Nutrition and Metabolism*, *55*(1-3), 123-139. https://doi.org/10.1159/000228999

Grundy, S. M. (1997). What is the desirable and monounsaturated ratio of saturated , polyunsaturated , fatty acids in the diet ? 1 ’ 2. *American Journal of Clinical Nutrition*, *66*(Suppliment), 988S-90S.

Harlioğlu, A. G., & Yilmaz, Ö. (2011). Fatty acid composition, cholesterol and fat-soluble vitamins of wild-caught freshwater spiny eel, Mastacembelus simack (Walbaum, 1792). *Journal of Applied Ichthyology*, *27*(4), 1123-1127. https://doi.org/10.1111/j.1439-0426.2011.01750.x

Hashempour-Baltork, F., Torbati, M., Azadmard-Damirchi, S., & Savage, G. P. (2018). Chemical, rheological and nutritional characteristics of sesame and olive oils blended with linseed oil. *Advanced Pharmaceutical Bulletin*, *8*(1), 107-113. https://doi.org/10.15171/apb.2018.013

Hashim, I. B., Koehlerv, P. E., Eitenmiller, R. R., & Kvien, C. K. (1993). Fatty Acid Composition and Tocopherol Content of Drought Stressed Florunner Peanuts, 21-24.

Hernández-martínez, M., Gallardo-velázquez, T., Osorio-, G., Castañeda-pérez, E., & Uribe-hernández, K. (2016). Characterization of Mexican Fishes According to Fatty Acid Profile and Fat Nutritional Indices Characterization of Mexican Fishes According to Fatty Acid Profile and Fat Nutritional Indices. *International Journal of Food Properties*, *19*(6), 1401-1412. https://doi.org/10.1080/10942912.2015.1079787

Hollis, J., & Mattes, R. (2007). Effect of chronic consumption of almonds on body weight in healthy humans. *The British Journal of Nutrition*, *98*(3), 651-656. https://doi.org/10.1017/S0007114507734608

Hopkins, C. Y., & Chisholm, M. . (1964). Fatty acid composition of some cucurbitaceae seed oils. *Canadian Journal of Chemistry*, *42*(7785), 560-564.

Iggman, D., & Risérus, U. (2017). Role of different dietary saturated fatty acids for cardiometabolic risk Role of different dietary saturated fatty acids for cardiometabolic risk. *Clinical Liipidology*, *4299*, 1758-4302.

Johnson, S., Saikia, N., Mathur, H. ., & Agarwal, H. . (2009). *Fatty acids profile of Edible Oils and Fats in India* (No. CSE/PML/PR-32/2009). *Centre for Science and Environment*. New Delhi.

Jumbe, T. J., Pickens, C. A., Valentini, K., Adjepong, M., Li, W., Kinabo, J. L., & Fenton, J. I. (2016). Evaluation of fatty acid and mineral content of Tanzanian seeds and oils. *Journal of Food Composition and Analysis*, *50*, 108-113. https://doi.org/10.1016/j.jfca.2016.05.016

Khetarpaul, N., Jood, S., & Goyal, R. (2007). Fatty acid composition and phisico-chemical chracteristics of cooking oils and thier blends. *Journal of Dairying, Foods and Homesciences*, *26*(3/4), 202-208.

Kostik, V., Memeti, S., & Bauer, B. (2013). Fatty acid composition of edible oils and fats. *Journal of Hygienic Engineering and Design*, 112-116.

Kou, L., Sabolová, M., Hor, P., & Rys, S. (2018). Lipid content , fatty acid pro fi le , and nutritional value of new oat cultivars. *Journal of Cereal Science*, *84*(September), 44-48. https://doi.org/10.1016/j.jcs.2018.09.012

Kris-Etherton, P. M. (1999). Monousaturated Fatty Acids and Risk of Cardiovascular Disease. *Circulation*, *8721*(71), 1253-1258. https://doi.org/10.1111/j.1751-7117.2009.00028.x

Kris-Etherton, P. M., Yu-Poth, S., Sabate, J., Ratcliffe, H. E., Zhao, G., & Etherton, T. D. (1999). Nuts and their bioactive constituents: effects on serum lipids and other factors that affect disease risk. *The American Journal of Clinical Nutrition*, *70*(3), 504S-511S. Retrieved from http://ajcn.nutrition.org/content/70/3/504s.short

Mancini, A., Imperlini, E., Nigro, E., Montagnese, C., Daniele, A., Orrù, S., & Buono, P. (2015). Biological and Nutritional Properties of Palm Oil and Palmitic Acid: Effects on Health. *Molecules*, *20*, 17339-17361. https://doi.org/10.3390/molecules200917339

Mente, A., Dehghan, M., Rangarajan, S., McQueen, M., Dagenais, G., Wielgosz, A., & Wang, Y. (2017). Association of dietary nutrients with blood lipids and blood pressure in 18 countries: a cross-sectional analysis from the PURE study. *The Lancet Diabetes & Endocrinology*, *5*(10), 774-787. https://doi.org/10.1016/S2213-8587(17)30283-8

Minzangi, K., Mpiana, P. T., Samvura, B., Kaaya, A. N., Bertrand, M., & Kadima, J. N. (2015). Composition of Fatty Acids and Tocopherols Content in Oilseeds of Six Wild Selected Plants from Kahuzi-Biega National Park/DR. Congo. *European Journal of Medicinal Plants*, *8*(3), 157-166. https://doi.org/10.9734/EJMP/2015/17297

Mugisha, J., Lwasa, S., & Mausch, K. (2014). *Value chain analysis and mapping for groundnuts in Uganda*.

Mukri, G., Adaf, H. ajisaheb L. N., Bhat, R. S. B., Gowda, M. V. C. G., Upadhyaya, H. ., & Sujay, V. (2012). Phenotypic and molecular dissection of ICRISAT mini core collection of peanut (Arachis hypogaea L .) for high oleic acid. *Plant Breeding*, *422*(131), 418-422. https://doi.org/10.1111/j.1439-0523.2012.01970.x

Nile, S. H., & Park, S. W. (2013). Fatty Acid Composition and Antioxidant Activity of Groundnut (Arachis hypogaea L .) Products. *Food Science and Technology Research*, *19*(6), 957-962. https://doi.org/10.3136/fstr.19.957

Ntare, B. R., Diallo, A. T., Ndjeunga, J., & Waliyar, F. (2008). *Groundnut Seed Production Manual*.

Nyagah, P. K. (2016). *The potential of Telfairia pedata for liquid biofuel and soap production*. Kenyatta University.

Okello, D. K., Biruma, M., & Deom, C. M. (2010). Overview of groundnuts research in Uganda: Past, present, and future. *African Journal of Biotechnology*, *9*(39), 6448-6459. https://doi.org/10.5897/AJB09.013

Okello, D. K., Deom, C. M., Puppala, N., Monyo, E., & Bravo-Ureta, B. (2016). Registration of 'Serenut 5R' Groundnut. *Journal of Plant Registrations*, *10*(2), 1-4. https://doi.org/10.3198/jpr2015.07.0041crc

Osmari, E. K., Cecato, U., Macedo, F. A. F., & Souza, N. E. (2011). Nutritional quality indices of milk fat from goats on diets supplemented with different roughages. *Small Ruminant Research*, *98*(1-3), 128-132. https://doi.org/10.1016/j.smallrumres.2011.03.030

Özcan, M. M. (2010). Some nutritional characteristics of kernel and oil of peanut (Arachis hypogaea L.). *Journal of Oleo Sci*, *59*(1), 1-5.

Pacheco, Y. M., Berm??dez, B., L??pez, S., Abia, R., Villar, J., & Muriana, F. J. G. (2006). Ratio of oleic to palmitic acid is a dietary determinant of thrombogenic and fibrinolytic factors during the postprandial state in men. *American Journal of Clinical Nutrition*, *84*(2), 342-349.

Patterson, E., Wall, R., Fitzgerald, G. F., Ross, R. P., & Stanton, C. (2012a). Health Implications of High Dietary Omega-6 Polyunsaturated Fatty Acids. *Nutrition and Metabolism*, *2012*, 1-16. https://doi.org/10.1155/2012/539426

Patterson, E., Wall, R., Fitzgerald, G. F., Ross, R. P., & Stanton, C. (2012b). Health Implications of High Dietary Omega-6 Polyunsaturated Fatty Acids, *2012*. https://doi.org/10.1155/2012/539426

Ramprasath, V. R., Jones, P. J. H., Buckley, D. D., Woollett, L. A., & Heubi, J. E. (2012). Decreased plasma cholesterol concentrations after pufa-rich diets are not due to reduced cholesterol absorption/synthesis.

Lipids, *47*(11), 1063-1071. https://doi.org/10.1007/s11745-012-3708-8

Ristić-Medić, D., Vučić, V., Takić, M., & Karadžić, Ivana and Glibetić, M. (2013). Polyunsaturated fatty acids in health and disease. *Journal of the Serbian Chemical Society*, *78*(9), 1269-1289. https://doi.org/10.2298/JSC130402040R

Rustan, A. C., & Drevon, C. A. (2005). Fatty Acids: Structures and Properties. *Encyclopedia of Life Sciences*, 1-7. https://doi.org/10.1038/npg.els.0003894

S˘imaT, V., Bogdanovic´, T., Poljak, V., & Petric˘evic´, S. (2015). Changes in fatty acid composition , atherogenic and thrombogenic health lipid indices and lipid stability of bogue (Boops boops Linnaeus , 1758) during storage on ice : Effect of fish farming activities. *Journal of Food Composition and Analysis*, *40*, 120-125. https://doi.org/10.1016/j.jfca.2014.12.026

Santos-Silva, J., Bessa, R. J. B., & Santos-Silva, F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs: II. Fatty acid composition of meat. *Livestock Production Science*, *77*(2-3), 187-194.

Shad, M. A., Pervez, H., Zafar, Z. I., Nawaz, H., & Khan, H. (2012). Physicochemical properties, fatty acid profile and antioxidant activity of Peanut Oil. *Pakistan Journal of Botany*, *44*(1), 435-440.

Shahidi, F. E. (2005). *Bailey's industrial oil and fat products: Volume 1, Edible Oil and Fat Products: Chemistry, Properties, and Health Effects.* (F. Shahidi, Ed.) (6th ed.). Hoboken, New Jersey: JOHN WILEY & SONS, INC.

Simopoulos, A. P. (2008). The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases. *Experimental Biology and Medicine*, *233*(6), 674-688. https://doi.org/10.3181/0711-MR-311

Simopoulos AP. (2002). The importane of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy*, *8*(56), 365-379.

Simopoulos, Artemis. P. (2010). The omega-6 / omega-3 fatty acid ratio : health implications. *Oilseeds and Fats, Crops and Lipids*, *17*(5), 267-275. https://doi.org/http://dx.doi.org/10.1051/ocl.2010.0325

Simopoulos, Artemis. P. (2016). An Increase in the Omega-6 / Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. *Nutrients*, *8*(3), 1-17. https://doi.org/10.3390/nu8030128

Simopoulos, Artemis P. (2004). Omega-6/Omega-3 Essential Fatty Acid Ratio and Chronic Diseases. *Food Reviews International*, *20*(1), 77-90. https://doi.org/10.1081/FRI-120028831

Sinanoglou, V. J., Batrinou, A., Mantis, F., Bizelis, I., & Miniadis-meimaroglou, S. (2013). Lipid quality indices : Differentiation of suckling lamb and kid breeds reared by traditional sheep farming. *Small Ruminant Research*, *113*(1), 1-10. https://doi.org/10.1016/j.smallrumres.2013.01.008

Strayer, D., Belcher, M., Fine, J., & Mcbrayer, T. (2006). *Food Fats* (9th ed.). Washington: Institute of Shortening and Edible Oils , Inc.

Turan, H., Sönmez, G., & Kaya, Y. (2007). Fatty acid profile and proximate composition of the thornback ray (Raja clavata , L . 1758) from the Sinop coast in the Black Sea. *Journal of Fisheries Sciences.Com*, *1*(2), 97-103. https://doi.org/10.3153/jfscom.2007012

Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: seven dietary factors. *The Lancet*, *338*(8773), 985-992.

Wen, N., & Chao, P. (1998). Effects of the Ratio of Polyunsaturated and Monounsaturated Fatty Acid to Saturated Fatty Acid on Rat Plasma. *Lipids*, *33*(5), 481-487.

Yang, S.-C., Lin, S.-H., Chang, J.-S., & Chien, Y.-W. (2017). High Fat Diet with a High Monounsaturated Fatty Acid and Polyunsaturated / Saturated Fatty Acid Ratio Suppresses Body Fat Accumulation and Weight Gain. *Nutrients*, *9*(10), 1-15. https://doi.org/10.3390/nu9101148

Yanishlieva, N. V., & Marinova, E. M. (2001). Stabilisation of edible oils with natural antioxidants. European journal of lipid science and technology. *European Journal of Lipid Science and Technology*, *103*(11), 752-767.

Yehuda, S. (2003). Omega-6/omega-3 ratio and brain-related functions. *In Omega-6/Omega-3 Essential Fatty Acid Ratio: The Scientific Evidence .Karger Publishers.*, *92*, 37-56. https://doi.org/10.1159/000073791

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Determination of Gamma-aminobutyric Acid (GABA) Content in Grains and Cruciferous Vegetable Seeds

Fadwa Al-Taher[1] & Boris Nemzer[1, 2]

[1]VDF FutureCeuticals, 2692 N. State Rte. 1-17, Momence, IL 60954, USA

[2]University of Illinois in Urbana-Champaign, Urbana, IL 60014, USA

Correspondence: Fadwa Al-Taher, VDF FutureCeuticals, 2692 N. State Rt. 1-17, Momence, IL 60954, USA. Tel: 1-815-507-1421. E-mail: Fadwa.Al-Taher@futureceuticals.com

Received: August 27, 2019 Accepted: October 10, 2019 Online Published: October 15, 2019

doi:10.5539/jfr.v8n6p49 URL: https://doi.org/10.5539/jfr.v8n6p49

Abstract

Gamma aminobutyric acid (GABA) has great physiological functions, mainly as a major inhibitory neurotransmitter in the brain, which makes it important for the development of functional foods. This study detected GABA in grains and cruciferous vegetable seeds by using HPLC after pre-column on-line derivatization with diode array detection (DAD) and fluorescence detection (FLD). The limit of quantitation was 2.94 and 2.86 μg/mL with DAD and FLD, respectively. GABA recoveries ranged from 98.8 to 111.2% on both detectors. Intra and inter-day precision showed relative standard deviations, generally, less than 10% for both DAD and FLD. GABA was determined in different grains (flaxseeds, white quinoa seeds, and buckwheat) and cruciferous vegetable seeds (broccoli, kale, daikon radish, mustard, cabbage, and brussels sprouts). Organic broccoli seeds contained the highest amount and mustard seeds the least amount of GABA in the Brassica family with none being detected in organic white quinoa and flaxseeds. A statistically significant difference ($p < 0.05$) exists between the various lots of the broccoli seeds. GABA is important as a natural source in functional foods.

Keywords: gamma-aminobutyric acid (GABA), cruciferous vegetable seeds, grains, germination, sonication, pre-column derivatization

1. Introduction

Gamma aminobutyric acid (GABA) is a four-carbon non-proteinogenic amino acid and a major inhibitory neurotransmitter in the brain. It has several physiological roles, mainly in the human central nervous system (Watanbe, Maemura, Kanbara, Tamayama & Hayasaki, 2002). Most recently, GABA as a bioactive compound has become popular for its functional and health effects because it has been recognized to improve brain function (Watanbe et al., 2002), lower blood pressure (Inoue et al., 2003), reduce insomnia (Plante, Jensen, Schoerning & Winkelman, 2012), induce relaxation and diminish anxiety (Abdou, Higashiguchi, Horie, Hatta, & Yokogoshi, 2006) and inhibit impulsivity (Silveri et al., 2013). It is also believed to help prevent diabetes (Taneera et al., 2012; Braun et al., 2010). Moreover, GABA can delay and/or prevent the spread of cancer cells and stimulate apoptosis in cancer cells. Additionally, it is shown to suppress tumors as well (Diana, Quílez, & Rafecas, 2014).

GABA is synthesized primarily from glutamate by the enzyme glutamate decarboxylase (GAD) with pyridoxal phosphate (the active form of vitamin B6) as a cofactor (Watanbe et al., 2002). GABA exists naturally in small quantities in many plant sources: vegetables (spinach, potatoes, cabbage, asparagus, broccoli and tomatoes), fruits (apples and grapes), and cereals (barley and maize). A high amount of GABA is found mainly in fermented products such as fermented dairy products, soy sauces and cheeses. Although the human body can generate its own GABA, it is sometimes hindered by a lack of estrogen, zinc or vitamins, or by a high amount of salicylic acid and food additives in the diet (Diana, Quílez, & Rafecas, 2014). With aging, ability to produce GABA in the cerebral cortex weakens (Leventhal, Wang, Pu, Zhou, & Ma, 2003). Therefore, it is worthwhile to consider dietary or nutritional supplements for GABA.

To promote and maintain the health of the consumers, it is pertinent to discover new natural food sources of GABA and development of functional foods. Particularly, grains and seeds will potentially be used as raw ingredients in nutritional GABA supplements and as raw materials for sprouts. Beans, rice, and wheat seeds have been used for germination studies to increase the content of GABA (Yang, Guo, & Gu, 2013; Ding et al., 2018a;

Ding et al., 2018b). GABA content for fava beans sprouted for five days under hypoxia was 2.21-fold higher than that of the control (Yang, Guo, & Gu, 2013). Hayat et al. (2015) determined controlled brown rice samples to be 47- 65 mg/kg, 115-935 mg/kg for germinated and 1032-1089 mg/kg for fermented samples. Ding et al. (2018a) noticed that GABA in red rice was 2.91 mg/100 g and increased after germination for 72 h and was 15.4 times higher than the un-germinated rice. GABA content in the wheat non-sprouting sample contained 14.68 ± 0.43 mg/100 g, which after a 72 h germination increased by 339% (Ding et al., 2018b). GABA content in grains and cruciferous vegetable seeds, which can be used as health promoting food ingredients such as flaxseeds, quinoa, barley and broccoli seeds, has not been established.

GABA content for germination of grains experiments has been tested primarily by using HPLC on a reversed phase column, C-8 or C18 after pre-column online derivatization with o-phthaldialdehyde (OPA) (Hayat et al., 2015; Ding et al., 2018a) or with addition of dansyl chloride to the sample (Ding et al., 2018b) and detection by an ultraviolet visible (UV-Vis) detector. Zhao et al. (2011) analyzed GABA in Chinese teas with HPLC-FLD using OPA for pre-column online derivatization. Ding et al. (2016) tested GABA in ungerminated and germinated dehulled rice under normoxic and hypoxic conditions by GC-MS using bismethylsilane trifluoracetamide (BSTFA) to derivatize samples. However, these analytical methods have not been used to determine GABA in grains and cruciferous vegetable seeds.

The objective of this study was to determine the amount of gamma aminobutyric acid (GABA) in grains and cruciferous vegetable seeds using high performance liquid chromatography with diode array detector (DAD) and a fluorescence detector (FLD). GABA content was also assessed in various lots of broccoli seeds.

2. Materials and Methods

2.1 Chemicals and Reagents

Sodium phosphate dibasic, (Na^2HPO4; purity ⩾ 99%), sodium tetraborate decahydrate ($Na^2B4O7.10\ H_2O$; purity ⩾ 99.5%), sodium azide (NaN_3; purity ⩾ 99.5%), gamma-aminobutyric acid (GABA; > 99% purity) and norvaline (internal standard; > 98% purity) were purchased from Millipore Sigma (St. Louis, MO, USA). All chemicals and reagents used were of analytical grade. Phosphoric acid, ACS grade and 36.5-38% HCl, ACS grade were obtained from VWR International (Radnor, PA, USA). Acetonitrile and methanol, both HPLC grade were acquired from EMD Millipore Corp. (Darmstadt, Germany). The solutions were prepared with ultra-pure water (Milli-Q system; Millipore, Bedford, MA, USA). Borate buffer (0.4 N, pH 10.2) and o-phthaldialdehyde (OPA) reagent (10 mg/mL) used for derivatization were purchased from Agilent Technologies (Santa Clara, CA, USA).

2.2 Material and Sample Preparation

A variety of grains and cruciferous vegetable seeds were collected from suppliers of VDF FutureCeuticals in Momence, IL. Caudill Seed Company (Louisville, KY, USA) supplied the organic (A) and conventional (A-E) broccoli seeds, organic buckwheat seeds, mustard seeds and organic daikon radish seeds. Organic white quinoa seeds were acquired from Colorexa (Deerfield Beach, FL, USA). Organic kale was purchased from Johnny's Seeds (Winslow, ME, USA). Organic broccoli seeds (B) and organic flaxseeds were obtained from Organic Quality Food (Spring, TX, USA). The collected grains and cruciferous vegetable seeds were then ground to a fine powder with an Economical Analytical Mill (IKA A11 basic, Germany) and stored at room temperature until they were used for extraction.

2.3 Extraction of GABA

Approximately 100 ± 10.0 mg ground grains and cruciferous vegetable seeds were extracted using 5 mL of Milli-Q water at room temperature (~23°C). Norvaline, added to a final concentration of 50 µg/mL was used as an internal standard. Each sample was shaken on a wrist shaker for 30 min, and sonicated in an ultrasonic bath for another 30 min. The solution was centrifuged at 8000 rpm for 10 min at room temperature (~23°C). The supernatant was filtered through a 0.45-µm nylon syringe filter into a 2 mL HPLC autosampler vial and the filtrate was used to determine the GABA content.

2.4 Analysis

Separation of GABA from the grains and cruciferous vegetable seeds was performed on an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) with an ultraviolet- visible diode array detector (DAD) (338 nm) and a fluorescence detector (FLD) (Excitation 230 nm and Emission 450 nm). The separation was achieved using a Zorbax Eclipse AAA C18 column (4.6 x 150 mm, 3.5 µm) from Agilent Technologies. Analysis was prepared according to Agilent's Application Note for amino acids (Henderson & Brooks, 2010).

Linearity of the method for GABA was determined to be in the concentration range of 4-120 µg/mL. The coefficient of determination (R2) was 0.99979 for the DAD and 0.99904 for the FLD indicating the goodness of the fit of the data to the regression line. The RSDs of the intraday (repeatability) results for GABA in organic white quinoa seeds were 6.0% and 5.5% for the 5 µg/mL level and 0.51% and 0.49% for 50 µg/mL, with the DAD and FLD, respectively. The RSDs of the interday (intermediate precision) results for GABA were 10.8% and 6.4% for the 5 µg/mL level, 3.8% and 3.6% for 50 µg/mL, and 1.4% and 3.1% for 100 µg/mL, with the DAD and FLD, respectively.

Limit of detection (LOD) was calculated as 3s and limit of quantitation (LOQ) as 10s, where s is the standard deviation. LOD for GABA was 0.881 and 0.857 µg/mL with DAD and FLD, respectively, whereas LOQ was found to be 2.94 and 2.86 µg/mL with DAD and FLD, respectively.

Trueness was evaluated by determining the average recoveries (%) of GABA in flaxseed and quinoa blank samples. No blank broccoli samples were found to perform this study. A broccoli sample containing GABA (1278 µg/g and 1331 µg/g as detected by DAD and FLD, respectively) was therefore chosen for the recovery study. Average Recoveries (%) of GABA in select grains/cruciferous vegetable seeds are shown in Table 1. Recoveries ranged from 100.0 to 110.5% with the DAD and 98.8 to 111.2% with the FLD detector, which established the accuracy of the method.

Table 1. Average GABA recoveries (%) ± %RSD in select grains and cruciferous vegetable seeds (n=4)

Grains/Seeds	**Flaxseeds**		**Quinoa**		**Broccoli**	
Concentration spiked (µg/mL)	**DAD %Rec ± %RSD**	**FLD %Rec ± %RSD**	**DAD %Rec ± %RSD**	**FLD %Rec ± %RSD**	**DAD %Rec ± %RSD**	**FLD %Rec ± %RSD**
5	107.9 ± 2.1	100.5 ± 5.7	101.1 ± 3.0	105.5 ± 2.7	104.8 ± 1.4	104.1 ± 3.9
50	100.0 ± 4.6	99.4 ± 0.54	101.0 ± 6.9	104.8 ± 6.8	102.5 ± 1.1	98.8 ± 0.65
100	101.8 ± 1.2	106.8 ± 1.0	106.7 ± 0.73	111.2 ± 1.1	110.5 ± 9.1	102.5 ± 0.78

2.5 Statistical Analysis

GABA identification and quantitation data generation for grains and cruciferous vegetable seeds was performed using Agilent Technology's Chemstation Openlab CDS Rev. C.01.05 (Santa Clara, CA, USA). Data analysis was performed to calculate the means, standard deviations and relative standard deviations using Microsoft Excel 365 ProPlus version 1903. Statistical analysis was performed with one-way analysis of variance (ANOVA) with significance set at p values of < 0.05 using SigmaPlot 14.0 (Systat Software; San Jose, CA, USA).

3. Results and Discussion

3.1 Extraction of GABA in Seeds

Zhao et al. (2011) extracted GABA from tea in hot water (85°C) for 2 hours. In this study, we observed that extraction of GABA with hot water did not work. Preliminary investigation was performed using ground organic broccoli seeds to determine the effectiveness of water extraction only (shaken for 30 min) versus water extraction (shaken for 30 min) and then sonication (30 min) at room temperature. Results showed that with sonication the level of GABA increased 32.2 % and 34.7 %, (n=4) with a significant difference ($p < 0.05$) when detected by the DAD and FLD, respectively.

Ultrasound increases extraction by additionally disrupting the plant tissue (Mason, Paniwnyk, & Lorimer, 1996). The increase in GABA content with sonication has been previously demonstrated. Yang, Gao, Yang & Chen (2015) found that ultrasound treatment increases the germination rate, sprout length, and GABA content of soybean sprouts. Ding et al. (2018a) showed that GABA content in red rice significantly increased after germination for 72 h and further increased at different stages during germination upon ultrasound treatment. In another study, Ding et al. (2018b) demonstrated that the GABA content in the ultrasound-treated soft white wheat was 30.7% higher than that without ultrasound treatment. Therefore, to optimize the extraction of GABA, water and sonication were used for the analyses of grains and cruciferous vegetable seeds. GABA was extracted from grains and cruciferous vegetable seeds with water at room temperature (~23°C) for 30 min. and sonication for 30 min.

3.2 Analysis of GABA in Grains and Cruciferous Vegetable Seeds

Select grains and seeds belonging to the genus Brassica were screened for GABA. Table 2 lists the mean GABA concentrations (n=4) and standard deviations (stdev) found in each of select grains and cruciferous vegetable seeds with mostly similar results obtained when identified using both detectors, DAD and FLD. It can be

observed that all of the Brassica vegetable seeds contained GABA ranging from 41.38 ± 2.67 mg/100 g to 116.6 ± 6.48 mg/100 g with DAD and 41.10 ± 2.46 mg/100g to 118.91 ± 4.06 mg/100g with FLD. Results showed that organic broccoli seeds contained the highest amount and mustard seeds the least amount of GABA in the Brassica family with none being detected in organic white quinoa and flaxseeds.

Table 2. Mean GABA concentration (mg/100g ± stdev) in grains and cruciferous vegetable seeds (n=4)

Grains/Cruciferous Seeds	**DAD**	**FLD**
	mg/100g ± stdev	**mg/100g ± stdev**
Conventional Broccoli seeds	104.1 ± 2.50	108.9 ± 3.47
Organic Broccoli seeds	116.6 ± 6.48	118.91 ± 4.06
Organic White Quinoa seeds	ND	ND
Organic Buckwheat seeds	10.58 ± 0.751	10.20 ± 0.686
Organic Flaxseeds	ND	ND
Organic Kale	57.39 ± 4.01	62.62 ± 3.79
Mustard seeds	41.38 ± 2.67	41.10 ± 2.46
Organic Daikon Radish	57.10 ± 4.75	54.16 ± 4.60
Organic Cabbage Dutch (green) seeds	83.93 ± 3.92	94.13 ± 4.48
Red Express Organic Cabbage (purple-red) seeds	70.86 ± 5.49	83.88 ± 7.20
Brussel Sprouts seeds	62.75 ± 13.5	70.61 ± 13.18

*ND-denotes not detected

Various lots of broccoli seeds, organic and conventional were assessed for GABA. Lot-to-lot variability of broccoli seeds was examined for GABA (n=4). Figure 1 depicts GABA content in the broccoli seeds lots analyzed. GABA concentrations ranged from 93.9 ± 13.4 mg/100 g to 131.9 ± 3.17 mg/100 g on the DAD and 96.03 ± 19.5 mg/100g to 129.7 ± 6.21 mg/100g on the FLD.

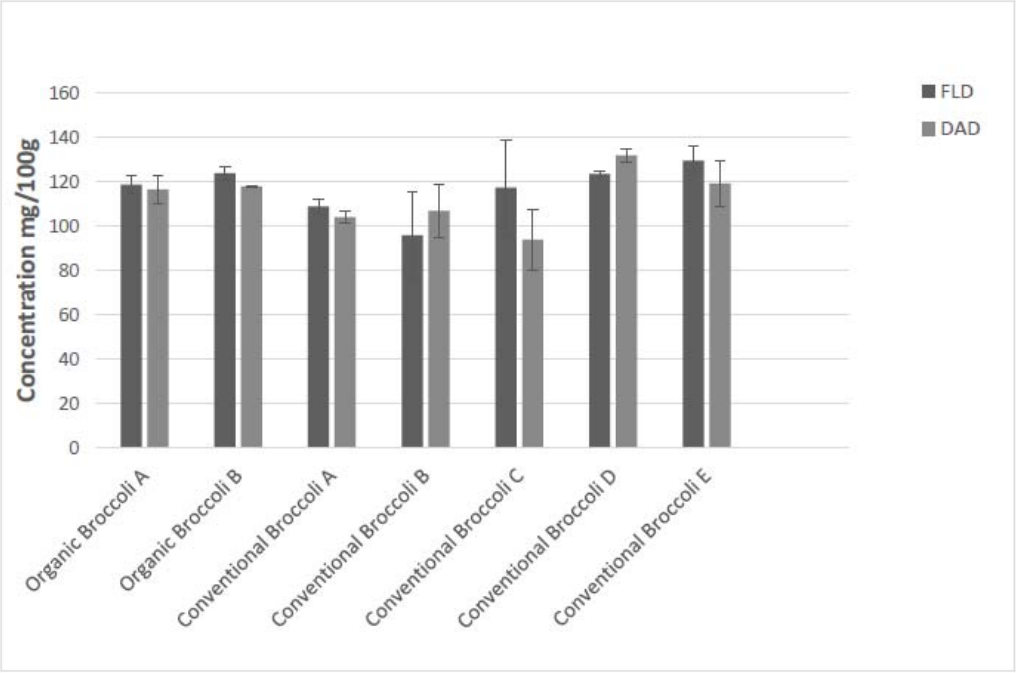


Figure 1. GABA content in various lots of organic and conventional broccoli samples

Although obtained from different suppliers, GABA content was similar in the two organic broccoli seeds and showed no significant difference ($p > 0.05$) with the DAD and ($p > 0.05$) with the FLD. Overall, there is a statistically significant difference for GABA results in conventional broccoli seeds observed by DAD ($p < 0.05$). Conventional Broccoli C is significantly different from Organic Broccoli A, Organic Broccoli B, Conventional Broccoli D and Conventional Broccoli E as detected on the DAD ($p < 0.05$). Conventional Broccoli D is also

significantly different from Conventional Broccoli A and Conventional Broccoli B with the DAD ($p < 0.05$).

In general, there is also a statistically significant difference ($p < 0.05$) for GABA results in broccoli seeds obtained with FLD. Conventional Broccoli B is significantly different ($p < 0.05$) from Organic Broccoli B, Conventional Broccoli D and Conventional Broccoli E with the FLD.

While broccoli samples were obtained mainly from the same supplier, a statistically significant difference does exist between the various lots of the broccoli seeds. There was not enough data for organic broccoli seeds to compare with the conventional broccoli seeds to draw a conclusion on the relative GABA content. However, it is evident from Figure 1 that broccoli seeds do contain a high GABA content.

4. Conclusion

This is the first time that results have been reported of GABA content in different grains (flaxseeds, white quinoa seeds, and buckwheat) and cruciferous vegetable seeds (broccoli, kale, daikon radish, mustard, cabbage, and brussels sprouts). The levels of GABA determined in this study indicate that broccoli seeds, or their short time germinated sprouts should be useful as a natural source of GABA in human diet.

References

Abdou, A. M., Higashiguchi, S., Horie, K., Hatta, H., & Yokogoshi, H. (2006). Relaxation and immunity enhancement effects of γ-Aminobutyric acid (GABA) administration in humans. *BioFactors, 26*, 201-208. https://doi.org/10.1002/biof.5520260305

Braun, M., Ramracheya, R., Bengtsson, M., Clark, A., Walker, J. N., Johnson, P. R., & Rorsman, P. (2010). γ-Aminobutyric acid (GABA) is an autocrine excitatory transmitter in human pancreatic ß-cells. *Diabetes, 59*, 1694-1701. https://doi.org/10.2337/db09-0797

Diana, M., Quílez, J., & Rafecas, M. (2014). Gamma-aminobutyric acid as a bioactive compound in foods: a review. *Journal of Functional Foods, 10*, 407-420. https://doi.org/10.1016/j.jff.2014.07.004

Ding, J., Ulanov, A. V., Dong, M., Yang, T., Nemzer, B. V., Xiong, S., & Feng, H. (2018a). Enhancement of gamma-aminobutyric acid (GABA) and other health-related metabolites in germinated red rice (*Oryza sativa* L.) by ultrasonication. *Ultrasonics Sonochemistry, 40,* 791-797. http://dx.doi.org/10.1016/j.ultsonch.2017.08.029

Ding, J., Hou, G. G., Nemzer, B. V., Xiong, S., Dubat, A., & Feng, H. (2018b). Effects of controlled germination on selected physiochemical and functional properties of whole-wheat flour and enhanced γ-aminobutyric acid accumulation by ultrasonication. *Food Chemistry, 243,* 214-221. http://dx.doi.org/10.1016/j.foodchem.2017.09.128

Ding, J., Yang, T., Feng, H., Dong, M., Slavin, M., Xiong, S., & Zhao, S. (2016). Enhancing contents of γ-Aminobutyric acid (GABA) and other micronutrients in dehulled rice during germination under normoxic and hypoxic conditions. *Journal of Agricultural and Food Chemistry, 64,* 1094-1102. http://dx.doi.org/10.1021/acs.jafc.5b04859

Hayat, A., Jahangir, T. M., Khuhawar, M. Y., Alamgir, M., Hussain, Z., Haq, F. U., & Musharraf, S. G. (2015). HPLC determination of gamma amino butyric acid (GABA) and some biogenic amines (BAs) in controlled, germinated, and fermented brown rice by pre-column derivatization. *Journal of Cereal Science, 64*, 56-62. http://dx.doi.org/10.1016/j.jcs.2015.04.014

Henderson, J. W., & Brooks, A. (2010). Improved amino acid methods using Agilent Zorbax Eclipse Plus C18 columns for a variety of Agilent LC instrumentation and separation goals (Application Note 5990-4547EN). Retrieved from https://www.agilent.com/cs/library/applications/5990-4547EN.pdf

Inoue, K., Shirai, T., Ochiai, H., Kasao, M., Hayakawa, K., Kimura, M., & Sansawa, H. (2003). Blood-pressure-lowering effect of a novel fermented milk containing γ-aminobutyric acid (GABA) in mild hypertensives. *European Journal of Clinical Nutrition, 57*, 490-495. https://doi.org/10.1038/sj.ejcn.1601555

Leventhal, A. G., Wang, Y., Pu, M., Zhou, Y., & Ma, Y. (2003). GABA and its agonists improved visual cortical function in senescent monkeys. *Science*, *300*, 812-815. https://doi.org/10.1126/science.1082874

Mason, T. J., Paniwnyk, L., & Lorimer, J. P. (1996). The uses of ultrasound in food technology. *Ultrasonics Sonochemistry, 3,* S253-S260. https://doi.org/10.1016/S1350-4177(96)00034-X

Plante, D. T., Jensen, J. E., Schoerning, L., & Winkelman, J. W. (2012). Reduced γ-Aminobutyric acid in occipital and anterior cingulate cortices in primary insomnia: a link to major depressive disorder? *Neuropsychopharmacology, 37*, 1548-1557. https://doi.org/10.1038/npp.2012.4

Silveri, M. M., Sneider, J. T., Crowley, D. J., Covell, M. J., Acharya, D., Rosso, I. M., & Jensen, J. E. (2013). Frontal lobe γ-Aminobutryic acid levels during adolescence: associations with impulsivity and response isolation. *Biological Psychiatry, 74,* 296-304. https://doi.org/10.1016/j.biopsych.2013.01.033

Taneera, J., Jin, Z., Jin, Y., Muhammed, S. J., Zhang, E., Lang, S., Salehi, A., Korsgren, O., Renström, E., Groop, L., & Birnir, B. (2012). γ-Aminobutyric acid (GABA) signaling in human pancreatic islets is altered in type 2 diabetes. *Diabetologia, 55*, 1985-1994. https://doi.org/10.1007/s00125-012-2548-7

Watanbe, M., Maemura, K., Kanbara, K., Tamayama, T., & Hayasaki, H. (2002). GABA and GABA receptors in the central nervous system and other organs. *International Review of Cytology, 213*, 1-47. https://doi.org/10.1016/S0074-7696(02)13011-7

Yang, H., Gao, J., Yang, A., & Chen, H. (2015). The ultrasound-treated soybean seeds improve edibility and nutritional quality of soybean sprouts. *Food Research International, 77,* 704-710. http://dx.doi.org/10.1016/j.foodres.2015.01.011

Yang, R., Guo, Q., & Gu, Z. (2013). GABA shunt and polyamine degradation pathway on γ-aminobutyric acid accumulation in germinating fava bean (*Vicia faba* L.) under hypoxia. *Food Chemistry, 136*, 152-159. http://dx.doi.org/10.1016/j.foodchem.2012.08.008

Zhao, M., Ma, Y., Wei, Z., Yuan, W., Li, Y., Zhang, C., Xue, X., & Zhou, H. (2011). Determination and comparison of γ-Aminobutyric acid (GABA) content in Pu-erh and other types of Chinese tea. *Journal of Agricultural and Food Chemistry*, *59*, 3641-3648. http://dx.doi.org/10.1021/jf104601v

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Nutritional Composition of the Green Leaves of Quinoa (*Chenopodium quinoa* Willd.)

Safiullah Pathan[1], Frieda Eivazi[1], Babu Valliyodan[1], Kamalendu Paul[2], Grato Ndunguru[1] & Kerry Clark[3]

[1]Department of Agriculture and Environmental Sciences, Lincoln University of Missouri, Jefferson City, MO 65101, USA

[2]Cooperative Extension, Lincoln University of Missouri, Jefferson City, MO 65101, USA

[3]Division of Applied Social Sciences, University of Missouri, Columbia, MO 65211, USA

Correspondence: Safiullah Pathan, Department of Agriculture and Environmental Sciences, Lincoln University of Missouri, Jefferson City, MO 65101, USA. Tel: 1-573-681-5963. E-mail: PathanS@LincolnU.edu

Received: August 31, 2019 Accepted: September 20, 2019 Online Published: October 16, 2019

doi:10.5539/jfr.v8n6p55 URL: https://doi.org/10.5539/jfr.v8n6p55

Abstract

Quinoa (*Chenopodium quinoa* Willd.) grain is often eaten worldwide as a healthy food, but consuming nutrient-rich quinoa leaves as a leafy green vegetable is uncommon. This study evaluated the potentiality of leafy green quinoa as a major source of protein, amino acids, and minerals in the human diet. Also, the study compared the nutrient content of quinoa leaves with those of amaranth and spinach leaves. The proximate analysis of quinoa dry leaves showed a higher amount (g/100 g dry weight) of protein (37.05) than amaranth (27.45) and spinach (30.00 g). Furthermore, a lower amount of carbohydrate (34.03) was found in quinoa leaves compared to amaranth (47.90) and spinach (43.78 g). A higher amount of essential amino acids was found in quinoa leaves relative to those of amaranth and spinach. The highest amounts (mg/100 g dry weight) of minerals in quinoa dry leaves were copper (1.12), manganese (26.49), and potassium (8769.00 mg), followed by moderate amounts of calcium (1535.00), phosphorus (405.62), sodium (15.12), and zinc (6.79 mg). Our findings suggest that quinoa leaves can be consumed as a green vegetable with an excellent source of nutrients. Therefore, we endorse the inclusion of quinoa in the leafy green vegetable group.

Keywords: amino acid, food nutrition, leafy green vegetable, mineral, protein, quinoa (*Chenopodium quinoa* Willd.)

1. Introduction

Green leafy vegetables (GLVs) or leafy greens are plants' young leaves, with or without petioles or shoots, which may be consumed raw (in a salad), stir-fried, stewed, or steamed. Leafy vegetables often contain high amounts of iron, magnesium, potassium, and calcium, and low amounts of sodium, carbohydrate, and fat and are lower in the glycemic index. Also, they contain a higher amount of folate, a B vitamin and antioxidants. The dietary guidelines for Americans recommend five servings of vegetables per day (based on an intake of 2,000 calories), with green leafy vegetables as one of the five recommended servings (HHS & USDA, 2015). Research has shown that more than 3 million children below five years of age die annually due to malnutrition globally (UNICEF, 2018). The most common and popular GLVs (traditional vegetables) are collard greens, kale, spinach, lettuce, and broccoli. Less popular and locally grown GLVs (non-traditional vegetables) are amaranth, Indian spinach, lamb's quarter, watercress, and moringa leaves. The GLVs are common ingredients in the diet of rural people of developing countries as these vegetables are inexpensive, locally grown, seasonally abundant, and often available year-round. A single variety cannot provide all the nutrients for adequate bodily functions; therefore, different types and amounts of vegetables are important for a healthy diet.

A number of researchers have investigated the nutritional quality and health benefits of traditional as well as non-traditional leafy vegetables, and they found that non-traditional leafy vegetables also contain higher amounts of nutrients (Abd El-Samad, Hussin, El-Naggar, El-Bordeny & Eisa, 2018; Adeyinka, Abolaji, & Olukemi, 2014; Akpana, Edward, Henry, & Joseph, 2017; Arowosegbe, Olanipekun, & Adeloye, 2018; Dias, 2012; Yadav, Tomar, Pachauri, & Jain, 2018). Therefore, it is clear that non-traditional leafy vegetables also play a significant role in

reducing the micronutrient deficiency of lower-income groups living in distant rural areas, mostly in Asia and Africa. The grain of quinoa (*Chenopodium quinoa* Willd.) is considered to be a novel, healthy food because of its nutritional properties and health benefits. However, the nutritional composition of the green leaves of quinoa as a vegetable are not yet fully known, only a few researchers have mentioned the benefits of consumption of green leaves of quinoa. Recently, Abd El-Samad et al. (2018) reported the suitability of potential use of the young quinoa plant as a new non-traditional leafy vegetable crop in Egypt.

The objective of this study was to determine the proximate, amino acid, and mineral composition of quinoa leaves, and to compare the values with those of spinach, a well-known, widely consumed GLV; and amaranth, a popular GLV of Asia and Africa. The present work highlights the potential benefits of considering quinoa leaves as a rich source of nutrients adequate for good human health. As of our knowledge, this study is the first of its kind in the USA to determine the nutritional components of green leafy quinoa.

2. Materials and Methods

2.1 Plant Materials

During the summer of 2018, three leafy vegetables, quinoa (*Chenopodium quinoa*) var. Ames 13739 (USDA – GRIN), amaranth (*Amaranthus viridis*) var. Green Callaloo (AM126, Baker Creek Heirloom Seed Company, Missouri, USA), and spinach (*Spinacia oleracea*) var. Bloomsdale (SP101, Baker Creek Heirloom Seed Company, Missouri, USA) seeds were planted and grown at the Lincoln University George Washington Carver Farm (38.96N, 92.36W) in Jefferson City, Missouri, United States. The soil at the experimental plots was silt loam with 20% clay content and 0.8% organic matter. Before sowing, the soil fertility was improved by incorporating the inorganic fertilizer NPK (12-12-12) at the rate of 35 lbs. per acre. Seeds of the three species were sown manually in 5-foot-long, four-row plots, with a plant-to-plant and row-to-row distance of 6 inches, in three replications. Plots were irrigated when required, and no pesticides were applied to the plots. During the growing period (first week of June to first week of July), day and night field average temperatures were 83.83 and 72.27°F; and the humidity was 69.93 and 87.56%, respectively.

2.2 Sample Preparation

Fresh leaves that were about a month old of each species (Figure 1A) from replicated plots were collected for nutritional analysis. The sampled leaves were thoroughly washed with distilled water and then left to dry at room temperature. The samples were later dried in an air-circulating oven (Imperial V Laboratory Oven, Barnstead Lab-line, Illinois, USA) at 65°C for 48 hours. Each sample was ground into powder using a grinder (Cyclotec Mill Foss 1093, FOSS A/S, Minnesota, USA). Ground powder was placed in plastic bags, coded for easy identification and stored in a cool dry place until analyses.

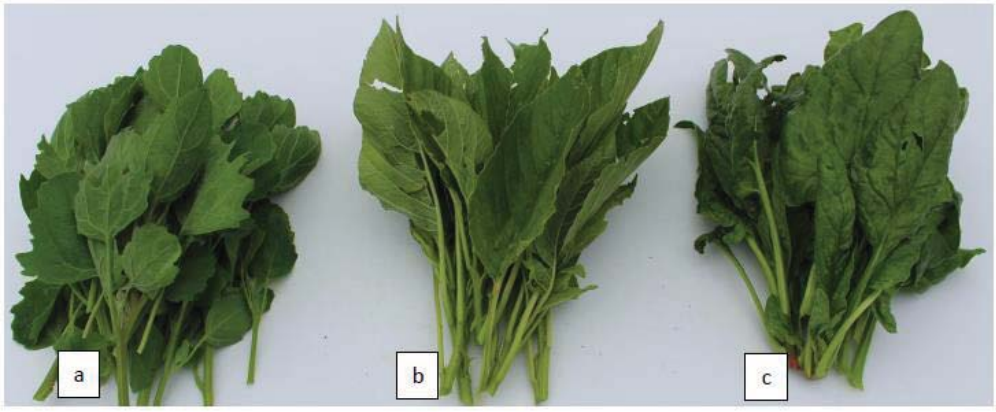


Figure 1A. Month-old fresh leaves of (a) quinoa, (b) amaranth, and (c) spinach harvested for drying

2.3 Proximate Analysis

Protein, fat, crude fiber, moisture, and ash in dried leafy vegetables were determined following the procedures described by the Association of Official Analytical Chemists (AOAC, 2006). The proximate analysis was carried out at the Experiment Station Chemical Laboratories (ESCL) of the University of Missouri, Columbia, Missouri, USA (https://aescl.missouri.edu/ContactUs.html). The sample moisture level was determined using AOAC Official Method 934.01. The ash content was determined following the AOAC procedure by the combustion of

samples at 600°C for 8 h. Total nitrogen content in the dried leaf samples was determined by the Kjeldahl method following AOAC method 984.13 (A-D). Crude fat and fiber were calculated following AOAC method 920.39 (A) and 978.10, respectively.

The carbohydrate content was estimated using the equation below:

$$Carbohydrate\ (\%) = 100\% - \%\ (crude\ protein + ash + crude\ fat + moisture) \quad (1)$$

The food value of each sample was determined by multiplying the protein, fat, and carbohydrate contents by the factors 4, 9, and 4, respectively, and adding all the values to get kcal per 100 g (Indrayan, Sharma, Durgapal, N. Kumar, & K. Kumar, 2005).

2.4 Amino Acid Analysis

The complete amino acid composition was analyzed using AOAC (2006) Official Method 982.30 E (a, b, c). Amino acid composition analysis was also carried out at the Experiment Station Chemical Laboratories (ESCL) of the University of Missouri, Columbia, Missouri, USA (https://aescl.missouri.edu/ContactUs.html). In brief, the protein samples were acid-digested into their constituent amino acids. The amino acids were then analyzed using a fully automated liquid chromatographic system. For cysteine and methionine determination, the acid hydrolysis was preceded overnight with cold acid oxidation and tryptophan determination involved alkaline hydrolysis.

2.5 Mineral Analysis

Powdered leaves of quinoa, amaranth, and spinach were digested using an SRC-UltraWAVE^{TM} microwave digestion system (Milestone Inc., Connecticut, USA). Standard reference material (SRM 1547 - peach leaves) was digested along with our samples. All samples, including SRM 1547, were digested in triplicate. For the digestion, an aliquot of each sample (< 0.5 g) was placed in acid-cleaned quartz tube followed by the addition of concentrated nitric acid (5 mL) and hydrogen peroxide (2 mL).

The determination of elements in all samples was performed using the Agilent 5110 inductively coupled plasma - optical emission spectrometer (ICP-OES) instrument (Agilent Technologies Inc., California, USA) equipped with a VistaChip II detector and configured with an SPS 4 autosampler (Kapadnis, Jain, & Vyas, 2016). The elemental concentrations from the ICP-OES were calculated with ICP Expert software (Version 7.4.1. 10449; Agilent).

The recovery (%) of elements in the reference materials was calculated using the following relationship:

$$Analyte\ recovery\ rate\ (\%) = Found\ value/Certified\ value\ x\ 100 \quad (2)$$

2.6 Data Processing

Triplicate measurements were conducted for all samples. The elemental concentrations in quinoa, amaranth, spinach, and the certified reference material were derived from external calibrations. The values were calculated using Agilent ICP Expert software (Agilent Inc., California, USA). The average ± standard deviation values were calculated with Microsoft® Excel 2010 (Microsoft Corporation, USA). All other (protein and amino acid) statistical analyses were performed using SAS® version 9.3 packages (SAS, 2011). Mean differences were determined using Fisher's least significant difference (LSD); a p-value of <0.05 was considered to be significant.

3. Results and Discussion

3.1 Proximate Analysis

The proximate analyses of the three studied GLVs are shown in Figure 1B. Crude protein (CP) content in the dry GLVs ranged between 27.45 and 37.05% on a dry weight (DW) basis. Quinoa leaves have a significantly higher amount of CP (37.05%) compared with 27.45% and 30.00% in amaranth and spinach leaves, respectively. The CP content was similar to those found in other leafy vegetables, such as *Chenopodium quinoa* (28.00 to 32.18%) (Abd El-Samad et al., 2018), *Moringa oleifera* (29.40%) (Gopalakrishnan, Doriya, & Kumar, 2016), and defatted leaves of *Amaranthus hybridus* (32.95%) (Iheanacho & Ubebani, 2009). However, a higher or lower amount of CP was reported in other leafy vegetables, such as Indian spinach (58.80%), *Amaranthus hybridus* (66.60%) (Asaolu, Adehemi, Oyakilome, Ajibulu, & M. Asaolu, 2012), and Amaranthus species (13.25 to 19.80% (Pradhan, Manivannan, & Tamang, 2015; Patricia, Zoue, Megnanou, Dou, Niamke, 2014). Any plant foods that contribute more than 12.00% calorific value from protein are considered a good source of protein (Aberoumand, 2009). Our result suggests that green leafy quinoa is an excellent protein source on par with the legume proteins (e.g. chickpea, lentils). Availability of high protein content play key role in alleviating nutritional deficiency of rural populations. There was a significant difference in crude fat among the studied GLVs and the fat content was

2.02%, 4.50%, and 5.39% in amaranth, quinoa, and spinach dry leaves, respectively. The lowest amount of fat was found in amaranth leaves and the highest amount in spinach. However, the values were within the range reported in other related vegetables including quinoa (Abd El-Samad et al., 2018; Gopalakrishnan et al., 2016; Onuminya, Shodiya, & Olubiyi, 2017; Pradhan & Tamang, 2015). Generally, leafy vegetables are considered poor sources of fat. Total carbohydrate content was lowest in quinoa, 34.03% and highest in amaranth, 47.90%. The carbohydrate amount in quinoa leaves was similar to Moringa leaves (37.55% to 41.20%) (Gopalakrishnan et al., 2016; Moyo, Masika, Hugo, & Muchenje, 2011) but lower than the amount of carbohydrate reported for amaranthus species (58.21 to 64.60%) (Pradhan & Tamang, 2015). In this study, a negative correlation was observed between protein and carbohydrate, where higher protein quinoa had a lower amount of carbohydrates. It is reported that higher plant based protein and lower carbohydrate intake helps in weight control in humans (Samaha et al., 2003). In addition, a moderate level of dietary fiber in quinoa, which is close to the spinach and moringa, contributes to the overall nutritional value. Availability of good sources of dietary fiber helps in many aspects of human health (Wang, Xu, Wang, & Galili, 2017). The energy expressed in terms of food value (kcal/100 g) for quinoa leaves was similar to amaranth and moringa leaves but lower than spinach.

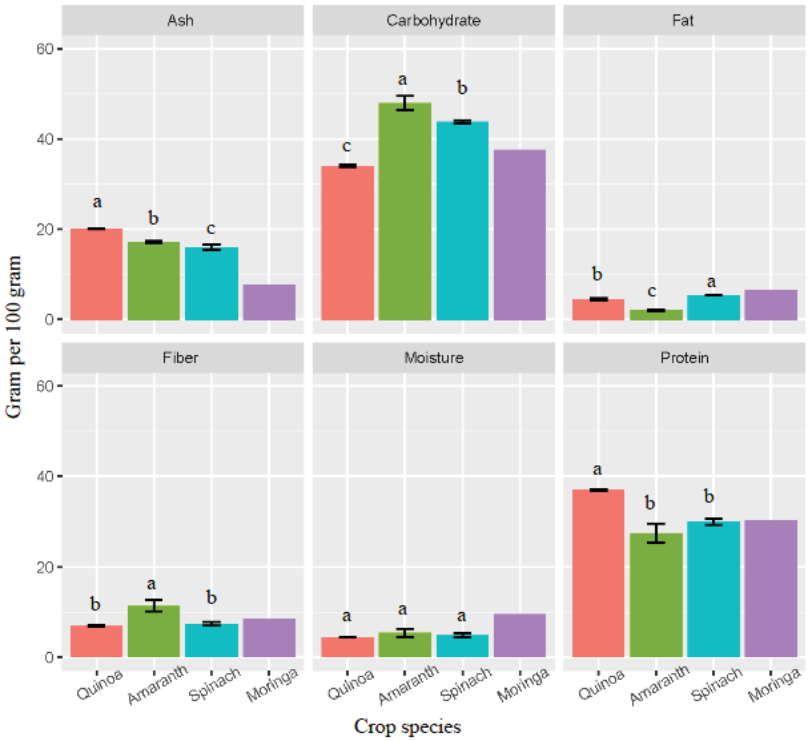


Figure 1B. Proximate analysis (g/100 g DW) of dry leaves of quinoa, amaranth, and spinach compared with moringa dry leaves (Moyoet al., 2011)

[a, b, c] = Different superscript letters for the same trait denote significant difference ($P < 0.05$)

3.2 Amino Acid Composition

The amino acid (AA) composition (g/100g) of dry leaves of quinoa, amaranth, and spinach and their nutritional evaluation are presented in Table 1. Out of the 18 AAs, the nine essential acids (EAAs) that humans cannot synthesize were found in high amounts in all samples. Nutritional quality of the protein is determined by the concentration of essential amino acids. The results showed that the total amino acid (TAA) content (g/100 g DW) was 29.04, 25.02, and 24.45 g in the leaves of quinoa, amaranth, and spinach, respectively. Interestingly, the highest amount of EAAs (13.76 g) and nonessential amino acids (NEAAs) (15.28 g) was found in quinoa leaves, among the studied leaves. The ratio between the EAAs and NEAAs was approximately 90% for all studied leaves. The most abundant EAAs were leucine (2.25 to 2.65 g), lysine (1.59 to 1.89 g), and valine (1.60 to 1.84 g). The most abundant NEAAs were glutamic acid (2.89 to 3.39 g) and aspartic acid (2.50 to 2.83 g), while cysteine was the least abundant in all the three vegetables analyzed (0.45 to 0.49 g). Methionine is generally deficient in green leaves but found in higher levels in quinoa than in amaranth and spinach. In the current work,

the values for methionine and cysteine were the least among all AAs. Our findings are in agreement with the results of previous studies on leafy vegetables (Omoyeni, Olaofe, & Akinyeye, 2015), moringa leaves (Moyo et al., 2011; Stadtlander & Becker, 2017), and quinoa grain (Barakat, Khalifa, Ghazal, Shams, & Denev, 2017). It is reported that the green leafy vegetables are deficient in the sulphur-amino acids (Gupta, Barat, Wagle & Chawla, 1989). Comparatively higher concentration of methionine in quinoa leaves helps to improve the protein quality and nutritive value. Methionine and cysteine are powerful antioxidants that help in the detoxification of harmful compounds and protection from radiation (Brisibe et al., 2009). Histidine, an EAA needed for the growth and development of infants, was lower in quinoa leaves than in hen's eggs but was similar to moringa leaves. The amount of histidine was higher in quinoa than in amaranth and spinach. Histidine was the most limited amino acid in all the studied vegetables and in hen's eggs (FAO, 1970). In addition, methionine was limited in all three vegetables studied.

Current results indicate that the amount of TAAs in quinoa leaves (29.04 g) was lower than the hen's egg standard (39.50 g) but was higher than in amaranth, spinach, and the published value of moringa leaves (24.45 to 25.95 g) (Moyo et al., 2011). However, the ratios between EAAs and TAAs, and NEAAs and TAAs were similar among quinoa, amaranth, and spinach as well as for the published results of moringa leaves and hen's eggs (Table 1). The EAA index (EAAI), an indicator of protein quality, was 77% for quinoa leaves, higher than for amaranth or spinach. The index was also lower for moringa leaves, at 60% (Moyo et al., 2011). Another parameter for protein quality determination is the ratio between the individual AA and the TEAA. Scores for tested AAs as well as that of the FAO, which uses the hen's egg as a standard (FAO, 1970) are presented in Table 2. Our results indicated that the amounts of EAAs in quinoa leaves were higher than in the other two vegetables (i.e., amaranth and spinach) but was lower than the scores of EAA in relation to the hen's egg standard.

Table 1. Amino acid composition (g/100 g DW in this study) of dry leaves of quinoa, amaranth, and spinach compared with dry leaves of moringa and hen's egg standard (published literatures)

	This study			Published literatures	
Amino acid	Quinoa	Amaranth	Spinach	Moringa[1]	Hen's egg[2]
Essential AA:					
Histidine (His)	0.70a	0.57c	0.62a	0.72	0.94
Isoleucine (Ile)	1.61a	1.43b	1.26c	1.18	2.44
Leucine (Leu)	2.65a	2.33b	2.25c	1.96	3.44
Lysine (Lys)	1.89a	1.84a	1.59c	1.64	2.70
Methionine (Met)	0.60a	0.53b	0.55c	0.30	1.31
Phenylalanine (Phe)	1.79a	1.45b	1.51c	1.64	2.25
Threonine (Thr)	1.45a	1.16b	1.23b	1.36	2.01
Tryptophan Trp)	1.23a	0.87c	1.00b	0.49	na3
Valine (Val)	1.84a	1.62b	1.60b	1.41	2.69
Non-essential AA:					
Alanine (Ala)	1.89a	1.74b	1.56c	3.03	2.31
Arginine (Arg)	1.71a	1.40b	1.44b	1.78	2.38
Aspartic acid (Asp)	2.83a	2.51b	2.50b	1.43	3.75
Cysteine (Cys)	0.49a	0.47a	0.45a	0.01	0.69
Glutamic acid (Glu)	3.39a	2.89b	2.94b	2.53	3.00
Glycine (Gly)	1.69a	1.45c	1.57b	1.53	1.31
Proline (Pro)	1.51a	1.24c	1.34b	1.20	1.63
Serine (Ser)	1.23a	1.16a	1.09a	1.09	5.00
Tyrosine (Tyr)	0.53a	0.36b	na3	2.65	1.60
Nutritional evaluation:					
EAA	13.76	11.81	11.58	10.70	17.81
NEAA	15.28	13.21	12.87	15.25	21.69
TAA	29.04	25.02	24.45	25.95	39.50
EAA/TAA%	47.38	47.20	47.36	40.12	45.0
NEAA/TAA%	52.62	52.80	52.64	58.77	54.91
EAA/NEAA%	90.05	89.40	89.98	70.16	82.21
EAAI%	77.26	66.31	65.02	60.08	100.00

[a, b, c] = Different superscript letters in the same row denote significant difference ($p < 0.05$);

[1] Moyo et al., 2011; [2] FAO, 1970; [3] na = not available;

EAA = essential amino acid; NEAA = non-essential amino acid; TAA = total amino acid;

EAAI = essential amino acid index [amount of AA in the test sample per unit protein (mg/g) / amount of AA in reference sample (hen's egg) unit protein (mg/g)]

Table 2. Assessment[1] of essential amino acid of quinoa, amaranth and spinach compared to reference amino acid in hen's egg protein (%)

	This study			Published literature
Amino acid	Quinoa	Amaranth	Spinach	Hen's egg (FAO, 1970)
His[2]	39.81	32.42	35.26	53.46
Ile	91.57	81.33	71.66	138.78
Leu	150.72	132.52	127.97	195.66
Lys	107.50	104.65	90.43	156.41
Met	34.13	30.14	31.28	114.32
Phe	101.81	82.47	85.88	127.97
Thr	82.47	65.98	69.96	114.32
Val	104.65	92.14	91.00	153.00
Met + Cys	62.00	56.88	63.13	113.75
First limiting AA	His	His	His	His
Second limiting AA	Met + Cys	Met + Cys	Met + Cys	Met + Cys

[1]Ratio between individual AA in mg to TEAA in hen's egg standard (FAO, 1970);

[2] See Table 1 for abbreviations

3.3 Mineral Analysis

3.3.1 Accuracy of the Results

The instrument readiness and method accuracy check was performed by analyzing SRM 1640a (trace elements in natural water) and SRM 1547 (peach leaves), respectively. The recovery rates of the elements were generally in good agreement with the certified values provided by the National Institute of Standards and Technology (NIST, 2010, 2017) except for the elevated value of only one element, potassium (122%). The recovery values for our independent calibration verification solution and a quality control sample (1 mg/L each of Ca, Cu, Fe, Mg, Mn, Na, and Zn; and 10 mg/L of K) ranged from 100% to 102% and 99% to 107%, respectively.

3.2.2 Mineral Composition Analysis

The average values (mg/100 g DW) of the essential minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn) are presented in Table 3. The concentration of different minerals varied among the leaf samples of quinoa, amaranth, and spinach. The most abundant minerals were K (ranged from 5,993.00 to 8,769.00 mg), Ca (1,168.00 to 2,597.00 mg) and magnesium (902.00 to 1,673.00 mg), and the lowest amount was Cu (0.89 to 1.12 mg). Quinoa had the highest concentration of the elements Cu (1.12 mg), K (8,769.00 mg), and Mn (26.49 mg). Ca (2,597.00 mg) and P (477.61 mg) were most abundant in amaranth, while Fe (23.65 mg), Mg (1,673.00 mg), Na (84.47 mg), and Zn (12.29 mg) were most abundant in spinach leaves. Alternately, the lowest concentration identified for Fe (11.55 mg) and Mg (902.00 mg) was in quinoa, while the lowest for Ca (1,168.00 mg) and P (313.25 mg) was in spinach leaves. Our results differed from other published values due to dissimilar growing environments and management practices across the geographical locations as well as the analytical methods used.

Table 3. Mineral composition (mg/100 g DW) in dry leaves of quinoa, amaranth, and spinach compared with published values for moringa and spinach

	This study			Published literatures	
Minerals	Quinoa	Amaranth	Spinach	Moringa[1]	Spinach[2]
Calcium (Ca)	1535.00b	2597.00a	1168.00c	1036	1110
Copper (Cu)	1.12a	1.12a	0.89b	na3	0.59
Iron (Fe)	11.55c	16.77b	23.65a	28.40	13.20
Magnesium (Mg)	902.00c	1171.00b	1673.00a	827	373
Manganese (Mn)	26.49a	23.47a	11.70b	na3	1.67
Phosphorous (P)	405.62b	477.61a	313.25c	513	632
Potassium (K)	8769.00a	5993.00b	6045.00b	5840	296
Sodium (Na)	15.12b	12.84b	84.47a	827	6.70
Zinc (Zn)	6.79b	6.74b	12.29a	5.50	3.10

[1]Edelman and Colt, 2016; [2] Stadtlander and Becker, 2017; [3] na: not available;

[a, b, c] = Different superscript letters in the same row denote significant difference ($P < 0.05$)

In this study, spinach had the highest amounts of Fe, Mg, Na, and Zn (Table 3). Amaranth had the highest amount of Ca and P, following a similar pattern to that reported earlier (Edelman & Colt, 2016; Stadtlander & Becker, 2017) but with different values. Quinoa leaves had the highest amount of K (8,769.00 mg), followed by spinach (6,045.00 mg). The K value of spinach was comparable with that reported by Edelman and Colt (2016). Na and K are important intracellular and extracellular cations, respectively. Potassium appears to improve endothelial, and increasing dietary potassium appears to improve vascular functions in humans (Blanch, Clifton, & Keogh, 2015). Potassium also helps to prevent muscle weakness, respiratory insufficiency, and hypertension in humans (Hathcock, 2014), while Na is involved in regulating the acid-base balance as well as nerve and muscle contractions (Akpanyung, 2005).

Edelman and Colt (2016) reviewed the nutritional compositions for seeds and green leaves that showed that GLVs contain higher amounts of minerals compared with cereal seeds. For example, leafy vegetables of this study have a higher amount of Ca (1,168.00 to 2,597.00 mg), and P (313.25 to 477.61 mg) compared with published results for cereal seeds Ca (6.40 to 34.0 mg), and P (100.00 to 332.00 mg/100 g). A balanced proportion of Ca and P needed in the body, and inadequate Ca-P balance can result in osteoporosis, arthritis, rickets, and tooth decay. A balanced proportion of Ca and P were found in quinoa. Minerals have greater stability during food processing as compared to vitamins and proteins (Kala & Prakash, 2004), but the mineral contents of leafy vegetables is highly dependent on fertilizer application, uptake of minerals by individual plants, and organic soil amendments (Anjorin, Ikokh, & Okolona, 2010).

In this study, concentrations of Fe (11.55 to 23.65 mg/100 g) and Zn (6.74 to 12.29 mg/100 g) were comparable to other leafy vegetables such as kale, spinach, and duckweed (Fe 25.70 to 28.40, Zn 5.50 to 15.00 mg/100 g) (Edelman & Colt, 2016), *Amaranthus viridis* and *Chenopodium album* (Fe 5.40 to 10.80, Zn 8.40 to 9.70 mg/100 g) (Pradhan & Tamang, 2015), and moringa leaves (Fe 13.20, Zn 3.10 mg/100 g) (Stadtlander & Becker, 2017). Wheat, corn, and rice grains have much lower concentration of Fe and Zn, while leafy vegetables, such as kale, spinach, and duckweed (Edelman & Colt, 2016) and the studied vegetables have higher concentrations of Fe and Zn (Table 3). Metal ions are needed by the human body and frequently serve as cofactors in enzymatic reactions and in maintaining protein structure. One-third of human proteins bind with metal ions, and over 10% of enzymes in the human body require Zn for activity (Azia, Levy, Unger, Edelman, & Sobolev, 2015). Iron is a vital trace element in the human body, controlling infection and cell-mediated immunity (Bhashkaran, 2001). Iron deficiency is the most prevalent nutritional deficiency, and anemia caused by iron deficiency is estimated to affect more than one billion people worldwide (Trowbridge & Martorell, 2002). Zinc is another essential micronutrient for growth and immune function, with an estimated 20% of the world population reported to be at risk of insufficient Zn intake (Black, 2003; Brown et al., 2004). Consumption of nutrient-rich leafy green quinoa may prevent nutritional deficiency caused by Fe and Zn.

Daily nutrient requirements for a child (Golden, 2009) and the necessary amount of dry matter intake are presented in Table 4. Quinoa leaves were excellent sources of protein and the macro-elements Ca, Mg, and P. As little as 3.35, 5.80, 1.30, and 16.60 g of leaf powder satisfies 15% of a child's daily protein, Ca, Mg, and P requirements (Tables 3 and 4), respectively. Both Ca and P are essential for a child's bone development for sustainable growth.

Table 4. Daily nutrient requirements[1] for a child and the necessary amount of dry matter intake (g) from quinoa, amaranth, and spinach leaves

				This study		
Nutrient	Unit	Requirement[1]		Quinoa	Amaranth	Spinach
		100%	15%			
Protein	g	22.3	3.35	9.03	12.19	11.15
Calcium	mg	595	89.25	5.81	3.44	11.15
Phosphorous	mg	450	67.50	16.64	14.13	21.55
Magnesium	mg	79	11.85	1.31	1.01	0.71
Potassium	mg	1099	164.85	1.88	2.75	2.73
Sodium	mg	978	146.70	970.23	1142.52	173.67
Iron	mg	17.8	2.67	23.12	15.92	11.29
Zinc	mg	12.5	1.87 2	7.61	27.82	15.26
Manganese	mg	1.2	0.18	0.68	0.77	1.54
Copper	µg	892	133.80	11.95	11.95	15.03
Histidine	mg	430	64.50	9.21	11.32	10.40
Isoleucine	mg	575	86.25	5.36	6.03	6.85
Leucine	mg	1245	186.75	7.05	8.01	8.30
Lysine	mg	116	178.50	9.44	9.70	11.23
Threonine	mg	655	98.25	6.78	8.47	7.99
Tryptophan	mg	175	26.25	2.13	3.02	2.63
Valine	mg	776	116.40	6.33	7.19	7.27

[1]Requirement values from Golden (2009), with data from FAO (1981) where available; otherwise, data from the Institute of Medicine (IMO, 2001)

4. Conclusions

Worldwide, quinoa grain is accepted and consumed as a nutrient-rich pseudocereal. However, the consumption of quinoa leaves is not well reported in the literature, and the nutrient composition is unknown. The current work investigated the nutritional (i.e., protein, amino acid, and mineral) content of the green leaves of quinoa and compared the dataset with those of amaranth and spinach leaves. The results showed that leafy green quinoa is nutritionally rich in protein, amino acids, and mineral content as well as food value. This indicates quinoa's potential as a noble nonconventional vegetable for human nutrition. Among the three studied vegetables, quinoa leaves contained a higher amount of crude protein, a lower amount of carbohydrate, all essential amino acids, and all key minerals. From our study, the nutrient composition of quinoa and amaranth leaves showed more resemblance in nutrient composition than spinach leaves. Due to its rich nutritional qualities, vegetable growers may add quinoa to their crop list as a new specialty GLV, thereby diversifying their farming pattern. Inclusion of vegetable quinoa in the crop list may lead to increased farm income, especially for small vegetable growers, and may help to overcome nutritional deficiencies in end users. Consequently, leafy vegetable quinoa can be considered an alternative to combat malnutrition and may be consumed directly by humans or indirectly by utilizing it as a feed for small ruminants, such as goats and sheep. The high nutritional content found in dried quinoa leaves indicates the usefulness of this vegetable as a potential animal feed that can facilitate both conservation and consumption when food is scarce or during the off-season. To the best of our knowledge, this is the first study of the nutritional components of green leafy quinoa in the US. Green leafy quinoa shows its outstanding potential as a nutrient-rich vegetable. Therefore, we recommend that it should be evaluated further for its nutritional benefits and other characteristics, such as potential anticancer and antidiabetic activity.

Conflicts of Interest Statement

The authors declare no conflicts of interest related to this article. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

The authors would like to thank the United States Department of Agriculture (USDA) - National Institute of Food and Agriculture (NIFA) (Evans-Allen grant to senior author, grant #1006493) and Organic Valley - Farmers Advocating for Organic (FAFO) for partial financial support and USDA - Germplasm Resources Information Network (GRIN) for providing quinoa seeds. We thank Cooperative Research Programs (Lincoln University of

Missouri) for the equipment and other resources used during the study. The authors greatly acknowledge Dr. Abua Ikem's technical support and advice. The contributions of Jimmie Garth, Marianne Dolan-Timpe, Allute Hamisi and Dr. Nancy Browning of Lincoln University and Dr. Hari Krishnan of USDA-ARS, Columbia, Missouri are greatly appreciated.

References

Abd El-Samad, E. H., Hussin, S. A., El-Naggar, A. M., El-Bordeny, N. E., & Eisa, S. S. (2018). The potential use of quinoa as a new non-traditional leafy vegetable crop. *Bioscience Research, 15*(4), 3387-3403.

Aberoumand, A. (2009). Proximate and mineral composition of the marchubeh (*Asparagus officinalis* L.) in Iran. *World Journal Dairy Food Science, 4*(2), 145-149.

Adeyinka, M. O., Abolaji, G. F., & Olukemi, R. A. (2014). Determination of trace element in raw leafy vegetables grown in selected local government of Osun state. *American Journal of Chemistry, 4*(1), 38-41. https://doi.org/10.5923/j.chemistry.20140401.06

Akpana, A. M., Edward, M. J., Henry, J., & Joseph, A. (2017). Mineral and vitamin composition of some lesser known leafy vegetables consumed in northern senatorial district of Cross River state, Nigeria. *American Journal of Food and Nutrition, 5*(2), 51-57. https://doi.org/10.12691/ajfn-5-2-1

Akpanyung, E. O. (2005). Proximate and mineral composition of bouillon cubes produced in Nigeria. *Pakistan Journal of Nutritio, 4*(5), 327-329. http://dx.doi.org/10.3923/pjn.2005.327.329

Anjorin, T. S., Ikokh, P., & Okolona, S. (2010). Mineral composition of moringa leaves, pods and seed from two region of Abuja, Nigeria. *International Journal of Agriculture and Biology, 12*(3), 431-434. Retrieved from http://www.parc.gov.pk/NARC/narc.html

AOAC (2006). *Official Methods of Analysis (18th ed.). Association of Official Analytical Chemist International,* Gaithersburg, MD, USA.

Arowosegbe, S., Olanipekun, M. K., & Adeloye, I. A. (2018). Ethnobotanical survey of indigenous leafy vegetables consumed in Ekiti state, Nigeria. *European Journal of Biology and Medical Science Research, 6*(1), 7-14. http://dx.doi.org/10.3923/sjsres.2018.8.13

Asaolu, S. S., Adefemi, O. S., Oyakilome, I. G., Ajibulu, K. E., & Asaolu, M. F. (2012). Proximate and mineral composition of Nigerian leafy vegetables. *Journal of Food Research, 1*(3), 214-218. https://doi.org/10.5539/jfr.v1n3p214

Azia, A., Levy, R., Unger, R., Edelman, M., & Sobolev, V. (2015). Genome-wide compuational determination of the human metalloproteome. *Proteins. 83*, 931- 939. https://doi.org/10.1002/prot.24790

Barakat, H., Khalifa, I., Ghazal, G. A., Shams, A., & Denev, P. N. (2017). Chemical composition and nutritional value of seeds from new quinoa accessions, cultivated in Egypt. *Bulgarian Chemical Communications, 49*(D), 231-238.

Bhashkaran, P. (2001). Immunobiology of mild nutrient deficiency. *British Journal of Nutrition, 85*, S75-S80. http://dx.doi.org/10.1079/BJN2000297

Black, R. E. (2003). Zinc deficiency, infectious disease and mortality in developing world. *J. Nutr. 133*, S1485-S1489. https://doi.org/10.1093/jn/133.5.1485S

Blanch, N., Clifton, P. M., & Keogh, J. B. (2015). A systematic review of vascular and endothelial function: effects of fruit, vegetable and potassium intake. *Nutrition Metabolism Cardiovascular Disease, 25*(3), 253-66. https://doi.org/10.1016/j.numecd.2014.10.001

Brisibe, E. A., Umoren, U. E., Brisibe, F., Magalhaes, P. M., Ferreira, J. F. S., Luthria, D., Wu, X., & Prior, R. L. (2009). Nutritional characterization and antioxidant capacity of different tissues of *Artmisia Annua* L. *Food Chemistry, 115,* 1240-1246. https://doi.org/10.1016/j.foodchem.2009.01.033

Brown, K. H., Rivera, J. A., Bhutta, Z., Gibson, R. S., King, J. C., Lönnerdal, B., Ruel, M. T., Sandtröm, B., Wasantwisut, E., & Hotz, C. (2004). International Zinc Consultative Group (IZINCG) Technical document no. 1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutrition Bulletin, 25*(1), S94-S203. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18046856

Dias, J. S. (2012). Nutritional quality and health benefits of vegetables: A review. *Food and Nutrition Sciences, 3*, 1354-1374. http://dx.doi.org/10.4236/fns.2012.310179

Edelman, M., & Colt, M. (2016). Nutrient value of leaf vs. seed. *Frontiers in Chemistry, 4*, 1-32.

https://doi.org/10.3389/fchem.2016.00032

FAO (1970). *Amino acid content of food and biological data on proteins.* FAO nutritional studies no. 24, Rome, Italy. Retrieved from: http://www.fao.org/3/ac854t/AC854T00.htm

FAO (1981). The joint FAO/WHO/UNU Expert Consultation on Energy and Protein Requirement: Infants, Children and Adolescents. Rome, Italy. Retrieved from: http://www.fao.org/3/AA040E/AA040E07.htm

Golden, M. H. (2009). Proposed recommended nutrient densities for moderately malnourished children. *Food Nutrition Bulletin, 30,* 267-342. https://doi.org/10.1177/15648265090303S302

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). *Moringa oleifera*: a review on nutritive importance and its medicinal application. *Food Science Human Wellness, 5*, 49-56. https://doi.org/10.1016/j.fshw.2016.04.001

Gupta, K., Barat, G. K., Wagle, D. S., & Chawla, H. K. L. (1989). Nutrient contents and antinutritional factors in conventional and non-conventional leafy vegetables. *Food Chemistry, 31*, 105-116. https://doi.org/10.1016/0308-8146(89)90021-6

Hathcock, J. N. (2014). *Vitamin and mineral safety* (3rd ed.). Council of Responsible Nutrition (CRN), Washington, DC, USA. pp.119-123.

HHS and USDA (U.S. Department of Health and Human Services and U.S. Department of Agriculture). (2015). *2015 – 2020 Dietary Guidelines for Americans* (8th eds). Retrieved from http://health.gov/dietaryguidelines/2015/

Iheanacho, K., & Ubebani, A. C. (2009). Nutritional composition of some leafy vegetables consumed in Imo-State, Nigeria. *Journal of Applied Science and Environmental Management, 13*(3), 35-38. https://doi.org/10.4314/jasem.v13i3.55349

Indrayan, A. K., Sharma, S., Durgapal, D., Kumar, N., & Kumar, M. (2005). Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Current Science, 89*, 1252-1255.

IOM (Institute of Medicine). (2001). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. The National Academies Press, Washington, DC, USA. Retrieved from https://www.nap.edu/read/10026/chapter/33

Kala, A., & Prakash, J. (2004). Nutrient composition and sensory profile of differently cooked green leafy vegetables. *International Journal of Food Properties, 7*(3), 659-669. https://doi.org/10.1081/JFP-200033079

Kapadnis, G., Jain, V., & Vyas, S. (2016). *Determination of elements in drinking* water *as per Bureau of Indian standards 10500, 14543 & 13428 using the Agilent 5100 ICP-OES.* Agilent Technologies India, 5991-6544EN (v2). Retrieved from https://www.agilent.com/cs/library/applications/5991-6631EN.pdf

Moyo, B., Masika, P. J., Hugo, A., & Muchenje, V. (2011). Nutritional characterization of moringa (*Moringa Oleifera* Lam.) leaves. *African Journal of Biotechnology, 10*, 12925-12933. http://dx.doi.org/10.5897/AJB10.1599

NIST (National Institute of Standards and Technology). (2010). *Certificate of analysis: Standard reference material (SRM) 1640a - trace elements in natural water*. NIST, Gaithersburg, Maryland, USA.

NIST (National Institute of Standards and Technology). (2017). *Certificate of analysis: Standard reference material (SRM) 1547 - peach leaves.* NIST, Gaithersburg, Maryland, USA.

Omoyeni, O. A., Olaofe, O., & Akinyeye, R. O. (2015). Amino acid composition of ten commonly eaten indigenous leafy vegetables of South-west Nigeria. *Journal of Nutrition and Health, 3*(1), 16-21. http://dx.doi.org/10.12691/jnh-3-1-3

Onuminya, T. O., Shodiya, O. E., & Olubiyi, O. O. (2017). Comparative proximate and phytochemical analyses of leafy vegetables of Lagos state. *Nigerian Journal of Pure and Applied Science, 30*(3), 3097-3103. http://dx.doi.org/10.19240/njpas.2017.C06

Patricia, O., Zoue, L., Megnanou, R-M., Dou, R., & Niamke, S. (2014). Proximate composition and nutritive value of leafy vegetables consumed in northern Cote D'ivoire. *European Scientific Journal, 10*(6), 212-227. http://dx.doi.org/10.19044/esj.2014.v10n6p%25p

Pradhan, S., Manivannan, S., & Tamang, J. P. (2015). Proximate, mineral composition and antioxidant properties of some wild leafy vegetables. *Journal of Scientific and Industrial Research, 74,* 155-159. http://hdl.handle.net/123456789/30726

Pradhan, S., & Tamang, J. P. (2015). Ethnobiology of wild leafy vegetables of Sikkim. *Indian Journal of Traditional Knowledge, 14*(2), 290-297. http://hdl.handle.net/123456789/32086

Samaha, F., Iqbal, N., Seshadri, P., Chicano, K., Daily, D., McGrory, J., … Stern, L. (2003). A low-carbohydrate as compared with a low-fat diet in severe obesity. *New England Journal of Medicine*, *348*, 2074-81. https://doi.org/10.1056/NEJMoa022637

SAS (2011). *SAS Software Version 9.3*. SAS Institute, Cary, NC, USA.

Stadtlander, T., & Becker, K. (2017). Proximate composition, amino and fatty acid profiles and element compositions of four different *Moringa* species. *Journal of Agricultural Science, 9*, 7. https://doi.org/10.5539/jas.v9n7p46

Trowbridge, F., & Martorell, M. (2002). Forging effective strategies to combat iron deficiency: Summary and recommendation. *Journal of Nutrition, 85*, 875-880. https://doi.org/10.1093/jn/132.4.875S

UNICEF (2018). Malnutrition rates remain alarming: stunting is declining too slowly while wasting still impacts the lives of far too many young children. Retrieved from http://data.unicef.org/topic/nutrition/malnutrition/#

Wang, G., Xu, M., Wang, W., & Galili, G. (2017) Fortifying Horticultural Crops with Essential Amino Acids: A Review. *International Journal of Molecular Sciences*, *18*(6), 1306. https://doi.org/10.3390/ijms18061306

Yadav, R. K., Tomar, B. S., Pachauri, N., & Jain, V. (2018). Studies of nutritional properties and antioxidant potential in green leafy vegetables. *Journal of the Science of Food and Agriculture, 2*(1), 7-13. https://doi.org/10.26855/jsfa.2018.01.001

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Extraction of Proteins and Pasting and Antioxidant Properties of Soybean Hulls

Sean X. Liu[1], Diejun Chen[1], Mukti Singh[1] & Jingyuan Xu[2]

[1]Functional Food Research Unit, National Center for Agricultural Utilization Research, USDA-ARS. 1815 N University Street, Peoria, IL 61604, USA

[2]Plant Polymer Research Unit, National Center for Agricultural Utilization Research, USDA-ARS. 1815 N University Street, Peoria, IL 61604, USA

Correspondence: Sean X. Liu, Functional Food Research Unit, National Center for Agricultural Utilization Research, USDA-ARS. 1815 N University Street, Peoria, IL 61604, USA. Tel: 1-309-681-6551. E-mail: Sean.liu@ars.usda.gov

Received: August 30, 2019 Accepted: October 5, 2019 Online Published: October 17, 2019

doi:10.5539/jfr.v8n6p66 URL: https://doi.org/10.5539/jfr.v8n6p66

Abstract

Soybean hulls are a by-product from soybean processing for oil and meal production which comprise approximately 8% of the whole seed. This study investigated water holding capacities and pasting properties, and first reported the phenolic contents and antioxidant activities from soybean hulls which are important to our long-term health. In addition, the conditions for extracting proteins from soybean hulls including optimum pH, as well as homogenizing and separation methods for extraction, were also studied. Higher protein content in extracts and recoveries was obtained with extraction at pH 9. Using sieve separation may be an effective way to extract proteins from hulls for industrial applications. The precipitated protein content increased from 51.52% to 59.29% after purification by washing with water once; however, after two washes, no further improvement was shown. The extracted proteins can be used for food applications. The ground hull powders (10% protein), dried supernatant (14% protein) and sediments (7-8% proteins) along with valuable fibers should be good food ingredients for several food categories. This research explored the great potential of converting the low value by-products into value-added functional food uses ~~along~~ with the benefit of reducing food and agricultural wastes.

Keywords: antioxidation, extracting, pasting, pH, proteins, soybean hull, water holding capacity, food product

1. Introduction

Soybean hull, a by-product of soybean processing for oil and meal production, comprises approximately 8% of the whole seed (Gnanasambandam & Proctor, 1999). It contains 12% crude protein, 2% fat, and 61% of neutral detergent fiber (Extension, 2019), and is currently used as supplemental feed for dairy cattle since they are low in lignin, their cellulose is highly digestible (85%) in the rumen, and fermentation rates are rapid (Stein et al., 2008). Soy hulls may also be used as energy supplements to high forage diets of lambs and steers (Anderson, et al., 1988).

The estimated yield of soybean hulls from a 60-pound bushel of soybeans is about three pounds, or approximately 5% of the original raw soybean weight. Based on this yield, the harvest projections for a one-year soybean crop in the state of Kansas could result in the production of almost 115,000 tons of soybean hulls (Kansas State University Agricultural Experiment Station and Cooperative Extension Service, 2019), indicating the great potential for this research. There is a great amount of information on separating protein from soybean seeds. Soy protein isolate is traditionally prepared from defatted soy meal using aqueous or mild alkali extraction (pH7-10) of proteins and soluble carbohydrates (Liu K., 1999). There is research on extracting protein using soybean hulls, such as a previous study in which the air classification of milled hulls was used to enrich these value-added proteins at various pH from 2.5 to 6.4 (Sessa, 2004). However, there was inadequate information on extracting protein from soybean hull. Furthermore, little was known on the commercial usage of soybean hulls for food beyond the use in animal feed.

As a low-value processing by-product containing valuable components, soybean hulls have the potential to be

turned into high value food and consumer products through innovative technologies. However, information on utilization of soybean hulls for food and non-food products is limited. This study investigated physicochemical properties of finely-milled soybean hulls that are important for developing food products and explored extraction methods for producing protein rich fractions for functional food ingredients based on particle size, pH value, and extraction solvent. Oxidative stress has been identified as the root cause for the development and progression of several diseases (Kasote et al., 2015). There is study reported on antioxidant activity of soybean but not on soybean hulls. Therefore, the phenolic contents and antioxidant activity in soybean hulls were examined in this study. The goal of this study is to reduce food and agricultural waste in soybean processing and enhance overall value of soybean crop by generating value-added products from the low value feedstock.

2. Materials and Methods

2.1 Materials

Organic soybean hulls (OSH) were obtained from Keystone Mills (Ephrata, PA, USA). Inorganic pelletized soybean hulls (ISH) (GMO) were obtained from Archer-Daniels-Midland (Quincy, IL, USA). Soybean flour was bought from Bob's Red Mill (Natural Foods, Inc., Milwaukie, OR, USA), and oat bran powder was purchased from Grain Millers (Eden Prairie, MN, USA). Sodium hydroxide was obtained from A division of EM industries, Inc., NJ, USA. Sodium hydroxide was purchased from Fisher Chemicals (Fair Lawn, NJ, USA).

2.2 Protein Extraction Procedures

Processing procedures were adopted from a previous method with modifications (Liu, 1999). The procedure is shown in Figure 1. We examined the effects of particle size of ground hulls, defatting, pH, type of alkali, and homogenization/separation methods on protein extraction.

2.2.1 Grinding Soy Hull

Soy hull were ground IKA mill (M20, IKA® Works, Inc., NC, USA), and passed through a sifter with a 50-mesh screen.

2.2.2 Defatting

The defatted soyhulls were accomplished using a Soxhlet extractor with hexane for 12 h.

2.2.3 Homogenization Methods

Ground soybean hull powder was mixed with water, or soybean hulls were homogenized using a commercial heavy-duty Waring blender (Model 37BL84, Dynamics Corporation of America, New Hartford, CT, USA) for 3 min, or a Polytron (PT6000, Kinematica AG, Littau, Switzerland) at about 3,000 rpm before increasing the rpm to about 5000 with water for 3 min.

2.2.4 pH Adjustment

Processing procedures were adopted from a previous method with modifications (Liu, 1999). The pH of the mixture was adjusted to pH 7.5, 9, 10, or 11 using Na(OH) solution, respectively, and stirred by a magnetic bar for 1 h.

2.2.5 Separation Methods

The extract was centrifuged for 10 minutes at 3000 rpm or separated by a sieve with 100 mesh screens. The sediments from centrifugation or sieve top were dried at 60 °C in an oven for further testing.

2.2.6 Protein Precipitation

The pH of the suspension was adjusted to 4.5 using HCl solution and centrifuged for 10 min at 3000 rpm. The precipitates and sediments were dried directly or washed 1-2 times at 40 °C in an oven.

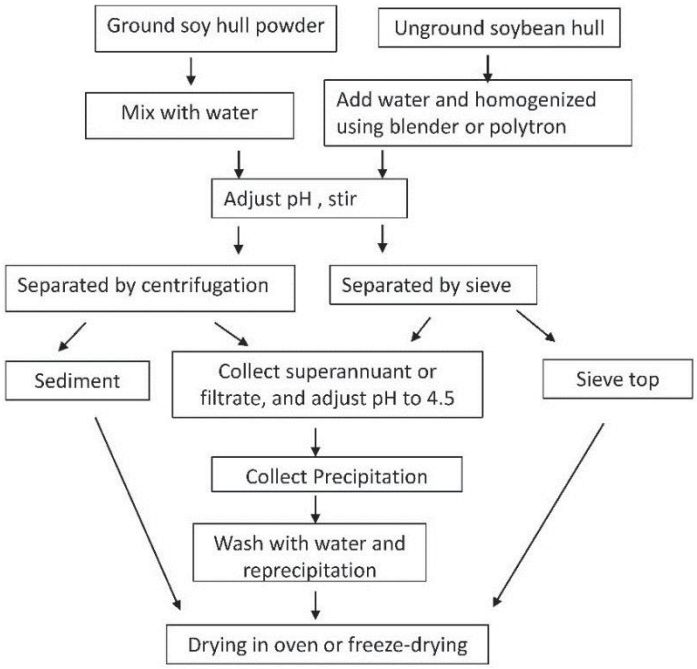


Figure 1. Flow chart of procedures for extracting protein from soybean hulls

2.3 Composition Determination

Total solid and moisture contents were determined by drying 5 g of sample at 105°C to a constant weight. Protein was determined by the combustion method using Leco CHN (St. Joseph, MI, USA) with a protein correction factor of %N × 6.25 (American Association of Cereal Chemists, 1995). Ash content was determined gravimetrically after combustion at 525°C for 12 h.

2.4 Total Phenolic Content

Phenolic content was determined by the Folin-Ciocalteau colourimetric method with minor modifications (Waterhouse, 2001; Yu and Zhou, 2004). To 100 μL of extract, 7.9 mL of deionized water and 0.5 mL of Folin-Ciocalteau reagent (F9252, Sigma Aldrich, St Louis, MO, USA) were added, mixed using a vortex mixer, and 1.5 mL of 1.85 *M* Na_2CO_3 was added after 15 min. Absorbance of samples was measured at 765 nm after 2 h. Gallic acid was used as a standard and results were expressed as mg of gallic acid equivalents per g (d.m.).

2.5 Antioxidant Activity

Antioxidant activity determination was conducted using a method reported by Yu et al. (2004) and Şensoy et al. (2006), which entailed reacting 0.5 mL of extract with 0.5 mL of 200μM 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) in a cuvette for 40 min in the dark. The cuvettes were converted after adding reagent and prior to reading the absorbance at 515 nm. Results were expressed as 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) equivalents per g (d.m.).

2.6 Water Holding Capacity

Water holding capacity of flours was determined according to the procedure of Ade-Omowaye et al. (2003) with some modifications. Briefly, flour samples (2 g) were mixed with 25 g of deionized water and vigorously mixed using a vortex to make a suspension, then held for 2 h, followed by centrifugation at 1,590 *g* for 15 min. Water was removed by pipette and measured. Each treatment was replicated twice. Water-holding capacity was calculated by the following equation:

Water holding capacity (%) = [Water added (g) -decanted water (g)] /dry sample weight g*100

2.7 Pasting Property Measurement

The pasting properties of samples were measured using a Rapid Visco Analyzer (RVA-4, Perten Scientific, Springfield, IL, USA) by a method described by Kaur and Singh (2006) with some modifications. Samples (2.8, 3.92, 4.48 g d.b.) were made up to a total weight of 28 g with distilled water in an RVA canister (10, 14, 16%

solids, w/w). The viscosity of the samples was monitored during the heating and cooling stages. Suspensions were equilibrated at 50°C for 1 min, heated to 95°C at a rate of 6.0°C/min, maintained at 95°C for 5 min, and cooled to 50°C at a rate of 6.0 °C/min, and held at 50°C for 2 min. For all test measurements, a constant paddle rotating speed (160 rpm) was used throughout the entire analysis except for 920 rpm in the first 10 s to disperse samples. Each sample was analyzed in duplicate.

2.8 Statistical Analysis

All data from triplicated samples were analysed with analysis of variance using Duncan's multiple comparison to determine significant differences ($P < 0.05$) between treatments (SAS Institute, 1999).

3. Results and Discussions

3.1 Compositions

Organic soybean hulls (OSH) contain water (6.56%), protein (9.96%), lipids (1.54%), and ash (4.87%), and the remaining content is estimated to contain fiber and carbohydrates (77.07%) (Figure 2). The protein content in OSH (9.96%) is higher than inorganic soybean hulls (ISH) (8. 50%,) in this study (Figure 3). The protein contents slightly increased after defatting (Figure 3). Research by Sessa (2004) found the finely ground fractions, with particle sizes ranging from<15 to 18 μm, were enriched with nitrogen and lipid when compared with the more coarsely ground fractions. Therefore, the compositions of soybean hulls could be affected by particle sizes.

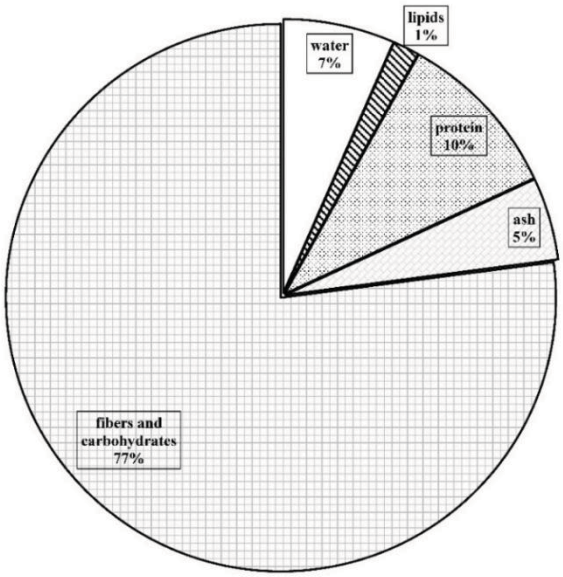


Figure 2. Compositions of organic soybean hulls

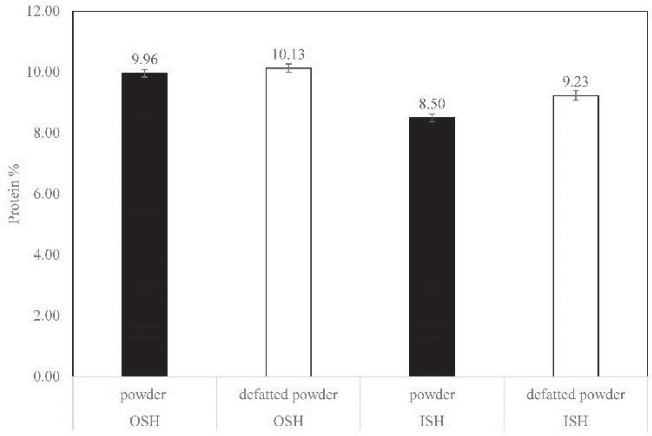


Figure 3. Protein contents of organic soybean hulls (OSH) and inorganic soybean hulls (ISH)

3.2 Antioxidant Activities

Phenolic acids are readily absorbed through the walls of human intestinal tract, and they may be beneficial to health because they work as antioxidants that prevent cellular damage due to free-radical oxidation reactions (Dyke & Rooney, 2007). They may also promote anti-inflammatory conditions in your body when people eat them regularly (Dyke & Rooney, 2007). Therefore, phenolic compounds in and antioxidant activities of soybean hulls were investigated. Oat bran was included in the test since it is also a seed coating and a well-known for its health-promotion properties. As a reference, oat bran, which is well known as a healthy food for reducing cholesterol, contain 0.93 mg/g phenolic compounds. Although the phenolic compounds (1.76 mg/g) in soybean hulls were much lower than soybean flour (3.65 mg/g), phenolic compounds in soybean hulls were significantly higher ($p < 0.05$) than oat bran (Figure 4). It may be that soy hulls contain less free phenolic compounds because phenolic compounds are tightly bound in and between fibers. On the other hand, soybean hulls show substantially higher ($p < 0.05$) antioxidant activity (3.06 μmol/g) than soybean flour (0.80 μmol/g) and oat bran (1.89 μmol/g) (Figure 5). The defatted hulls had slightly lower antioxidant activity (2.69 μmol/g) than undefatted hulls, suggesting some liposoluble antioxidants were removed by the defatting procedure. Foods rich in antioxidants provide a promising way of combating the undesirable effects of reactive oxygen species induced oxidative damage in human body (Kasote et al., 2015). Thus, soybean hulls should be useful as functional food ingredients because they not only contain proteins and valuable fibers, but also exhibit good antioxidant activity.

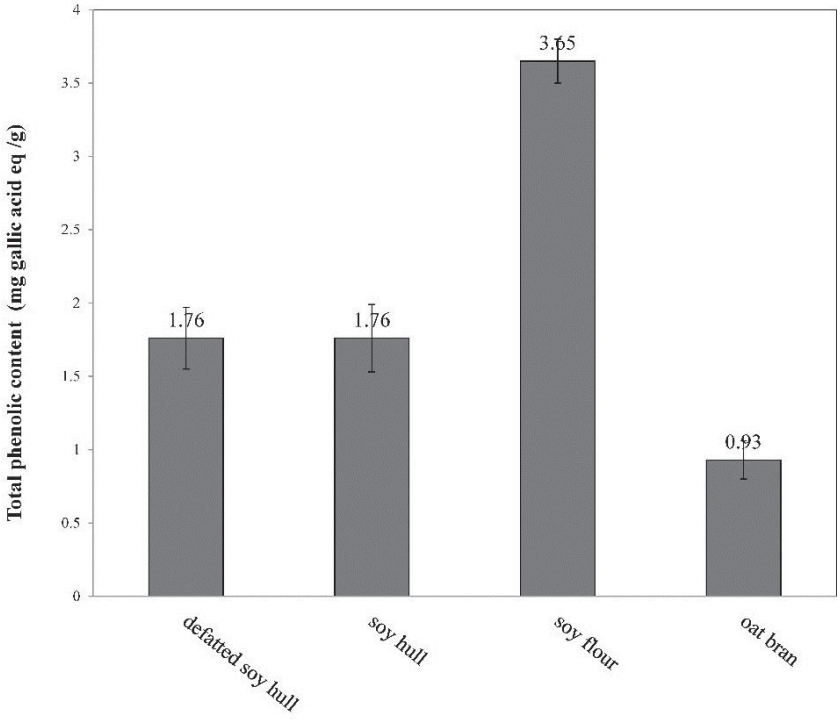


Figure 4. Phenolic contents of organic soybean hulls, soy flour and oat bran

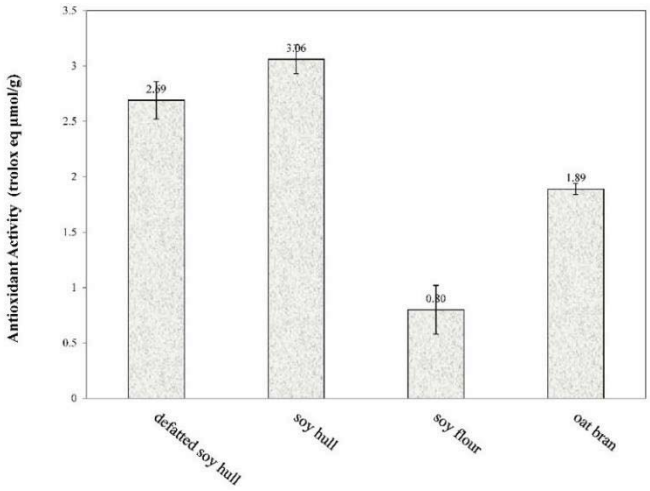


Figure 5. Antioxidant activities of organic soybean hulls, soy flour, and oat bran

3.3 Water Holding Capacity (WHC)

Water holding capacity (WHC) is an important property related to many quality traits of an emulsion, such as viscosity and stability. We found that WHC of soybean hulls was affected by soy hull particle size and acidic/alkali treatment of hulls (pH). The WHC of 20 mesh soybean hulls was somewhat higher (483.1 g/100 g) than that of 50 mesh soybean hulls (405.4 g/100 g) (Table 1). Water holding capacities became lower when the sizes of soybean hulls were smaller. This result suggested that WHC could be influenced by fiber structures and composition of the soy hull fractions. The water may reside inside fiber pores or bound to cellulose fibrils or proteins through hydrogen bonding. The high WHC might be attributed to the loose fibril arrangement, branched structures, large pore size, high hydrophilic surface area per unit mass or high hydrophilic nature (Qui et al., 2017). The large pores formed with fibers and branched structures were damaged by grinding, resulting in low water holding capacities.

Correspondingly, considerable reductions of 48%, and 39% were observed for 50, and 20 mesh hulls with acidic treatments (Table 1). This is consistent with a study reported by Marshall et al. (2000) where soy hulls were treated for absorption of metal ions. For soybean hulls, a 26% increase in adsorption capacity was detected after NaOH treatment compared with water washing. However, the capacity was significantly reduced (about 78%) after washing with HCl (Marshall et al., 2000). Acid-washed hulls caused the adsorbent surface to be protonated (Marshall et al., 2000) reducing the likelihood of hydrogen bond-like binding between electronegative atom and H atoms. Hydrogen bonds are not just formed between water molecules; they can form, to varying degrees, between H atoms and electronegative atoms such as O, N, F, and Cl (Israelachvili, 2011). A clear trend was observed for the water solubility. Water solubility was very low for soybean hulls. The soluble solids in 50 mesh and 20 mesh soybean hulls were unmeasurable under test conditions in this study, indicating high content of insoluble fibers and proteins in soybean hulls.

Table 1. Water holding capacities of soybean hulls with different sizes and pH

	Water holding capacity[1] (%)
20 mesh-pH7	483.10 ± 3.88 [a]
20 mesh-pH4.5	443.96 ± 0.09 [b]
50 mesh-pH7	405.36 ± 0.80 [c]
50 mesh-pH4.5	357.32 ± 7.69 [d]

[1]Means followed by the same letter within the same column are not significantly different ($P<0.05$).

3.4 Pasting Properties

Pasting properties of soybean meal were frequently reported. However, there is no literature focused on pasting properties of soybean hulls. The rapid pasting viscosity (RVA) data were useful since it could provide information for food handling during food processing. RVA pasting data disclosed the changes of sample viscosities during heating and cooling. Pasting properties are displayed in Figure 6. In general, the viscosity of

soybean hulls suspension increased as the amount of soybean hulls in the pasting increased. The viscosity for 10% soybean hull suspension for all sizes remained essentially unchanged during heating and shearing, exhibited a flat pasting curve, and had lower final viscosity compared to the viscosities for 14% and 16% soybean hulls. The viscosities of samples containing 14% and 16% soybean hulls increased gradually during heating and shearing and showed high final viscosity at 50°C after cooling. This increased viscosity could be due to interaction of swollen fibers and proteins during cooling to form a stable matrix with greater stability under heating and shearing. In general, viscosities for all pasting with 20 mesh soybean hulls were found to be much greater than that of 50 mesh soybean hulls. The fiber structures of soybean hulls likely attributed to these higher viscosities. The trend of final peaks from pasting appeared to be related to their water holding capacities. The above results suggested that processing 50 mesh soybean hulls would consume lower energy.

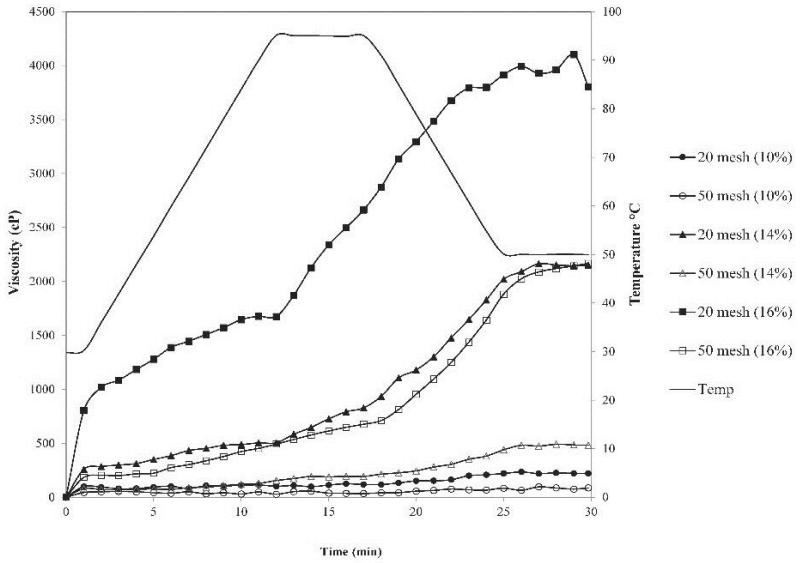


Figure 6. Pasting properties of organic soybean hulls with different sizes and solids

3.5 Extraction of Protein-Rich Products

3.5.1 Effect of pH and Water Temperature

It was previously found the preferred pH for protein extraction from soybean flour was between pH 7.5 and 9.0 (Berk, 1992). Proteins retained their native conformation in films at pH 8 but were partially or extensively denatured at pH 11 and 2 (Mauri & Añón, 2006). Thus, soybean hulls were extracted using water at pH 7.5, 9, 10, and 11 (Table 2). The soybean hulls extracted at pH 9 had the highest protein content (T3: 52.58%) and the hulls at pH 10 was the second highest protein content (T4: 51.52%), while the hulls extracted at pH 7.5 was the lowest (T1: 38.54%). Overall, pH 9-10 is the ideal pH condition, resulting in higher protein contents (about 52%) in the extracted solids from the starting soybean hulls containing 10% protein (Table 2). Although the soybean hull powders extracted at 9 pH had the lowest protein content (T2: 30.35%), they had the highest protein recovery (24.64%) among all extractions using soybean hull powder due to the highest solids obtained from hot extraction. This implies that hot water probably increased the solubility, and the protein recoveries were the functions of both extracted solids and protein content. The suspension of defatted soybean hulls with double hot water extractions could not be separated after pH was adjusted to 4.5. The reasons are probably due to the hull suspension that was emulsified by double hot extractions, and particles with proteins could not precipitate during centrifugation (Table 2, T7).

3.5.2 Effect of Defatting

The extracted protein content (Table 2, T6: 52.75%) with recovery (26.38%) from defatted hull powders was the highest protein content among the extractions, which may be contributed to easy separation without interference of emulsification by lipids. However, defatting incurs additional energy and costs. Also, residual solvent from defatting could be a problem if soybean hulls are used as food ingredients. Overall, for protein extraction from soybean hulls, defatting is a costly unnecessary step.

3.5.3 Hulls versus Powders

When extracting protein from hulls, soybean hulls were soaked for 1 h and blended for 3 min before stirring and pH adjusted to the desired value. In Table 2, the protein content (T8: 45.85%) from soybean hulls was lower than soybean powder at the same pH (pH=9) (Table 2, T3: 52.58%). However, extracted soybean hulls at pH 9 accomplished the highest protein recovery (T8: 28.59%) due to the high solids collected (0.53 g). Grinding hulls required a lot of energy and labor, and lowered the antioxidant activity as shown in Figure 5. The same trend was observed for soybean hulls as shown in Table 3. The protein contents were similar when compared ground hulls to unground hulls (C: 51.46% versus ground hulls G: 51.52%). The recoveries using powders from acidic precipitates (G: 24.05%), sediments (G: 68.92%) and total recovery (G: 92.97%) were slightly higher than from blended hulls (C: 22.99, 65.62, and 88.51%, respectively). Directly using soybean hulls could be an alternative way to extract proteins in some situations.

3.5.4 Homogenizing Methods

Three homogenizing methods including polytron, blender and stirring were used for soybean hulls (Table 3). Polytron is a homogenizer that was tested in this study because the rotor/stator principle of polytron sounds like a good idea for grinding tough tissues by sonically disrupting the cells as well as mechanically. However, solids from polytron (Table 3A) were not easily separated after acidification possibly due to the hulls that were homogenized into very fine particles not separated by centrifugation. The stirred powdered sample recovered low solids (C: 0.89 g) but had higher protein content (C: 51.46%) than blended hulls stirred overnight (B: 43.06%). However, the hull sample by blending had higher recovery (B: 25.73%) than the one using stirring because the blended sample resulted in higher solids (B: 1.19 g) than the sample with stirring (C: 0.89 g). Thus, protein recovery is the function of dry weight and protein content. Results in this study indicate that overworked soybean hulls using polytron will affect protein separation, similar to double hot water extractions. Blending hulls could be an optimum way to process soybean hulls for extracting proteins.

3.5.5 Separation Methods

Two separation methods, centrifugation and sieve, were compared (Table 3, D and E). Soybean hulls using centrifugation had higher protein content (E: 47.02%) than that from sieve separation (D: 34.56%) in dried acid precipitates. However, protein recovery from acid precipitates (D: 28.46%) using sieve method was higher than that using centrifugation (E: 16.05%) due to high yield (D: 1.64 g) from sieve separation. On the other hand, protein recovery from sediments (D: 64.12%) using sieve method was lower than that using centrifugation method (E: 75.71%). This indicated that centrifugation included some insoluble protein into the sediments by gravity. Total recovery using sieve separation (D: 92.58%) was slightly higher than that of centrifuge separation (E: 91.76%). Thus, sieve separation could be an effective way to extract protein from hulls for industrial application.

3.5.6 Times for Purification

Acid precipitation can be washed using water to gain higher protein content. In Table 3, G, H, and I were without washing, and washing once or twice, respectively (Table 3). The protein contents significantly increased from 51.52% (G) to 59.29% (H) by washing once but no improvement was seen when washing twice (I: 58.67%). Without washing (G: 24.05 %) and washing once (H: 24.11%) had similar recoveries that were higher than from washing twice (I: 12.66%). The total recoveries were decreased with the increasing wash times. This suggests that washing precipitates can increase the protein content, but the total recoveries decreased, indicating that some soluble proteins were lost during washing.

3.5.7 NaOH Versus $Ca(OH)_2$

Besides NaOH, $Ca(OH)_2$ could be used for increasing pH and digesting hulls. In Table 3, using NaOH had higher protein content (C: 51.46%), protein recovery (C: 22.99%) from acidic precipitates, and total recovery (C: 88.51%) than using $Ca(OH)_2$, which had protein content (F: 40.83%), protein recovery (F: 16.4%) from precipitates, and total recovery (F: 75.91%). These results were consistent with a previous observation (Paker, et al., 2017). In their study, the protein solubility was greater when Ca $(OH)_2$ was used compared to NaOH; also, using $Ca(OH)_2$ as the processing base yielded the greatest lipid recovery ($p < 0.05$) at 77 g 100 g^{-1} , whereas the greatest protein recovery ($p < 0.05$) was recorded as 53 g 100 g^{-1} protein using NaOH. However, using $Ca(OH)_2$ gave lighter color for products, and increased Ca content which is a valuable nutrient for health.

Table 2. Comparisons of pH, temperature, defatting, and hull versus powder for extracting value-added products from soybean hulls (10 g)

T #	materials	pH	procedure description	dried precipitates dry weight (g)	protein %	recovery %	dried sediments dry weight (g)	protein %	recovery %	total recovery %
1	powder	7.5	cold, single, stir	0.38 ^{d}	38.54 ^{e}	22.09 ^{f}	-	-	-	-
2	powder	9	hot, single, stir	0.69 ^{a}	30.35 ^{f}	24.64 ^{c}	-	-	-	-
3	powder	9	cold, single, stir	0.37^{d}	52.58 ^{a}	22.89 ^{e}	-	-	-	-
4	powder	10	cold, single, stir	0.47 ^{c}	51.52 ^{b}	24.05 ^{d}	8.12	8.46	68.92	92.97
5	powder	11	cold, singly, stir	0.39 ^{d}	48.44 ^{c}	22.09 ^{f}	7.99	6.19	58.16	80.26
6	defatted powder	10	cold, single, stir	0.46 ^{c}	52.75 ^{a}	26.38 ^{b}	8.29	6.02	54.25	80.63
7	defatted powder	10	hot, double, stir	0 ^{e}	0 ^{g}	0 ^{g}	7.69	8.73	72.96	72.96
8	hull	9	cold, single, blend	0.53 ^{b}	45.85 ^{d}	28.59 ^{a}	-	-	-	-

Means followed by the same letter within the same column were not significantly different ($p<0.05$).

Table 3. Comparisons of homogenization, separation and purification methods for extracting value-added products from soybean hulls (20 g) at pH10

T #	materials	procedure description	dried precipitates dry weight (g)	protein (%)	recovery %	dried sediments dry weight (g)	protein %	recovery %	total recovery %
A	hull	polytron, stir overnight	1.75 ^{a}*	13.71 ^{h}	12.06 ^{h}	14.98 ^{f}	8.88 ^{b}	66.72 ^{c}	78.78 ^{h}
B	hull	blend, stir overnight	1.19 ^{c}	43.06 ^{e}	25.73 ^{b}	15.99 ^{e}	7.88 ^{g}	63.21^{g}	88.94 ^{d}
C	hull	blend, stir, purified once	0.89 ^{d}	51.46 ^{c}	22.99 ^{d}	15.94 ^{e}	8.19 ^{e}	65.52 ^{e}	88.51 ^{e}
D	hull	blend, stir, sieve	1.64 ^{b}	34.56 ^{g}	28.46 ^{a}	14.16 ^{g}	9.02 ^{a}	64.12 ^{f}	92.58 ^{b}
E	hull	blend, stir, centrifuge	0.68 ^{f}	47.02 ^{d}	16.05 ^{f}	17.36 ^{a}	8.69 ^{c}	75.71 ^{a}	91.76 ^{c}
F	hull	blend, stir, $Ca(OH)_2$	0.80 ^{e}	40.83 ^{f}	16.40 ^{e}	16.12 ^{d}	7.35 ^{h}	59.51 ^{h}	75.91 ^{i}
G	powder	stir	0.93^{d}	51.52 ^{c}	24.05 ^{c}	16.23 ^{c}	8.46 ^{d}	68.92 ^{b}	92.97 ^{a}
H	powder	stir, purified once	0.81 ^{e}	59.29 ^{a}	24.11 ^{c}	15.94 ^{e}	7.06 ^{i}	56.51 ^{i}	80.62 ^{f}
I	powder	stir, purified twice	0.43 ^{g}	58.67^{b}	12.66 ^{g}	16.59 ^{b}	7.96 ^{f}	66.28 ^{d}	78.94 ^{h}

*Dried whole supernatant.

Means followed by the same letter within the same column are not significantly different ($p<0.05$).

3.6 Value-added Products

3.6.1 Extracted Protein

The extracted protein (50-60%) can be used protein-rich food products or used in other applications (Photo 1).

3.6.2 Ingredients Rich in Proteins, Fibers, Minerals and Phytosterols

The finely ground power from soybean hulls, dried sediments and whole freeze-dried supernatants may be good food ingredients for various purposes (Photo 1). The ground powder from hulls and the sediments from protein extractions containing 8-9% proteins are excellent food ingredients that can be directly used in baking products, such as cookies and energy bars. In addition, neutralized whole supernatant from extraction after alkane treatment can be freeze-dried (Photo 1), and would be an excellent soluble ingredient with about 13% protein that was higher than in the starting materials (9-10%). The freeze-dried whole supernatant can be used in food products, such as dressings or toppings since it is water soluble. Furthermore, soybean hulls may be developed into valuable supplements with high protein, dietary fiber, minerals, and phytosterols due to their excellent antioxidant activity and nutritional value.

In this primary study, about 25-29% protein was able to be extracted from soybean hulls, regardless of pH, homogenization and separation methods. This could be due to the protein in hulls tightly bound with fibers and lowere water solubility as shown in Table 1. An earlier study found a lower recovery protein from plants which likely due to binding to fibers and varied among plant parts (Rothman, et al., 2008). Therefore, the proportion of fiber-bound protein should be considered when estimating protein, and further research is needed.

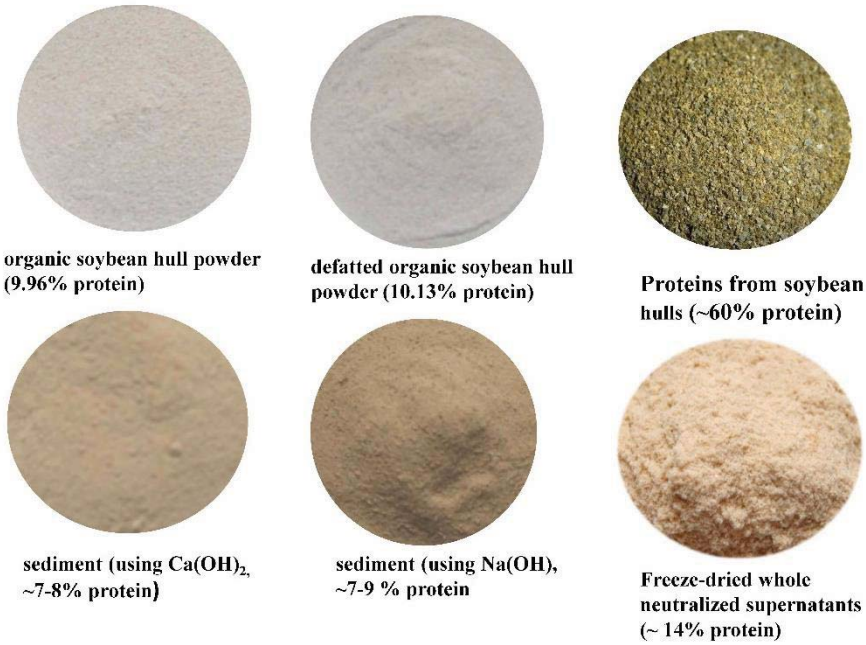


Figure 7. Ground soybean hulls and potential value-added products

4. Conclusion

This research unveiled the nutritional value of soybean hulls and first reported antioxidant activity, pasting and water properties of soybean hulls. Also, various protein extraction methods were studied for valuable products from soybean hulls. The extraction at pH 9-10 had higher protein recoveries compared to pH 7.5 and 11. Total recovery using sieve separation (92.58%) is slightly higher than that of centrifugation (91.76%). Using sieve separation could be an effective way to extract protein from soybean hulls for industrial application. The precipitated protein content from extracted supernatant (51.52%) increased to 59.29% after purification by washing with water once. No further improvement was shown with two washings.

Several potential products were revealed in this research. The purified proteins can be used for food or industrial applications. The ground hull powders (10% protein), dried supernatant (14% protein), and sediments (7-8% protein) along with antioxidant activity and valuable fibers will be tested to see if they can be good food ingredients in a future study. This research explored the great potential to covert soybean hulls from low value byproducts into value-added functional food or industrial products along with the benefit of reducing waste in the environment

Acknowledgement

Mention of trade names or commercial products in this article is solely for the purpose of providing scientific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

References

Ade-Omowaye, B. I. O., Taiwo, K. A., Eshtiaghi, N. M., Angersbach, A., & Knorr, D. (2003). Comparative Evaluation of the Effects of Pulsed Electric Field and Freezing on Cell Membrane Permeabilisation and Mass Transfer during Dehydration of Red Bell Peppers. *Innovative Food Science & Emerging Technologies, 4*, 177-188. https://dx.doi.org/10.1016/S1466-8564(03)00020-1

American Association of Cereal Chemists. (1995). Crude protein – Combustion method. In: *American Association of Cereal Chemists Official Methods, 9th edition*. St. Paul, MN: The Association, method *46*-30.01

Anderson, S. J., Merrill, J. K., McDonnell, M. L., & Klopfenstein, T. J. (1988). Digestibility and utilization of mechanically processed soybean hulls by lambs and steers. *Journal of Animal Science, 66*(11), 2965-2976. https:// dx.doi.org/10.2527/jas1988.66112965x

Berk, Z. (1992). Isolated soybean proteins (ISP). Chapter 6. In: Technology of production of edible flours and protein products from soybeans. FAO Agricultural Service Bulletin. No.97. Food and Agriculture Organization of the United Nations. Rome, Italy. Retrieved from https://www.fao.org/3/t0532e/t0532e07.htm

Dyke, L., & Rooney, L. W. (2007). Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World*, *52*, 105-111. https://doi.org/10.1094/CFW-52-3-0105QD1004316358

Extension. (2019). Retrieved from https://articles.extension.org/pages/39695/what-are-soybean-hulls.

Gnanasambandam, R., & Proctor, A. (1997). Soy hull as an adsorbent source in processing soy oil. *JAOCS, Journal of the American Oil Chemists' Society*, *74*, 685-692. https://dx.doi.org/10.1007/s11746-997-0201-2

Israelachvili, J. N. (2011). Intermolecular and Surface Forces. 3rd Edition. Academic Press, an Imprint of Elsevier, Waltham, MA. pp. 152.

Kansas State University Agricultural Experiment Station and Cooperative Extension Service. (2019). Soybean Hulls: Composition and Feeding Value for Beef and Dairy Cattle. https://www.bookstore.ksre.ksu.edu/pubs/MF2438.pdf

Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of biological Sciences*, *11*, 982-991. https://dx.doi.org/10.7150/ijbs.12096

Kaur, M., & Singh, N. (2006). Relationships between selected properties of seeds, flours, and starches from different chickpea cultivars. *International Journal of Food Properties, 9*, 597-608. https://dx.doi.org/10.1080/10942910600853774

Liu, K. (1999). Soybeans. Chemistry, Technology, and Utilization. Aspen publishers Inc., Gaithersburg, MD. pp. 387

Marshall, W. E., Wartelle, L. H., Boler, D. E., & Toles, C. (2000). Metal ion adsorption by soybean hulls modified with critic acid: a comparative study. *Environmental Technology*, *21*, 601-607. https://dx.doi.org/10.1080/09593332108618075

Mauri, A. N., & Añón, M. C. (2006). Effect of solution pH on solubility and some structural properties of soybean protein isolate films. *Journal of the Science of Food and Agriculture*, *86*, 1064-1072. https://dx.doi.org/10.1002/jsfa.2457

Paker, I., Jaczynski, J., & Matak, K. E. (2017). Calcium hydroxide as a processing base in alkali-aided pH-shift protein recovery process. *Journal of the Science of Food and Agriculture, 97*, 811-817. http://dx.doi.org/10.1002/jsfa.7800.

Qiu, S., Yadav, M. P., & Yin, L. (2017). Characterization and functionalities study of hemicellulose and cellulose components isolated from sorghum bran, bagasse and biomass. *Food Chem., 230*, 225-233. https://doi.org/10.1016/j.foodchem.2017.03.028.

Rothman, J. M., Chapman, C. A., & Pell, A. N. (2008). Fiber-bound nitrogen in gorilla diets: implications for estimating dietary protein intake of primates. *American Journal of Primatology, 70*, 690-4. http://dx.doi.org/10.1002/ajp.20540

SAS Institute Inc. The SAS® system for Windows®, version 8e. Cary, NC. 1999.

Şensoy, I., Rosén, R. T., Ho, C. T., & Karwe, M. V. (2006). Effect of Processing on Buckwheat Phenolics and Antioxidant Activity. *Food Chemistry*, *99*, 388-393. http://dx.doi.org/10.1016/j.foodchem.2005.08.007

Sessa, D. J. (2004). Processing of soybean hulls to enhance the distribution and extraction of value-added proteins. *Journal of the Science of Food and Agriculture*, *84*, 75-82. https://doi.org/10.1002/jsfa.1612

Stein, H. H., Berger, L. L., Drackley, J. K., Fahey, G. C., Jr., Hernot, D. C., & Parsons, C. M. (2008). Nutritional Properties and Feeding Values of Soybeans and Their Coproducts. In L. A. Johnson, P. J. White, & R. Galloway (Eds.), *Soybeans: Chemistry, Production, Processing, and Utilization*. (pp. 613-660). AOCS Press, Urbana IL. https://doi.org/10.1016/B978-1-893997-64-6.50021-4

Waterhouse, A. L. (2001). Determination of total phenolics. In: R. E. Wrolstad, *Current Protocols in Food Analytical Chemistry,* I1.1.1-I1.1.8. New York: John Wiley & Sons.

Yu, L., & Zhou, K. (2004). Antioxidant properties of bran extracts from Platte wheat grown at different locations. *Food Chemistry, 90*, 311-316. https://dx.doi.org/10.1016/j.foodchem.2004.04.007

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

In vitro Antioxidant Activities of Natural Polysaccharides: An overview

Boris V. Nemzer[1, 2], Diganta Kalita[1], Alexander Ya Yashin[3], Nikolay E. Nifantiev[4] & Yakov I. Yashin[3]

[1]Department of Research & Development, VDF FutureCeuticals, Inc., Momence, IL 60954, USA

[2]Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

[3]International Analytical Center of Zelinsky Institute of Organic Chemistry, Moscow 119991, Russia

[4]Laboratory of Glycoconjugate Chemistry of Zelinsky Institute of Organic Chemistry, Moscow 119991, Russia

Correspondence: Boris V. Nemzer, Department of Research & Development, VDF FutureCeuticals, Inc., Momence, IL 60954, USA. Tel: 1-815-507-1427. E-mail: bnemzer@futureceuticals.com

Received: August 30, 2019 Accepted: September 24, 2019 Online Published: October 29, 2019

doi:10.5539/jfr.v8n6p78 URL: https://doi.org/10.5539/jfr.v8n6p78

Abstract

Polysaccharides are naturally occurring biomacromolecules composed of carbohydrate molecules linked by glycosidic bonds. A number of polysaccharides are known to possess beneficial therapeutic effects against inflammation, diabetes, cardiovascular diseases, and cancers. Indeed, polysaccharides are reportedly effective free radical scavengers and antioxidants, thereby playing a critical role in the prevention of damage to living organisms under oxidative stress. In this review we provide an overview of the sources, extraction, and antioxidant activities of some natural polysaccharides.

Keywords: polysaccharide, antioxidant activity, health benefits

1. Introduction

Polysaccharides are naturally occurring biomacromolecules composed of carbohydrate molecules linked by glycosidic bonds. These molecules play an important role in the development of pharmaceuticals, food, nutritional products, and biodegradable packaging materials (Yu, Shen, Song, & Xie, 2018; Copeland, Blazek, Salman, & Tang, 2009; Yang & Zhang, 2009; Cazon, Velazquez, Ramirez, & Vazquez, 2017). They are commonly derived from plants, fruits, vegetables, herbs, algae, mushrooms, and microorganisms (Kardasova & Machova, 2006; Zhang et al., 2019; Maity, Maity, & Dasgupta, 2019; Han et al., 2019; Hajji et al., 2019, Liu, Ooi, & Chang, 1997; Huang, Siu, Wang, Cheeung, & Wu, 2013; Hu, Gen, Zhang, & Jiang, 2001; Guo et al., 2010; Kodali & Sen, 2008).

Polysaccharides can be either linear or branched and they can be classified as homopolymers if the polymer composed of identical monosaccharides, or heteropolymers, if the polysaccharide is composed of two or more different monosaccharides. The structural composition of polysaccharides is rather complex since the monosaccharide units linked through glycosidic linkages can be composed of different sequences, degrees of polymerization, and branching. As such, the structures of these compounds are determined using various sophisticated analytical and spectroscopic techniques, such as fourier transform infrared spectroscopy (FT-IR), ultraviolet spectrophotometry (UV-Vis), nuclear magnetic resonance (NMR) spectrophotometry, gas chromatography-mass spectrophotometry (GC-MS), and liquid chromatography-mass spectrophotometry (LC-MS). It has also been found that their structural properties vary based on the natural sources, processing methods, extraction methods, and variety of agricultural or botanical species (Wang, Hu, Nie, Yu, & Xie, 2016; Wang et al., 2013; Cui, 2005).

One of the major applications of polysaccharides is their uses as a staple food source for humans. For example, starch is key ingredient in various foods supplying 50–70% of the energy in a human diet. Upon digestion, starch can be hydrolyzed to glucose, which is an integral part of the development of brain and red blood cells. The rate of digestibility of polysaccharides has an active role in determining the glycemic index (GI), i.e., the level of glucose in the blood. More specifically, a rapid rate of digestibility results in a higher GI and can leads to complications such as type II diabetes and obesity. However, polysaccharides that exhibit beneficial health

properties are of great interest in the food industry as a number of these compounds have been shown to exhibit anti-inflammatory, antidiabetics, and anticancer properties, in addition to preventing cardiovascular diseases (Yu, et al., 2018; Copeland et al., 2009; Yang & Zhang, 2009; Cazon et al., 2017; Kardasova & Machova, 2006). Polysaccharides are also used in many biomedical and biological applications, such as tissue engineering, drug delivery, wound healing, and biosensors (Yu, Shen, Song, & Xie, 2018; Copeland, Blazek, Salman, & Tang, 2009; Kardasova & Machova, 2006; Zhanget al., 2019). More importantly, some have been employed as antioxidants both *in vitro* and *in vivo* due to their free radical scavenging properties that play a critical role in the prevention of damage from oxidative stress. Thus, in this review, we provide an overview of the sources, extraction, in vitro antioxidant properties of some natural polysaccharides.

2. Sources of Polysaccharide

2.1 Plant Polysaccharides

Among the various natural polysaccharides, the major polysaccharides tend to be extracted from plant materials. Several polysaccharides isolated from plant sources have been reported to exhibit significant antioxidant activities both *in vivo* and *in vitro*. For example, two polysaccharides isolated from *Rosa rugosa petals* (RRPS, namely RRPS-1 and RRPS-2) were analyzed for their antioxidant activities and it was found that RRPS-2 had a good radical scavenging potential (Zhang et al., 2019). In addition, a water soluble arabinoxylan (APPS), with a molecular weight of $\sim 1.49 \times 10^5$ Da was isolated from the green stem of *Andrographis paniculate* and showed strong antioxidant activity against superoxide and hydroxyl free radicals (Maity, Maity, & Dasgupta, 2019). Kiwifruit polysaccharides also exhibited high antioxidant activities (Han et al., 2019). Furthermore, Hajji et al., (2019) isolated a novel water-soluble polysaccharide (PLP1) from the root barks of *Periploca laevigata* by hot water extraction and further purification by diethylaminoethyl cellulose (DEAE)-sepharose chromatography, and this compound exhibited a strong antioxidant activity. Recently, Wei et al., (2019) isolated polysaccharides from seabuckthorn berries (PSB) and investigated the role of microwave power on their antioxidant activities. Furthermore, a water-soluble polysaccharide (MWP) with a galactose-, glucose-, mannose-, fructose with 1:33.2:8.4:7.2 was successfully isolated from Malus micromalus Makino fruit (Hui, Jun, & Chuang, 2019) and this polysaccharide showed degrees of scavenging effects on hydroxyl, DPPH (2,2-ddiphenyl-2-picrylhydrazyl), and superoxide radicals in a dose dependent manner. Zhu et al., (2009) isolated two major polysaccharide fractions composed of arabinose, galactose, and glucose, which were associated with protein portions from Huaza No. 4 rapeseed meal using column chromatography and ethanol precipitation, and this compound also exhibited a significant antioxidant activity (Zhu and Wu, 2009). In addition, three polysaccharides with molecular weights of 218, 178, and 60 kD were extracted from the roots of *Arctium lappa L.* (ALPs) and were reported to exhibit strong scavenging activities for DPPH, hydroxyl, and superoxide radicals both *in vitro* and *in vivo* (Jiang et al., 2019). Furthermore, Litchi pulp polysaccharide extracted from Litchi *Chinensis Sonn*. showed a dose dependent antioxidant activity against DPPH, hydroxyl, and superoxide radicals, (Kong et al., 2010) while Ginkgo biloba leaves (GBLs), a common herbal remedy, exhibited noticeable scavenging effects on superoxide radicals and ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radicals (Ren et al., 2019).

2.2 Fungal Polysaccharide

Fungal cell walls are composed of polysaccharides such as glucans, chitin, and mannans, which contain glucose, N-acetylglucosamine, and mannose repeating units, respectively. However, the structures of these species also differ; for example glucans are composed of glycosidic linkages including branched glucans and linear glucans, while chitin is a water insoluble polymer composed of β1,4-linked N-acetylglucosamine units. Studies into the beneficial effects of these polysaccharides indicated that mushrooms polysaccharides tend to exhibit significant antioxidant properties. For example, *Inonotus oblique* mushrooms produce a crude extract polysaccharide, which is widely used in Russian folk medicine and is reported to have significant medicinal and antioxidant properties (Mu et al., 2012). In addition, a polysaccharide from *Hericium erinaceus* mushrooms exhibits a strong *in vitro* antioxidant activity in addition to neuroprotective effects on β-induced neurotoxicity in neurons (Cheng, Tsai, Lien, Lee, & Sheu, 2016). Furthermore, polysaccharides extracted from the edible mushroom *Tricholoma mongolicum* exhibited *in vitro* antioxidant activities in a dose-dependent manner (Zhao et al.,2016) while fucogalactomannan obtained from the *Tylopilus ballouii* mushroom inhibited superoxide and hydroxyl radicals with IC_{50} values of 1.25 and 1.6 mg/mL, respectively (Lima et al., 2016). Moreover, a water-soluble β-glucan isolated from the hot water extract of the edible mushroom *Entoloma lividoalbum* (Kühner & Romagn) Kubička stimulated the production of macrophages, splenocytes, and thymocytes, in addition to exhibiting hydroxyl and superoxide radical scavenging activities and reducing properties (Maity, et al., 2015). Indeed, it has been reported that antioxidant activities of these fungi originated from their component polysaccharides and

polysaccharide -protein complexes (Tseng, Yang, & Mau, 2008; Wu & Wang, 2009; Alzorqi, Sudheer, Lu, Manickam, 2016; Chen, Zhong, Zeng, & Ge, 2008). In this context, Chen et al., (2008) reported that the administration of polysaccharides lentinans from *Lentinus edodes* could decrease the oxidative stress induced by a high-fat diet in rats (Chen, Zhong, Zeng, & Ge, 2008).

2.3 Bacterial Polysaccharide

Bacterial polysaccharides are biomacromolecules that are anchored to the cell surface by lipid and exist as extracellular polysaccharides, peptidoglycans, lipopolysaccharides, capsules and exopolysaccharides. Bacterial stains such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Bordetella bronchiseptica*, *Actinobacillus pleuropneumoniae*, and *Yersinia pestis* contains high level of polysaccharides in the form of extracellular polysaccharides (EPS). More specifically, Guo et al., (2010) reported that two polysaccharide isolated from the marine bacterium *Edwardsiella tarda* exhibited a good antioxidant activity against hydroxyl and DPPH radicals, while Kodali et al., (2008) isolated an EPS from the probiotic bacterium *Bacillus coagulans* and confirmed that it exhibited a significant antioxidant activity (Guo et al., (2010); Kodali & Sen, 2008). Moreover, Raza et al., (2011) reported that an EPS from *Paenibacillus polymyxa* possessed a good superoxide scavenging activity in addition to a moderate inhibition of lipid peroxidation (Raza, Makeen, Wang, Xu, & Qirong, 2011).

2.4 Animal Polysaccharide

Animal polysaccharides as glycogens composed of α-1,4-glycosidic bonds with branched α-1,6 bonds present at approximately every tenth monomer. These polysaccharides are used to store energy and are produced by the liver and the muscles during glycogenesis. Some examples of polysaccharides found in the human body are hyaluronic acid, chondoitrin sulfate, dermatan sulfate, keratan sulfate and heparin. A study into the antioxidant activity of a polysaccharide from *Hyriopsis cumingii* (HCp) showed both in *vitro* and *in vivo* free radical scavenging, in addition to an increase in the activities of antioxidant enzymes in a dose-dependent manner (Qiao et al., 2009). A free neutral polysaccharide was extracted from the mucus of the loach (*Misgurnus anguillicaudatus*) and their effects on the removal of reactive oxygen species were examined. Indeed, the efficiently removal of •O2, •OH, H_2O_2 and other active oxygen compounds were confirmed and these polysaccharides were found to significantly protect DNA chains from damage by free radicals (Zhang, Wang, & Dong, 2011). Chitin is one of the main components of cell walls in fungi, radulas of mollusks and is a linear homopolysaccharide comprising N-acetyl-glucosamine. Ngo et al., (2008) employed acidic hydrolysis for the isolation of a polysaccharide from crab chitin and upon investigation of its cellular antioxidant effects, they observed free radical scavenging effects (Ngo, Kim, & Kim, 2008).

2.5 Algal Polysaccharide

Fucoidan is a natural sulfated polysaccharide that contains fucose and is known to exhibit numerous beneficial health properties due to the presence of the sulphate ester group. Algal polysaccharides are generally found in the form of alginates, fucans, laminarans, cellulose, and sulfated galactans. More specifically, Zhang et al., (2010) found that fucoidan had promising antioxidant activity toward superoxide radicals rather than hydroxyl and DPPH radicals and brown algae containing sulfated polysaccharides showed significant antioxidant properties (Zhang et al., 2010). In addition, Sokolvoa et al., (2011) reported that sulfated polysaccharides, namely carrageenans, isolated from red algae possessed strong antioxidant activities both *in vivo* and *in vitro* due to stimulation of the catalytic activity of superoxide dismutase (SOD) (Sokolova et al., 2011). Both the molecular mass and sulfate content in fucoidan play a significant role in determining its radical scavenging activity and, a positive correlation with the sulfate content has been demonstrated. It was also reported that the sulfated and acetylated derivatives of polysaccharides extracted from the red algae Gracilariopsis lemaneiformis exhibited strong antioxidant activities, although certain derivatives exhibited stronger activities than the unmodified compounds (Wang et al., 2019).

3. Polysaccharide Extraction, Isolation and Characterization

Water is commonly employed as a solvent for the extraction of polysaccharides. However hot water was found to be better extracting solvents than the cold water. Several polysaccharides were extracted from various sources as listed in the table 1 by using water with temperatures 50-100 ˚C. Higher temperature improved the extraction of polysaccharide. In most of the cases the polysaccharides have been isolated using chromatography such as anion chromatography, DEAE column chromatography and gel filtration after ethanol precipitation, and deproteination. Figure 1 represents a general strategic extraction and purification of polysaccharide form various polysaccharide sources. Pure Garlic polysaccharide was extracted by using hot water. These polysaccharides were isolated and separated by column chromatography on Sephadex G200 and washed with distilled water and then

characterised by ^{13}C and ^{31}P NMR spectroscopy (Chen & Huang, 2019). Pure pumpkin polysaccharides were extracted by hot water after purification by ethanol precipitation and dialysis. Further sulfated and phosphorylated pumpkin polysaccharides were characterized by IR and ^{13}C NMR spectroscopy (Chen & Huang, 2019). Cushaw polysaccharide have been extracted with water at 100 ˚C after ethanol precipitation followed by dialysis and sulfated and phosphorylated polysaccharide have been prepared with different degrees of substitution. Substituted polysaccharides have been characterized by IR and NMR spectroscopy (Chen & Huang, 2018). Chen et al., (2019) extracted and isolated polysaccharide with potential antioxidant activity from native ginseng by hot water and characterized by IR and NMR spectroscopy (Chen & Hunag, 2019).

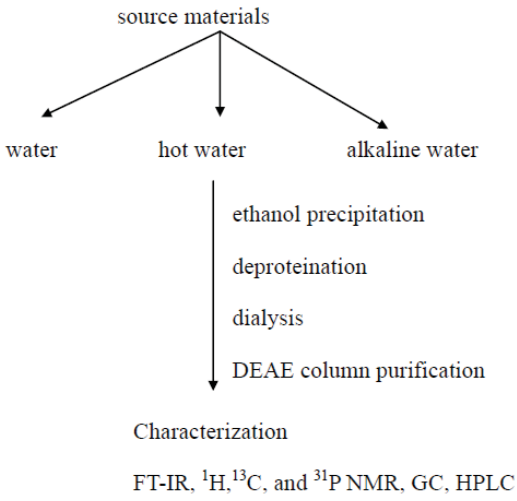


Figure 1. Schematic representation of extraction and characterization of polysaccharides

It has been observed that some advanced treatments such microwave irradiation, sonication, and other irradiation treatments have also been adopted to optimize polysaccharide extraction (Table 1). Furthermore, the extracted crude polysaccharides have been successfully purified by column chromatography.

Table 1. Sources, extraction methods, purifications, characterizations for some polysaccharides

sources	solvent	treatment and/or processing	characterisation	ref
Polygonatum sibiricum	water	microwave, ethanol precipitation, diethylaminoethyl cellulose-52 anion-exchange chromatography	high performance gel chromatography (HPGC)	Zhang et al., (2019)
Fritillaria pallidiflora	water	anion-exchange and gel-permeation chromatography	FT-IR, NMR, SEM	Rozi et al., (2019)
kiwifruit	water	microwave, ultrasound	FT-IR, high performance size exclusion chromatography (HPSEC), HPLC	Han et al., (2019)
Periploca laevigata	water	DEAE-sepharose chromatography	UHPLC, GC-MS, FT-IR, ^{1}H NMR and ^{13}C NMR	Haaji et al., (2019)
Ginkgo biloba leaves	water	DEAE (diethylaminoethanol) Sepharose Fast Flow column	high performance liquid chromatography (HPLC), IR	Ren et al., (2019)
Inonotus obliquus	water	anion-exchange DEAE cellulose column and Sephadex G-200 gel	IR, Liquid Chromatography, HPGPC,	Hu et al., (2016)
Mung beans	alkaline water	purified and separated by anion exchange and gel filtration	HPSEC, GC	Yao et al., (2016)
Anji Baicha	hot water	-	-	Zhang et al., (2016)
Paecilomyces hepiali HN1	hot water	-	HPLC	Wu et al., (2016)
Poria cocos	hot water	ultrasonic, microwave, enzyme assisted	IR	Wang et al., (2016)
Artemisia sphaerocephala	water	ultrasound assisted, sulfated with triphenylchloromethane	HPLC, IR	Zheng et al., (2016)
Tangerines peel	water	microwave assisted, purified by anion exchange and gel filtration	IR	Chen et al., (2016)
Blackcurrant fruit	water	pressurised water extraction	GC, HPLC, and IR	Xu et al., (2016)
Dipsacus asperoides	water	ultrasonic power		Tan et al., (2015)
Chrysanthemum morifolium	hot water	anion exchange, DEAE -cellulose column, gel filtration	partial acid hydrolysis and NMR	Zheng et al., (2015)
Rana chensinesis	water		HPLC, GC, IR	Wang et al., (2015)
Stachys sieboldii Miq	water	DEAE Sepharose, Sepharose CL-6B	HPLC, GC	Feng et al., (2015)
Fortunella margarita	water	ultrasonic power, purified DEAE Sepharose, Sepharose CL-6B	UV-Vis, HPLC, NMR	Zeng et al., (2015)
Rana chensinesis	water	DEAE Sepharose, Sepharose CL-6B	-	Wang et al., (2015)
Radix Cyathulae officinalis	water	Water soluble	IR, GCMS, GPC	Han et al., (2015)
Cucurbita moschata	water	ultrasound and enzyme	-	Wu et al., (2014)
Isochrysis galbana	water	anion exchange chromatography and gel	IR, ESI-MS, NMR	Sun et al., (2014)
Tremella fuciformis	water	Fe^{2+}/Vc/H_2O_2	UV-Vis, IR, HP-GC	Zhang et al., (2014)
garlic	petroleum ether, acetone, methanol	cellulase assisted	-	Pan et al., (2014)
Ficus microcarpa	water	ultrasound	IR spectroscopy	Jiang et al., (2014)

4. Mechanisms of Antioxidant Properties of the Polysaccharide

4.1 Antioxidant Activities by Crude Polysaccharide Extract and Polysaccharide Conjugates

Numerous literatures exist regarding the health benefits of polysaccharides, where most of the studies were carried out using crude polysaccharide extracts. Interestingly, the presence of proteins, amino acid, peptides, organic acid, and other components appeared to enhance the antioxidant properties since, the purified polysaccharides tended to exhibit significantly lower antioxidant activities than the crude polysaccharides. For example, Ennaifer et al., (2018) reported an antioxidant-rich decoction *Pelargonium graveolens* containing a water-soluble polysaccharide (Ennaifer, Bouzaine, Chouaibi, & Hamdi, 2018). Also, Zhang et al., (2019) isolated three polysaccharide extract with and without proteins and found that the protein-containing polysaccharide extract exhibited the highest antioxidant activity (Zhang et al., 2019). In another study, Huang et al., (2003) reported that polysaccharides isolated from *Cordyceps sinesis* showed no antioxidant activity following separation of the protein from the crude (Huang, Liang, Guo, Zhou, & Cheng, 2003). In addition, Siu et al., (2016) investigated the antioxidant activities of crude polysaccharide from mushrooms (*L. edodes, G. frondosa,* and *Zt. Versicolor*) and found that their antioxidant activities were positively correlated with the contents of phenolic acids and proteins (Siu, Xu, Chen, & Wu, 2016). Indeed, the formation of polysaccharide complexes with other components such as amino acids, proteins, and lipids are particularly common, yielding polysaccharide conjugates. Interestingly, majority of reports indicate that the polysaccharide conjugates exhibit higher antioxidant activities than the free polysaccharides or bioactive compounds. It has been suggested that polysaccharide-polyphenols and polysaccharide-protein conjugates are formed via hydrophobic interactions and hydrogen bonding. Recently Barmudez et al., (2019) reported that interactions between polysaccharides and phenolic compounds contributed to a strong free radical scavenging activity through a combination of covalent (ester bonds) and non-covalent (hydrogen bonding and electrostatic interactions) interactions (Barmuez et al., 2019). It was found that the interactions after drying were stronger than those found in the simple mixture. For example, phenolic acids (secondary metabolites in many plant species) form polysaccharide conjugates that exhibit significant antioxidant activities. Indeed, Wang et al., (2012) investigated the antioxidant activities of oolong tea and found no antioxidant activities from the phenolic acid-free and protein-free extracts (Wang, Yang, & Wei, 2012).

Polyphenol associated polysaccharides are also known to contribute significantly to the antioxidant activity determined by using the DPPH assay. In some cases, polyphenol-free tea polysaccharides have also been found to exhibit a strong antioxidant activity. Thus, to examine the role of polysaccharide polyphenol complexes in determining the antioxidant activity of such species, Wu et al., (2011) carried out a comparative study using tea polyphenols, β-glucan, and the corresponding mixture and complex, and found that the complex exhibited the highest antioxidant activity against hydroxyl free radicals (Wu et al., 2011). However, inconsistent results were found for the mixture, thereby demonstrating that a complex structure of tea polyphenols and β-glucan plays a critical role in determining the antioxidant activity. Ferulic acid polysaccharide complexes have also been found to exhibit strong antioxidant activities. Moreover, Shu et al., (2019) investigated the role of particle size in green tea powder and found that the antioxidant activity could be improved by reducing the particle size (Shu, Li, Yang, Dong, Wang, 2019).

Selenium is a biologically relevant element found in soil and agricultural products. However, organoselenium compounds have been reported to exhibit more beneficial properties than free selenium species. To mimic the enhanced bioavailability and biological properties of organoselenium compounds, a series of selenium compounds have been synthesized using biologically relevant molecules such as proteins, amino acids, alkaloids and polysaccharides. For example, Wei et al. (2012) reported that a selenylated polysaccharide (Se- RHP) synthesized from *Radix hedysari* exhibited an enhanced antioxidant activity compared to the free polysaccharide (Wei, Cheng, Wei, & Zhang, 2012). Similar polysaccharide selenium complexes have been synthesized by Wang et al., (2012) using a polysaccharide extracted from *Artemisia sphaerocephala* (Wang, Zhao, Wang, Yao, & Zhao, 2012). In addition, in the case of *Potentilla anserina L.* Zhao et al., (2013) successfully improved the antioxidant activity compared to the native polysaccharides through the formation of a complex with selenium (Zhao et al., 2013). The higher antioxidant activity of the polysaccharide was attributed to conformational changes in the polymers containing a higher number of hydroxyl groups. Furthermore, Yu et al., (2007) reported that Se-enriched green tea had a higher antioxidant activity than the regular tea due to the formation of polysaccharide-Se complexes, while polysaccharides isolated from Se-enriched *G. lucidum* were more effective in reducing the production of superoxide radicals (Yu , Sheng, Xu, An, & Hu, 2007; Zhao et al., 2008). Moreover, Mao et al., (2014) and Cheng et al., (2013) studied the polysaccharide isolated from *G. frondosa* and found that the Se-enriched polysaccharide (Se-GP) was a stronger scavenger for DPPH, ABTS, and hydroxyl

radicals (Mao et al., 2014; Cheng et al., 2013). However, no significant differences were observed between these species in terms of the polysaccharide content and the molecular weight, with the Se content being the only variable. Interestingly, Guo et al., (2013) reported that a selenium-polysaccharide synthesized by using selenium chloride oxide ($SeCl_2O$) and the polysaccharide also exhibited a higher total antioxidant capacity, superoxide radical scavenging effect, and hydroxyl radical scavenging effect (Guo et al., 2013).

4.2 Antioxidant Properties of Processed Polysaccharide

The biological properties of polysaccharide are dependent on their structural composition, conformation, and type of glycosidic linkage. Moreover, the sources, extraction, and processing methods, significantly influence the polysaccharide structure and conformation, which can subsequently alter the chemical and biological properties. Interestingly, number of reports have indicated a correlation between the molecular weight and radical scavenging activity of polysaccharides (Liu, Wang, Pang, Yao, & Gao, 2010; Xing et al., 2005; Zha et al., (2009); Sun, Wang, Shi, & Ma, 2009; Zhang, Wang, Mo, & Qi, 2013, Choi et al., 2009). It was hypothesized that polysaccharides with low molecular weights would possess a greater number of reductive hydroxyl group terminals to accept and eliminate free radicals, since the number of hydroxyl or amino groups in polysaccharide molecules such as chitosan greatly influence the free radical scavenging activity. For example, Zhang et al., (2019) isolated four polysaccharide fractions (P-1: 71.40%, P-2: 1.95%, P-3: 1.14%, and P-4: 1.64%) from crude Polygonatum sibiricum polysaccharide (PSP), and the antioxidant capacity was P-4 > P-3 > P-2 > PSP > P-1 (Zhang et al., 2019). In addition, two low molecular weight polysaccharides were isolated from *G. lucidum* by Liu et al., (2010) and these were reported to display significant antioxidant activities (Liu, Wang, Pang, Yao, & Gao, 2010). Similarly, Xing et al., (2005) observed higher scavenging activities by polysaccharides with lower molecular weights, which was partly attributed to differences in their structural composition compared with higher molecular weight species (Xing et al., 2005). Furthermore, Zha et al.(2009) obtained three polysaccharide fractions from rice bran with molecular weights ranging from 1.2×10^5 to 6.3×10^6 Da (PW1), 3.5×10^4 to 7.4×10^4 Da (PW2), and 5.3×10^3 to 2.3×10^4 Da (PW3), and they found that PW3 exhibited the best reducing power, chelating metal ion potential, and scavenging ability against DPPH and ABTS radicals (Zha et al., 2009).

To improve the taste and quality of raw materials for foodstuffs, processing is commonly carried out using a range of methods, including cooking. During such processing, polysaccharides are transformed or degraded; for example, treatment with γ-rays enhances the antioxidant activity as the polysaccharide structure is retained, but fragmentation to lower molecular weight polysaccharides took place (Choi et al., 2009). Treatment with ultrasonic waves also degrades the polysaccharide structure and enhances the antioxidant activity (Zhou, Wang, Ma, & He, 2008). In addition, various chemical treatment methods have been adopted for the processing of foods, including the use of ascorbic acid and hydrogen peroxide to degrade the polysaccharide obtained from *Enteromorpha linza*. (Zhang, Wang, Zhao, & Qi, 2014). However, contrasting results were obtained. More specifically, although Sun et al., (2009) reported that high molecular weight polysaccharides exhibited no significant antioxidant activities, and low molecular weight polysaccharides displayed strong antioxidant activities (Sun, Wang, Shi, & Ma, 2009). Cheng et al., (2013) found that higher molecular weight polysaccharides from *Epimedium acuminatum* exhibited higher antioxidant activities than the lower molecular weight polysaccharides (Cheng et al., 2013). In contrast, some other studies have suggested that the effect of molecular weight was not significant based on the similar antioxidant levels of polysaccharides and oligosaccharides (Kardosova & Machov, 2006).

4.3 Antioxidant Activities of Functionalized Polysaccharides and Polysaccharides Bearing Different Functional Groups

The structural characteristics of a polysaccharide play a significant role in determining its potential antioxidant activity. Although polysaccharides bearing only an oxygen heteroatom do not exhibit significant antioxidant properties derivatization through sulfation, carboxylation, phosphorylation, benzoylation, acetylation, or oxidation produces promising antioxidants. For example, sulfated polysaccharides are major derivatives that exhibit significant antioxidant activities and protect against lipid peroxidation. Yang et al., (2011) confirmed that the sulfated polysaccharides from *Corallina officinalis* displayed a higher antioxidant potential than the desulafated species (Yang, Liu, Wu, & Chen, 2011). A similar observation was reported by Hu et al., (2010) for the polysaccharides extracted from *Undaria pinnatifida*, while Wang et al., ((2009) reported that native sulfated polysaccharides isolated from *Laminaria japonica* showed a significant antioxidant activity (Hu, Liu, Chen, Wu, 2010; Wang et al., 2009). Indeed, it has been reported that the antioxidant capacity of a sulfated polysaccharide depends on the degree of substitution on the polysaccharide backbone, since the degree of substitution has a critical impact on polymer conformational and structural changes. In addition, Qi et al., (2015) isolated four different polysaccharides with varied molecular weight and reported that low molecular weight polysaccharides

had a higher reducing power (Qi et al., 2005). Furthermore, Zhang et al., (2011) reported a strong antioxidant activity for the sulfated polysaccharide isolated from fresh persimmon (*Diospyros kaki* L.) fruit, while Wu et al., (2013) reported the development of acetylated, phosphorylated, and benzoylated levan-type exopolysaccharide from *Paenibacillus polymyxa* EJS-3. Moreover, Wei et al., (2012) modified phosphorylated polysaccharides from *Radix hedysari,* while Qi et al., (2006) acetylated and benzoylated derivatives from the polysaccharides of *Ulva pertusa*. (Qi et al., 2006; Wei, Cheng, Wei, & Zhang, 2012).

Pectic acid, chitosans and alginates are some other form of polysaccharides that exhibit high antioxidant activities. However due to the cationic or anionic nature of these polysaccharides, the presence of sulfated or phosphorylated glucans resulted in different antioxidant potentials (Qi et al., 2005). For example, Chen et al. (2009) reported that agar bearing a sulfate group, and chitosan bearing an amino group exhibited antioxidant activities, while starch bearing a hydroxyl group did not (Chen, Tsai, Huang & Chen, 2009). In a similar study Rao and Muralikrishna observed that glucose and soluble starch, laminarin, did not show any antioxidant activity (Rao & Muralikrishna, 2006). In addition, acidic polysaccharides containing uronic acid displayed a higher antioxidant activity due to the presence of electrophilic keto or aldehyde groups (Chen, Zhang, & Xie, 2004). Interestingly, uronic acid polysaccharides exhibit a different levels of antioxidant properties, decreasing in the order polygalacturonic acid > glucuronic acid > galacturonic acid. This difference was attributed to the nature of the carbonyl group (i.e., chain or ring). Furthermore, Li et al. (2011) reported that polysaccharides isolated from *Zizyphus Jujuba* cv. *Jinsixiaozao* exhibited different antioxidant activities due to the presence of varying levels of uronic acid with polysaccharide fractions containing higher uronic acid contents giving stronger free radical scavenging activities than fractions containing no uronic acid (Li, Liu, Fan, Ai, & Shan, 2011).

Fucoidan is a natural sulfated polysaccharide found in algae and it has been shown to have number of health benefits. Wang et al. (2009) extracted fucoidan from *Laminaria japonica* and derivatized it to its sulfated, phosphorylated, benzoylated and aminated forms, all of which exhibited strong antioxidant activities (Wang et al., 2009). In addition, Feng et al., (2010) modified the lentinan polysaccharide found in mushrooms to give its sulfated derivative, which again exhibited a significant antioxidant activity (Feng, Li, Wu, He, & Ma, 2010). Compared to the native polysaccharide extracted from *Portulaca oleracea* L. Chen et al., (2014) reported that phosphorylated polysaccharides produced higher antioxidant activities (Chen et al., 2014). In addition, Wang et al., (2014) phosphorylated the galactomannan polysaccharide and found that a higher degree of phosphorylation correlated positively with the antioxidant activity (Wang et al., 2014). Furthermore, Jung et al., (2011) reported higher antioxidant activity upon increasing the degree of sulfation, while Wang et al., (2013) found that a higher degree of sulfation in the polysaccharide isolated from the green algae *Enteromorpha linza* gave a higher DPPH scavenging activity (Jung, Bae, Lee, & Lee, 2011; Wang et al., 2013). In contrast, Xie et al., (2015) found that sulfated polysaccharides with the highest degree of substitution did not produce the highest antioxidant activities.

Based on these results for functionalized polysaccharides it was hypothesized that substitution on a polysaccharide chain weakens the dissociation energy of the hydrogen bonds, and so the hydrogen donating ability of the polysaccharide derivatives is greater than that of the unmodified species. Alternatively, abstraction from the anomeric carbon may be promoted. However, chemical modification is often accompanied by a decrease in molecular weight, thereby improving the antioxidant potentials of the polysaccharides. In terms of sulfated polysaccharides, free radicals are often trapped in an electrostatic manner since the sulfate groups tend to generate a highly acidic environment, while substitution by sulfur may also weaken the hydrogen bonding between polysaccharides (Wang et al., 2016). Furthermore, the types of substitution and the degree of substitution appeared to influence the physicochemical properties (e.g. molecular weight, polarity, solubility, and charge density) and conformation of native polysaccharides. The degree of substitution may also affect the activity through interruption of the inter and intramolecular hydrogen bonds (Wang et al., 2016). It is also possible that saccharides (carbohydrates) can become antioxidants in alkaline media at pH values >9. In such a medium, an amperometric sensor can directly detect saccharides without the requirement for derivatization, thereby selecting only the target antioxidants (Wang, Zhang, Yao, Zhao, & Qi, 2013). Moreover, in an alkaline medium, dissociation occurs through the removal of hydrogen from the hydroxyl groups of saccharides. This does not take place in other media (acid and/or neutral), and so in such cases, the amperometric sensor does not detect the presence of any saccharides.

Antioxidant activities of polysaccharide have been associated with diverse forms of polysaccharide including polysaccharide conjugate, polysaccharide complex, functionalized polysaccharide and degraded products of polysaccharide (Figure 2). In addition to the significant antioxidant properties of polysaccharides, these macromolecular systems also exhibit anticancer and antiviral properties, immune regulation and other biological activities. More specifically, the anti-cancer properties of polysaccharides have been reported to occur through

either the induction of cellular apoptosis and cell cycle arrest, or by inhibiting tumor invasion, adhesion and metastasis. In addition, synergistic effects have been reported in combination treatment with conventional anti-cancer drugs (Chen & Huang, 2018; Lovegrove et al., 2017). Polysaccharides are known to play a significant role in human health, since the rate of digestion, adsorption, and metabolism of polysaccharides is regulated in the stomach, small intestine, and large intestine thereby controlling colonic health and vascular function. Dietary polysaccharides therefore have the potential to regulate the intestinal microenvironmentand stimulate macrophages or lymphocytes in the gut tissues to prevent cancers (Huang, Nie, & Xie, 2017; Laurienzo, 2010). Dietary fiber and resistant forms of starch therefore play a positive role in reducing risk factors for chronic diseases, including cardiovascular disease and certain types of cancers.

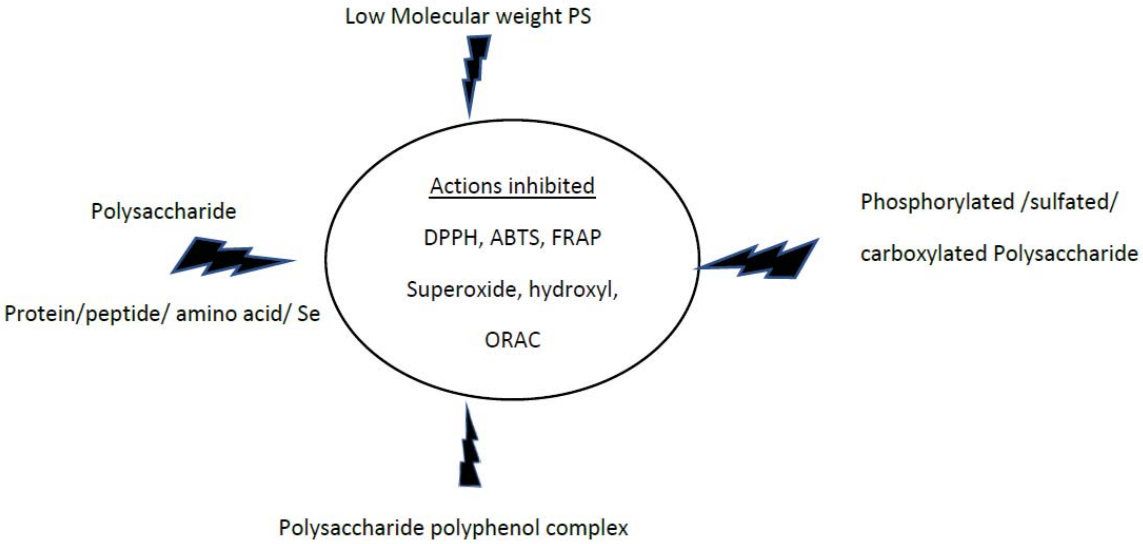


Figure 2. Polysaccharide species for antioxidant activities

5. Conclusion

Natural antioxidants exhibiting strong antioxidant activities are commonly employed to protect against oxidative damage, yet little is known regarding the antioxidant effects of polysaccharides. Although polysaccharide-rich foodstuffs assigned a high glycemic index (i.e. rapidly digested to give glucose) suspected to have links with obesity, diabetes, and high blood pressure, recent findings into the physiological effects of polysaccharides, polysaccharide conjugates, and polysaccharide complexes with biorelevant molecules results in slower glucose release and provides physiological health benefits. Therefore, modified polysaccharide and slowly digested polysaccharides are the most desirable types of polysaccharides from a nutritional standpoint. We expect that recent findings into polysaccharides exhibiting antioxidant properties may lead to a better understanding of the potential of natural polysaccharides to act as functional antioxidants, due to their high antioxidant activities. This area is therefore in need of further exploration in future studies.

References

Alzorqi, I., Sudheer, S., Lu, T. J., & Manickam, S. (2016). Ultrasonically extracted β-D-glucan from artificially cultivated mushroom, characteristic properties and antioxidant activity. *Ultrasonics Sonochemistry, 35*, 531-540. https://doi.org/10.1016/j.ultsonch.2016.04.017

Bermúdez-Oria, A., Rodríguez-Gutiérrez, G., Fernández-Prior, Á., Vioque, B., & Fernández-Bolaños, J. (2019). Strawberry dietary fiber functionalized with phenolic antioxidants from olives. Interactions between polysaccharides and phenolic compounds. *Food Chemistry, 280*, 310-320. https://doi.org/10.1016/j.foodchem.2018.12.057

Cazón, P., Velazquez, G., Ramírez, J. A., & Vázquez, M. (2017). Polysaccharide-based films and coatings for food packaging: A review. *Food Hydrocolloids, 68*, 136-148.

Chen, L., & Huang, G. (2018). Antitumor activity of polysaccharides- An overview. *Current Drug Targets*, *19*, 89-96. https://doi.org/10.2174/1389450118666170704143018

Chen, X., Zhong, H., Zeng, J., & Ge, J. (2008). The pharmacological effect of polysaccharides from *Lentinus*

edodes on the oxidative status and expression of VCAM-1mRNA of thoracic aorta endothelial cell in high-fat-diet rats. *Carbohydrate Polymers, 74*(3), 445-450.

Chen, J., & Huang, G. (2019). Antioxidant activities of garlic polysaccharide and its phosphorylated derivative. *International Journal of Biological Macromolecules, 125*, 432-435. https://doi.org/10.1016/j.ijbiomac.2018.12.073

Chen, L., & Huang, G. (2019). Antioxidant activities of sulfated pumpkin polysaccharides. *International Journal of Biological Macromolecules, 126*, 743-746. https://doi.org/10.1016/j.ijbiomac.2018.06.148

Chen, F., & Huang, G. (2018). Extraction and antioxidant activities of cushaw polysaccharide. *International Journal of Biological Macromolecules*, 1646-1649. https://doi.org/10.1016/j.ijbiomac.2018.09.200

Chen, Y., Zhang, H., Wang, Y., Nie, S., Li, C., & Xie, M. (2014). Acetylation and carboxymethylation of the polysaccharide from Ganoderma atrum and their antioxidant and immunomodulating activities. *Food Chemistry, 156*, 279-288. https://doi.org/10.1016/j.foodchem.2014.01.111

Chen, H. X., Zhang, M., & Xie, B. J. (2004). Quantification of uronic acids in tea polysaccharide conjugates and their antioxidant properties. *Journal of Agricultural and Food Chemistry, 52*, 3333-3336. https://doi.org/10.1021/jf0349679

Chen, F., & Huang, G. (2019). Antioxidant activity of polysaccharides from different sources of ginseng. *International Journal of Biological Macromolecules, 125*, 906-908. https://doi.org/10.1016/j.ijbiomac.2018.12.134

Chen, R., Jin, C., Tong, Z., Lu, J., Tan, L., Tian, L., & Chang, Q. (2016). Optimization extraction, characterization and antioxidant activities of pectic polysaccharide from tangerine peels. *Carbohydrate Polymers, 136*, 187-197. https://doi.org/10.1016/j.carbpol.2015.09.036

Chen, S. K., Tsai, M. L., Huang, J. R., & Chen, R. H. (2009). *In vitro* antioxidant activities of low-molecular-weight polysaccharides with various functional groups. *Journal of Agricultural and Food Chemistry, 57*, 2699-2704. https://doi.org/10.1021/jf804010w

Cheng, H., Feng, S., Shen, S., Zhang, L., Yang, R., Zhou, Y., & Ding, C. (2013). Extraction, antioxidant and antimicrobial activities of *Epimedium acuminatum* Franch. Polysaccharide. *Carbohydrate Polymers, 96*, 101-108. https://doi.org/10.1016/j.carbpol.2013.03.072

Cheng, J. H., Tsai, C. L., Lien, Y. Y., Lee, M. S., & Sheu, S. C. (2016). High molecular weight of polysaccharides from *Hericium erinaceus* against amyloid β-induced neurotoxicity. BMC Complementary and *Alternative Medicine*, *16*, 170. https://doi.org/10.1186/s12906-016-1154-5

Choi, J. I., Kim, H. J., Kim, J. H., Byun, M. W., Soo, C. B., Hyun, A. D., Hwang, Y., J., Kim, D. J., & Lee, J., W. (2009). Application of gamma irradiation for the enhanced physiological properties of polysaccharides from seaweeds. *Applied Radiation and Isotopes, 67*, 1277-1281. https://doi.org/10.1016/j.apradiso.2009.02.027

Copeland, L., Blazek, J., Salman, H., & Tang, M. C. (2009). Form and functionality of starch. *Foods Hydrocolloids, 23*(6), 1527-1534. https://doi.org/10.1016/j.foodhyd.2008.09.016

Cui, S. W. (2005). Food Carbohydrates: chemistry, physical properties, and applications, CRC Press.

Ennaifer, M., Bouzaiene, T., Chouaibi, M., & Hamdi, M. (2018). *Pelargonium graveolens* aqueous decoction: a new water-soluble polysaccharide and antioxidant-rich extract. *Biomedical Research International, 12*, 2691513. https://doi.org/10.1155/2018/2691513

Feng, K., Chen, W., Sun, L., Liu, J., Zhao, Y., Li, L., Wang, Y., & Zhang, W. (2015). Optimization extraction, preliminary characterization and antioxidant activity in vitro of polysaccharides from *Stachys sieboldii Miq*. tubers. *Carbohydrate Polymers, 125*, 45-52. https://doi.org/10.1016/j.carbpol.2015.02.026

Feng, Y., Li, W., Wu, X., He, L, & Ma, S. (2010). Rapid and efficient microwave-assisted sulfate modification of lentinan and its antioxidant and antiproliferative activities in vitro. *Carbohydrate Polymers, 82*, 605-612. https://doi.org/10.1016/j.carbpol.2010.05.025

Guo, S., Mao, W., Han, Y., Zhang, X., Yang, C., Chen, Y., Chen, Y., Xu, J., Li, H., Qi, X., & Xu, J. (2010). Structural characteristics and antioxidant activities of the extracellular polysaccharides produced by marine bacterium *Edwardsiella tarda. Bioresource Technology, 101*(12), 4729-4732. https://doi.org/10.1016/j.biortech.2010.01.125

Guo, Y., Pan, D., Li, H., Sun, Y., Zeng, X., & Yan, B. (2013). Antioxidant and immunomodulatory activity of

selenium exopolysaccharide produced by Lactococcus lactis subsp. Lactis. *Food Chemistry, 138*, 84-89. https://doi.org/10.1016/j.foodchem.2012.10.029

Han, X., Shen, S., Liu, T., Du, X., Cao, X., Feng, H., & Zeng, X. (2015). Characterization and antioxidant activities of the polysaccharides from Radix Cyathulae officinalis Kuan. *International Journal of Biological. Macromolecules, 72*, 544-552. https://doi.org/10.1016/j.ijbiomac.2014.09.007

Han, Q. H., Liu, W., Li, H. Y., He, J. L., Guo, H., Lin, S., Zhao, L., Chen, H., Liu, Y. W., Wu, D. T., Li, S. Q., & Qin, W. (2019). Extraction optimization, physicochemical characteristics, and antioxidant activities of polysaccharides from Kiwifruit (*Actinidia chinensis* Planch.). *Molecules,* 24(3), 461. https://doi.org/10.3390/molecules24030461

Hajji, M., Hamdi, M., Sellimi, S., Ksouda, G., Laouer, H., Li, S., & Nasri, M. (2019). Structural characterization, antioxidant and antibacterial activities of a novel polysaccharide from Periploca laevigata root barks. *Carbohydrate Polymers, 206*, 380-388. https://doi.org/10.1016/j.carbpol.2018.11.020

Huang, Q. L., Siu, K. C., Wang, W. Q., Cheung, Y. C., & Wu, J. Y. (2013). Fractionation, characterization and antioxidant activity of exopolysaccharides from fermentation broth of a *Cordyceps sinensis* fungus. *Process Biochemistry, 48*(2), 380-386. https://doi.org/10.1016/j.procbio.2013.01.001

Huang, L. F., Liang, Y. Z., Guo, F. Q., Zhou, Z. F., & Cheng, B. M. (2003). Simultaneous separation and determination of active components in *Cordyceps sinensis* and *Cordyceps militaris* by LC/ESI-MS. *Journal of Pharmaceutical and Biomedical Analysis, 33*, 1155-1162. https://doi.org/10.1016/s0731-7085(03)00415-1

Huang, X., Nie, S., & Xie, M. (2017). Interaction between gut immunity and polysaccharides. *Critical Reviews in Food Science and Nutrition, 57*, 2943-2955. https://doi.org/10.1080/10408398.2015.1079165

Hu, J. F., Gen, M. Y., Zhang, J. T., & Jiang, H. D. (2001). An *in vitro* study of the structure-activity relationships of sulfated polysaccharide from brown algae to its antioxidant effect. *Journal of Asian Natural Product Research, 3*(4), 353-358. https://doi.org/10.1080/10286020108040376

Hui, Y., Jun-Li, H., & Chuang, W. (2019). Anti-oxidation and antiaging activity of polysaccharide from *Malus micromalus Makino* fruit wine. *International Journal of Biological. Macromolecules, 121*, 1203-1212. https://doi.org/10.1016/j.ijbiomac.2018.10.096

Hu, C., Li, J., Yang, D., Pan, Y., & Wan, H. (2010). A neuroprotective polysaccharide from *Hyriopsis cumingii. Journal of Natural Products, 73*(9), 1489-1493. https://doi.org/10.1021/np1001847

Hu, Y., Sheng, Y., Yu, M., Li, K., Ren, G., Xu, X., & Qu, J. (2016). Antioxidant activity of *Inonotus obliquus* polysaccharide and its amelioration for chronic pancreatitis in mice. *International Journal of Biological. Macromolecules, 87*, 348-56. https://doi.org/10.1016/j.ijbiomac.2016.03.006

Hu, T., Liu, D., Chen, Y., Wu, J., & Wang, S. (2010). Antioxidant activity of sulfated polysaccharide fractions extracted from *Undaria pinnitafida in vitro*. *International Journal of Biological Macromolecules, 46*, 193-198. https://doi.org/10.1016/j.ijbiomac.2009.12.004

Jiang, Y. Y., Yu, J., Li, Y. B., Wang, L., Hu, L., Zhang, L., & Zhou, Y. H. (2019). Extraction and antioxidant activities of polysaccharides from roots of *Arctium lappa L. International Journal of Biological Macromolecules, 123*, 531-538. https://doi.org/10.1016/j.ijbiomac.2018.11.087

Jiang, C., Li, X., Jiao, Y., Jiang, D., Zhang, L., Fan, B., & Zhang, Q. (2014). Optimization for ultrasound-assisted extraction of polysaccharides with antioxidant activity in vitro from the aerial root of *Ficus microcarpa*. *Carbohydrate Polymers, 110*, 10-17. https://doi.org/10.1016/j.carbpol.2014.03.027

Jung, H. Y., Bae, I., Lee, Y. S., & Lee, H. G. (2011). Effect of the degree of sulfation on the physicochemical and biological properties of *Pleurotus eryngii* polysaccharides. *Food Hydrocolloids, 25*, 1291-1295. https://doi.org/10.1016/j.foodhyd.2010.12.002

Kardoˇsov´a, A., & Machov´a, E. (2006). Antioxidant activity of medicinal plant polysaccharides. *Fitoterapia,* 77, 367-373. https://doi.org/10.1016/j.fitote.2006.05.001

Kodali, V. P., & Sen, R. (2008). Antioxidant and free radical scavenging activities of an exopolysaccharide from a probiotic bacterium. *Biotechnology Journal, 3*(2), 245-251.

Kong, F., Mingwei, Z., Sentai, L., Shujuan, Y., Jianwei, C., & Zhencheng, W. (2010). Antioxidant activity of polysaccharide-enriched fractions extracted from pulp tissue of *Litchi Chinensis Sonn*. *Molecules, 15*(4), 2152-2165. https://doi.org/10.3390/molecules15042152

Laurienzo, P. (2010). Marine Polysaccharides in Pharmaceutical Applications: An Overview. *Marine Drugs, 8*(9), 2435-2465. https://doi.org/10.3390/md8092435

Lima, A. T., Santos, M. N., de Souza, L. A., Pinheiro, T. S., Paiva, A. A., Dore, C. M., … Araujo, R. M. (2016). Chemical characteristics of a heteropolysaccharide from Tylopilus ballouii mushroom and its antioxidant and anti-inflammatory activities. *Carbohydrate Polymers, 144*, 400-409. https://doi.org/10.1016/j.carbpol.2016.02.050

Liu, F., Ooi, V. E. C., & Chang, S. T. (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sciences, 60*, 763-771. https://doi.org/10.1016/s0024-3205(97)00004-0

Liu, W., Wang, H., Pang, X., Yao, W., & Gao, X. (2010). Characterization and antioxidant activity of two low-molecular-weight polysaccharides purified from the fruiting bodies of *Ganoderma lucidum*, *International. Journal of Biological Macromolecules, 46*, 451-457. https://doi.org/10.1016/j.ijbiomac.2010.02.006

Li, J., Liu, Y., Fan, L., Ai, L., & Shan, L. (2011). Antioxidant activities of polysaccharides from the fruiting bodies of *Zizyphus Jujuba* cv. *Jinsixiaozao*. *Carbohydrate Polymers*, *84*, 390-394. https://doi.org/10.1016/j.carbpol.2010.11.051

Lovegrove, A., Edwards, C. H., De Noni, I., Patel, H., El, S. N., Grassby, T., … Shewry, P. R. (2017). Role of polysaccharides in food, digestion, and health*, Critical Reviews in Food Science and Nutrition, 57*, 237-253. https://doi.org/10.1080/10408398.2014.939263

Maity, G. N., Maity, P., Dasgupta, A., Acharya, K., Dalai, S., & Mondal, S. (2019). Structural and antioxidant studies of a new arabinoxylan from green stem Andrographis paniculata (Kalmegh). *Carbohydrate Polymers, 212*, 297-303. https://doi.org/10.1016/j.carbpol.2019.02.051

Maity, P., Sen, I. K., Maji, P. K., Paloi, S., Devi, K. S. P., Acharya, K., Maiti, T. K., & Islam, S. S. (2015). Structural, immunological, and antioxidant studies of β-glucan from edible mushroom *Entoloma lividoalbum*. *Carbohydrate Polymers, 123*, 350-358. https://doi.org/10.1016/j.carbpol.2015.01.051

Mao, G., Zou, Y., Feng, & W., Wang, W., Zhao, T., Ye, C., Zhu, Y., Wu, X., Yang, L., & Wu, X. (2014). Extraction, preliminary characterization and antioxidant activity of Se-enriched Maitake polysaccharide. *Carbohydrate Polymers, 101*, 213-219. https://doi.org/10.1016/j.carbpol.2013.09.034

Mu, H., Zhang, A., Zhang, W., Cui, G., Wang, S., & Duan, J. (2012). Antioxidative properties of crude polysaccharides from Inonotus obliquus. *International Journal of Molecular Sciences, 13*, 9194-9206. https://doi.org/10.3390/ijms13079194

Ngo, D. N., Kim, M. M., & Kim, S. K. (2008). Chitin oligosaccharides inhibit oxidative stress in live cells. *Carbohydrate Polymers, 74*(2), 228-234. https://doi.org/10.1016/j.carbpol.2008.02.005

Pan, S., & Wu, S. (2014). Cellulase-assisted extraction and antioxidant activity of the polysaccharides from garlic. *Carbohydrate Polymers, 111*, 606-609. https://doi.org/10.1016/j.carbpol.2014.05.022

Qiao, D., Ke, C., Hu, B., Luo, J., Ye, H., Sun, Y., Yan, X., & Zeng, X. (2009). Antioxidant activities of polysaccharides from *Hyriopsis cumingii. Carbohydrate Polymers, 78*(2), 199-204. https://doi.org/10.1016/j.carbpol.2009.03.018

Qi, H., Zhang, Q., Zhao, T., Hu, R., Zhang, K., & Li, Z. (2006). In vitro antioxidant activity of acetylated and benzoylated derivatives of polysaccharide extracted from Ulva pertusa (Chlorophyta). *Bioorganic and Medicinal Chemistry Letters, 16*, 2441-2445. https://doi.org/10.1016/j.bmcl.2006.01.076

Qi, H., Zhao, T., Zhang, Q., Li, Z., Zhao, Z., & Xing, R. (2005). Antioxidant activity of different molecular weight sulfated polysaccharides from *Ulva pertusa Kjellm* (Chlorophyta). *Journal of Applied Physics, 17,* 527-534. https://doi.org/10.1007/s10811-005-9003-9

Raza, W., Makeen, K., Wang, Y., Xu, Y., & Qirong, S. (2011). Optimization, purification, characterization and antioxidant activity of an extracellular polysaccharide produced by *Paenibacillus polymyxa* SQR-21. *Bioresource Technology, 102*(10), 6095-6103. https://doi.org/10.1016/j.biortech.2011.02.033

Rao, R. S. P., & Muralikrishna, G. (2006). Water soluble feruloyl arabinoxylans from rice and ragi: changes upon malting and their consequence on antioxidant activity. *Phytochemistry*, *67*, 91-99. https://doi.org/10.1016/j.phytochem.2005.09.036

Ren, Q., Chen, J., Ding, Y., Cheng, J., Yang, S., Ding, Z., Dai, Q., & Ding, Z. (2019). In vitro antioxidant and immunostimulating activities of polysaccharides from Ginkgo biloba leaves. *International Journal of*

Biological Macromolecules, 124, 972-980. https://doi.org/10.1016/j.ijbiomac.2018.11.276

Rozi, P., Abuduwaili, A., Mutailifu, P., Gao, Y., Rakhmanberdieva, R., AkberAisa, H., & Yili, A. (2019). Sequential extraction, characterization and antioxidant activity of polysaccharides from *Fritillaria pallidiflora* Schrenk, *Intenational Journal of Biological Macromolecules, 131*, 97-106. https://doi.org/10.1016/j.ijbiomac.2019.03.029

Shu, Y., Li, J., Yang, X., Dong, X., & Wang, X. (2019). Effect of particle size on the bioaccessibility of polyphenols and polysaccharides in green tea powder and its antioxidant activity after simulated human digestion. *Journal of Food Science and Technology, 56*(3), 1127-1133. https://doi.org/10.1007/s13197-019-03573-4

Siu, K. C., Xu, L., Chen, X., & Wu, J. Y. (2016). Molecular properties and antioxidant activities of polysaccharides isolated from alkaline extract of wild *Armillaria ostoyae*mushrooms. *Carbohydrate Polymers, 137*, 739-746. https://doi.org/10.1016/j.carbpol.2015.05.061

Sokolova, E. V., Barabanova, A. O., Homenko, V. A., Solov'eva, T. F., Bogdanovich, R. N., & Yermak, I. M. (2011). *In vitro* and *ex vivo* studies of antioxidant activity of carrageenans, sulfated polysaccharides from red algae. *Bulletin of Experimental Biology and Medicine, 150*(4), 426-428. https://doi.org/10.1007/s10517-011-1159-5

Sun, Y., Wang, H., Guo, G., Pu, Y., & Yan, B. (2014). The isolation and antioxidant activity of polysaccharides from the marine microalgae Isochrysis galbana. *Carbohydrate Polymers*, *113*, 22-31. https://doi.org/10.1016/j.carbpol.2014.06.058

Sun, L., Wang, C., Shi, Q., & Ma, C. (2009). Preparation of different molecular weight polysaccharides from *Porphyridium cruentum* and their antioxidant activities. *International Journal of Biological Macromolecules, 45*, 42-47. https://doi.org/10.1016/j.ijbiomac.2009.03.013

Tan, L. H., Zhang, D., Yu, B., Zhao, S. P., Wang, J. W., Yao, L., & Cao, W. G. (2015). Antioxidant activity and optimization of extraction of polysaccharide from the roots of Dipsacus asperoides. *International Journal of Biological Macromolecules, 81*(10), 332-339. https://doi.org/10.1016/j.ijbiomac.2015.08.022

Tseng, Y. H., Yang, J. H., & Mau, J. L. (2008). Antioxidant properties of polysaccharides from *Ganoderma tsugae*. *Food Chemistry, 1072*, 732-738. https://doi.org/10.3923/ijp.2011.58.65

Wang, J., Yang, T., Tian, J., Zeng, T., Wang, X., Yao, T., Zhang, J., & Lei, Z. (2014). Synthesis and characterization of phosphorylated galactomannan: the effect of DS on solution conformation and antioxidant activities. *Carbohydrate Polymers, 113*, 325-335. https://doi.org/10.1016/j.carbpol.2014.07.028

Wang, J., Wang, F., Zhang, Q., Zhang, Z., Shi, X., & Li, P. (2009). Synthesized different derivatives of low molecular fucoidan extracted from Laminaria japonica and their potential antioxidant activity in vitro. *International Journal of Biological Macromolecules, 44*, 379-384. https://doi.org/10.1016/j.ijbiomac.2009.02.001

Wang, J., Hu, S., Nie, S., Yu, Q., & Xie, M. (2016). Reviews on mechanisms of in vitro antioxidant activity of polysaccharides Oxidative *Medicine and Cellular Longetivity,* 1-13. https://doi.org/10.1002/biot.200700208

Wang, H., Liu, Y. M., Qi, Z. M., Wang, S. Y., Liu, S. X., Li, X., Wang, H. J., & Xia, X. C. (2013). An overview of natural polysaccharides with antioxidant properties. *Current Medicinal Chemistry, 20*, 2899-2913

Wang, N., Zhang, Y., Wang, X., Huang, X., Fei, Y., Yu, Y., & Shou, D. (2016). Antioxidant property of water-soluble polysaccharides from Poria cocos Wolf using different extraction methods. *International Journal of Biological Macromolecules, 83*, 103-110. https://doi.org/10.1016/j.ijbiomac.2015.11.032

Wang, X., Zhang, Z., Wu, Y., Sun, X., & Xu, N. (2019). Synthesized sulfated and acetylated derivatives of polysaccharide extracted from *Gracilariopsis lemaneiformis* and their potential antioxidant and immunological activity. *International Journal Biological Macromolecules, 124*, 568-572. https://doi.org/10.1016/j.ijbiomac.2018.11.244

Wang, X., Zhang, Z., Yao, Z., Zhao, M., & Qi, H. (2013). Sulfation, anticoagulant and antioxidant activities of polysaccharide from green algae Enteromorpha linza. *International Journal of Biological Macromolecules, 58*, 225-230. https://doi.org/10.1016/j.ijbiomac.2013.04.005

Wang, Z., Zhao, Y., Su, T., Zhang, J., & Wang, F. (2015). Characterization and antioxidant activity in vitro and in vivo of polysaccharide purified from *Rana chensinensis* skin. *Carbohydrate Polymers, 126*, 17-22. https://doi.org/10.1016/j.carbpol.2015.03.031

Wang, Z., Zhao, Y., & Su, T. (2015). Extraction and antioxidant activity of polysaccharides from *Rana chensinensis* skin. *Carbohydrate Polymers, 115*, 25-31. https://doi.org/10.1016/j.carbpol.2014.08.082

Wang, Y., Yang, Z., & Wei, X. (2012). Antioxidant activities potential of tea polysaccharide fractions obtained by ultrafiltration. *International Journal of Biological Macromolecules, 50*, 558-564, 69. https://doi.org/10.1016/j.ijbiomac.2011.12.028

Wang, J., Zhao, B., Wang, X., Yao, J., & Zhang, J. (2012). Synthesis of selenium-containing polysaccharides and evaluation of antioxidant activity *in vitro*. *International Journal of Biological Macromolecules, 51*(5), 987-991. https://doi.org/10.1016/j.ijbiomac.2012.08.011

Wei, E., Yang, R., Zhao, H., Wang, P., Zhao, S., Zhai, W., Zhang, Y., & Zhou, H. (2019). Microwave-assisted extraction releases the antioxidant polysaccharides from seabuckthorn (*Hippophae rhamnoides L.*) berries. *International Journal of Biological Macromolecules, 123*, 280-290. https://doi.org/10.1016/j.ijbiomac.2018.11.074

Wei, D., Cheng, W., Wei, Y., & Zhang, L. (2012). Phosphorylated modification and *in vitro* antioxidant activity of *Radix hedysari* polysaccharide. *Glycoconjugate Journal, 29*(4), 167-172. https://doi.org/10.3390/molecules22030501

Wu, Y., & Wang, D. (2009). A new class of natural glycopeptides with sugar moiety-dependent antioxidant activities derived from *Ganoderma lucidum* fruiting bodies. *Journal of Proteome Research, 8*(2), 436-442. https://doi.org/10.1021/pr800554w

Wu, H., Zhu, J., Diao, W., & Wang, C. (2014). Ultrasound-assisted enzymatic extraction and antioxidant activity of polysaccharides from pumpkin (*Cucurbita moschata*). *Carbohydrate Polymers, 113*, 314-324. https://doi.org/10.1016/j.carbpol.2014.07.025

Wu H., T. Min, X., Li, X. F., Li, L., Lai, F., Tang, Y. Q., & Yang, X. H. (2013). Physicochemical properties and antioxidant activities of acidic polysaccharides from wampeeseeds. *International Journal of Biological Macromolecules, 59*, 90-95. https://doi.org/10.1016/j.ijbiomac.2013.04.020

Wu, Z., Zhang, M., Xie, M., Dai, Z., Wang, X., Hu, B., Ye, H., & Zeng, X. (2016). Extraction, characterization and antioxidant activity of mycelial polysaccharides from *Paecilomyces hepiali* HN1. *Carbohydrate Polymers, 137*, 541-548. https://doi.org/10.1016/j.carbpol.2015.11.010

Wu, Z., Ming, J., Gao, R., Wang, Y., Linag, Q., Yu, H., & Zhao, G. (2011). Characterization and antioxidant activity of the complex of tea polyphenols and oat β-glucan, *Journal of Agricultural and Food Chemistry, 59*(19), 10737-10746. https://doi.org/10.1021/jf202722w

Xie, J., Wang, Z., Shen, M., Nie, S. P., Gong, B., Li, H. S., Zhou, Q., Li, W. J., & Xie, M. Y. (2015). Sulfated modification, characterization and antioxidant activities of polysaccharide from Cyclocarya paliurus. *Food Hydrocolloids.* https://doi.org/10.1016/j.foodhyd.2015.02.018

Xing, R., Liu, S., Guo, Z, Yu, H., Wang, P., Li, C., Li, Z., & Li, P. (2005). Relevance of molecular weight of chitosan and its derivatives and their antioxidant activities *in vitro*. *Bioorganic and Medicinal Chemistry, 13*,1573-1577. https://doi.org/10.1016/j.bmc.2004.12.022

Xu, Y., Cai, F., Yu, Z., Zhang, L., Li, X., Yang, Y., & Liu, G. (2016). Optimisation of pressurised water extraction of polysaccharides from blackcurrant and its antioxidant activity. *Food Chemistry, 194*, 650-658. https://doi.org/10.1016/j.foodchem.2015.08.061

Yao, Y., Zhu, Y., & Ren, G. (2016). Antioxidant and immunoregulatory activity of alkali-extractable polysaccharides from mung bean. *International Journal of Biological Macromolecules, 84*, 289-94. https://doi.org/10.1016/j.ijbiomac.2015.12.045

Yang, L., & Zhang, L. (2009). Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources. *Carbohydrate Polymers, 76*(3), 349-361. https://doi.org/10.1016/j.carbpol.2008.12.015

Yu, Y., Shen, M., Song, Q., & Xie, J. (2018). Biological activities and pharmaceutical applications of polysaccharide from natural resources: A review. *Carbohydrate Polymers, 183,* 91-101. https://doi.org/10.1016/j.carbpol.2017.12.009

Yu, F., Sheng, J., Xu, J., An, X., & Hu, Q. (2007). Antioxidant activities of crude tea polyphenols, polysaccharides and proteins of selenium-enriched tea and regular green tea. *European. Food Research and Technology, 225*, 843-848. https://doi.org/10.1007/s00217-006-0490-y

Zeng, H., Zhang, Y., Zhao, Y., Tian, Y., Miao, S., & Zheng, B. (2015). Extraction optimization, structure and antioxidant activities of Fortunella margarita Swingle polysaccharides. *International Journal of Biological Macromolecules, 74*, 232-242. https://doi.org/10.1016/j.ijbiomac.2014.12.013

Zha, X. Q., Wang, J. H., Yang, X. F., Liang, H., Zhao, L. L., Bao, S. H., Luo, J. P., Xu, Y. Y., & Zhou, B. B. (2009). Antioxidant properties of polysaccharide fractions with different molecular mass extracted with hot-water from rice bran. *Carbohydrate Polymers, 78*, 570-575. https://doi.org/10.1016/j.carbpol.2009.05.020

Zhang, C., Zhao, F., Li, R., Wu, Y., Liu, S., & Liang, Q. (2019). Purification, characterization, antioxidant and moisture-preserving activities of polysaccharides from Rosa rugosa petals. *International Journal of Biological Macromolecules, 124*, 938-945. https://doi.org/10.3390/molecules24071354

Zhang, L. S., Wang, X., & Dong, L. L. (2011). Antioxidation and antiglycation of polysaccharides from *Misgurnus anguillicaudatus*. *Food Chemistry, 124*(1), 183-187.

Zhang, Z. S., Wang, F., Wang, X. M., Lie, X. L., Hou, Y, & Zhang, Q. B. (2010). Extraction of the polysaccharides from five algae and their potential antioxidant activity in vitro. *Carbohydrate Polymers, 82*, 118-121. https://doi.org/10.1016/j.carbpol.2010.04.031

Zhang, H., Cai, X. T., Tian, Q. H., Xiao, L. X., Zeng, Z., Cai, X. T., Yan, J. Z., & Li, Q. Y. (2019). Microwave-assisted degradation of polysaccharide from Polygonatum sibiricum and antioxidant activity. *Journal of Food Science, 84*(4), 754-76. https://doi.org/10.1111/1750-3841.14449

Zhang, Z., Wang, X., Li, J., Wang, G., & Mao, G. (2016). Extraction and free radical scavenging activity of polysaccharide from 'Anji Baicha' (Camellia sinensis (L.) O. Kuntze). *International Journal of Biological Macromolecules, 84*,161-165. https://doi.org/10.1016/j.ijbiomac.2015.12.016

Zhang, Z., Wang, X., Zhao, M., & Qi, H. (2014). Free-radical degradation by Fe2+/Vc/H2O2 and antioxidant activity of polysaccharide from Tremella fuciformis. *Carbohydrate Polymers, 112*, 578-582. https://doi.org/10.1016/j.carbpol.2014.06.030

Zhang, J. Q., Li, C., Huang, Q., You, L. J., Chen, C., Fu, X., & Liu, R. H. (2019). Comparative study on the physicochemical properties and bioactivities of polysaccharide fractions extracted from Fructus Mori at different temperatures. *Food and Function, 22*, 10(1), 410-42. https://doi.org/10.1039/C8FO02190B

Zhang, Y., Lu, X., Fu, Z., Wang, Z., & Zhang, J. (2011). Sulphated modification of a polysaccharide obtained from fresh persimmon (Diospyros kaki L.) fruit and antioxidant activities of the sulphated derivatives. *Food Chemistry, 127*, 1084-1090. https://doi.org/10.1016/j.foodchem.2011.01.100

Zhang, Z., Wang, X., Mo, X., & Qi, H. (2013). Degradation and the antioxidant activity of polysaccharide from *Enteromorpha linza*. *Carbohydrate Polymers, 92*(2), 2084-2087. https://doi.org/10.1016/j.carbpol.2012.11.096

Zhao, B., Zhang, J., Yao, J., Song, S., Yin, Z., & Gao, Q. (2013). Selenylation modification can enhance antioxidant activity of *Potentilla anserina* L. polysaccharide. *International Journal of Biological Macromolecules, 58*, 320-328. https://doi.org/10.1016/j.ijbiomac.2013.04.059

Zhao, Y. M., Song, J. H., Wang, J., Yang, J. M., Wang, Z. B., & Liu, Y. H. (2016). Optimization of cellulase-assisted extraction process and antioxidant activities of polysaccharides from Tricholoma mongolicum Imai. *Journal of the Science of Food and Agriculture, 96*, 4484-4491. https://doi.org/10.1002/jsfa.7662

Zhao, L., Zhao, G., Du, M., Zhao, Z., Xia, L., & Hu, X. (2008). Effect of selenium on increasing free radical scavenging activities of polysaccharide extracts from a Se-enriched mushroom species of the genus Ganoderma. *European Food Research and Technology, 226*, 499-505. https://doi.org/10.1007/s00217-007-0562-7

Zheng, Q., Ren, D., Yang, N., & Yang, X. (2016). Optimization for ultrasound-assisted extraction of polysaccharides with chemical composition and antioxidant activity from the Artemisia sphaerocephala Krasch seeds. *International Journal of Biological Macromolecules, 91*, 856-866. https://doi.org/10.1016/j.ijbiomac.2016.06.042

Zheng, C., Dong, Q., Chen, H., Cong, Q., & Ding, K. (2015). Structural characterization of a polysaccharide from Chrysanthemum morifolium flowers and its antioxidant activity. *Carbohydrate Polymers, 130*, 113-121. https://doi.org/10.1016/j.carbpol.2015.05.004

Zhou, C., Wang, Y., Ma, H., & He, R. (2008). Effect of ultrasonic degradation on *in vitro* antioxidant activity of polysaccharides from *Porphyra yezoensis* (Rhodophyta). *Food Science and Technology International, 14*, 479-486. https://doi.org/10.1177/1082013208100665

Zhu, J., & Wu, M. (2009). Characterization and free radical scavenging activity of rapeseed meal polysaccharides WPS-1 and APS-2. *Journal of Agricultural and Food Chemistry, 57*, 812-819. https://doi.org/10.1021/jf802687t

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Evaluation of Phenolic Content and Free Radical Scavenging Activity of Indonesia Wild Honey Collected from Seven Different Regions

Y. Riswahyuli[1, 3], Abdul Rohman[2], Francis M. C. S. Setyabudi[1] & Sri Raharjo[1]

[1]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora 1, Bulaksumur, Sleman, Yogyakarta, Indonesia

[2]Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

[3]National Agency of Drug and Food Control (BPOM RI), Jakarta, Indonesia

Correspondence: Sri Raharjo, Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora 1, Bulaksumur, Sleman, Yogyakarta, Indonesia. Tel: 62-274-589-797.

Received: September 12, 2019 Accepted: October 20, 2019 Online Published: October 29, 2019

doi:10.5539/jfr.v8n6p94 URL: https://doi.org/10.5539/jfr.v8n6p94

Abstract

Several wild honey samples collected from seven different regions in Indonesia were investigated to determine their total phenolic content, flavonoid content, and free radical scavenging activity by analyzing the 1,1-diphenyl-2-picrylhydrazyl (DDPH) radical and phenolic profile. Rutin, (+)-catechin, ferulic acid, and galangin were found to be the major phenolic compounds of Indonesia wild honey. The total phenolic content significantly correlated with the total flavonoid content (p=0.000) and the percentage of DPPH radical scavenging activity (p=0.000). Results indicated that there are different polyphenol profiles among the different regions.

Keywords: wild honey, antioxidant, polyphenolic, flavonoid

1. Introduction

Honey is a natural food produced by honey bees using flower nectar or tree and plant exudates. Recently, honey has become extremely popular because of its potential beneficial effects for human health. It has been used as a common traditional drug since ancient times for treating burns, gastrointestinal problems, asthma, infection and wounds, skin ulcers, and cataracts and other eye diseases (Osés, et al., 2016; Ferreira, et al., 2009). The beneficial effects of honey can be partly attributed to its antibacterial and antioxidant activities. Studies have shown that honey exhibits activity against oxidation reactions in food such as enzymatic browning, lipid oxidation, and food deterioration(Ardehali, et al., 2017; Ferreira, et al., 2009; Antony, et al., 2000; Chen, et al., 2000).

The antioxidant activity of honey is due to a combination of compounds such as phenolic acids, flavonoids, ascorbic acid, carotenoids, enzymes, amino acids, and products derived from the Maillard reaction (Gül & Pehlivan, 2018; Moniruzzaman, et al. 2014, Khalil, et al. 2011, and Gheldof, et al. 2002).Recently, there has been a significant increase in research on the characterization of natural polyphenols especially their identification and quantification (Pyrzynska, & Biesaga, 2009). This research direction has been largely oriented toward identifying a promising marker compound (Kus, et al., 2014; Kaškoniene, & Venskutonis, 2010), and such studies have reported more than 150 polyphenol compounds from honey bee products (Ferreira, et al., 2009).

The concentration and the class of antioxidants are highly dependent on the floral source and the total capacity of the antioxidants, which comprises a combination of activity of compounds found in honey (Gheldof, et al., 2002). Earlier studies have demonstrated that the capacity of antioxidants in honey obtained from various flower sources significantly correlated with its phenolic and flavonoid contents and the origin of the flower (Alvarez-Suarez, et al., 2009; Baltrušaityte, et al., 2007; Gheldof, et al., 2002; Anklam, 1998). One study showed that the important components, including phenolic derivatives, present inplant nectar are transferred to honey (Idris, et al., 2011). The primary factor responsible for the antioxidant activity of honey is the substituent group present in its phenolic components, e.g., hydroxyl, methyl, acyl, or glycosyl groups (Gašic et al., 2014).

Regarding the flavonoids present in honey, previous studies have reported that pinobanksin, pinocembrin, quercetin, chrysin, galangin, luteolin, and kaempferol are the major ones, whereas luteolin, quercetin, 8-methoxykaempferol, is or hamnetin, kaempferol, and galangin are present in small concentrations in Manuka honey (Alvarez-Suarez, et al., 2014; Chan, et al., 2013; Kaškonienand Veskutonis, 2010). This characterization of polyphenols was useful for differentiating the source of honey and could be used as a botanical marker (Spilioti, et al., 2014; Alvarez-Suarez, et al 2014; Anklam, 1998; Tomas-Barberan, et al., 1993). Caffeic acid and *p*-coumaric acid found in chestnut honey and protocatechuic acid found in conifer tree honey have been used as flower markers in some previous studies (Haroun, et al., 2012; Tomas-Barberan, 2001).

Today, several methods are used to explore the antioxidant activity of honey, such as determination of active oxygen species, radical scavenging activity (RSA) (Alvarez-Suarez, et al., 2009; Gheldof, et al., 2005; Meda, et al., 2005; Gheldof, et al., 2002; Nagai, T, et al., 2001),measurement of the inhibition of lipid peroxidation by enzymatic or nonenzymatic reactions (Nagai, et al., 2001; Chen, et al., 2000), and ferric reducing antioxidant power assay (Bertoncelj, et al., 2007; Aljadi & Kamarudin,2004).

Chromatographic fingerprinting is an efficient and widely used method for determining the content of polyphenols or antioxidant compounds (Zhao, et al., 2016; Sun et al., 2014; Kuś, et al., 2014). Solid-phase extraction techniques have been successfully applied,followed by the identification of the compounds by capillary electrophoresis (CE), gas chromatography, or high-performance liquid chromatography (HPLC).The use ofdiode array detector (DAD) and mass spectrometry (MS) demonstrated greater effectiveness, especially withHPLC in cases of flavonoids and with GC in cases of phenolic acids (Alvarez-Suarez, et al., 2009). Several researchers have demonstrated that HPLC with DAD and GC-MS are useful for the characterization of honey from plant sources (Kus, et al., 2014; Soria, et al., 2009).Till date, only a few studies have reported about the determination of total phenolic content (TPC), antioxidant activity, and the phenolic profile of honey in Indonesia (Chayati, 2008; Kartika & Bertoni, 2014; Kustiawan 2014). Therefore, we conducted this study to determine the TPC, the total flavonoid content (TFC), the (RSA) (DPPH), and the phenolic profiling by HPLC – DAD using wild honey collected from seven different regions or islands in Indonesia.

2. Material and Method

2.1 Honey Samples

Indonesia wild honey (IWH) samples were collected between 2016 and 2018 from seven different regions in seven different islands in Indonesia. The regions wereas follows: Bangka Belitung, Sulawesi, Kalimantan, Sumatra, West Nusa Tenggara, East Nusa Tenggara, and Java (Figure 1). Their authenticity and freshness wereassured by collecting them directly from the forest areas, local beekeepers under the Indonesia Wild Honey Association, and/or from well-traced suppliers. The samples were collected in sealed glasscontainers and kept in dark conditions.

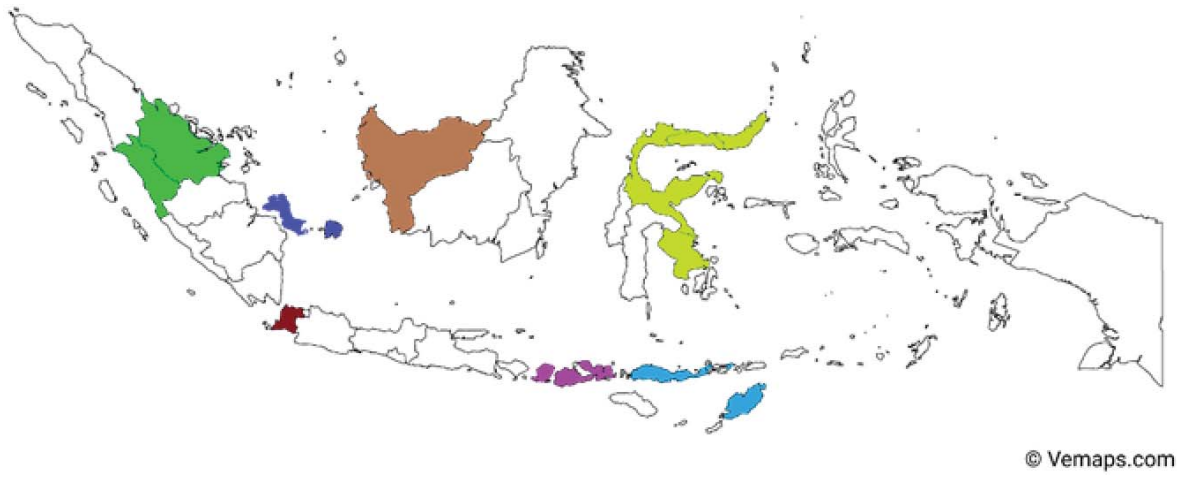


Figure 1. Sampling area for Wild Honey (= Sumatera island (Riau& Jambi) =Bangka & Belitung Island = Java (Banten province) = Kalimantan Island (West Kalimantan Province = Sulawesi Island (South Sulawesi, Central Sulawesi, Gorontalo & North Sulawesi) = West Nusa Tenggara (Sumbawa Province) =East Nusa Tenggara (Kupang, Timor Tengah Selatan , Flores Timur &Sikka)

2.2 Analysis of TPC

TPC was determined using the Folin–Ciocalteu method at a wavelength of 750 nm with gallic acid as the standard (Ferreira, et al 2009; Socha, et al., 2007; Prior, et al., 2005; Singleton, et al., 1999; Singleton, et al., 1965). About 0.5 mL of honey solution (100 mg/mL) that was previously homogenized was added to 0.3 mL of 10% Folin–Ciocalteu reagent. Then, 2 mL of 15% sodium carbonate solution (Na_2CO_3) was added after 6 min and the volume was made up to 5 mL using distilled water. This solution was incubated for 20 min in the dark before measuring using a spectrophotometer. A calibration curve was plotted at a predetermined concentration of 0–300 mg/L, and the results were expressed in milligrams of gallic acid equivalent (mg/kg GAE).

2.3 Analysis of TFC

The TFC was measured using the method developed by Zhishen et al. (1999). Briefly, 2 mL of honey solution in water (1 g/4 mL) was mixed with 0.3mL of 5% $NaNO_2$ (w/v). After 5 min, 0.3 mL of 10% w/v $AlCl_3$ was added to the solution, followed by the addition of 2mLofNaOH and then 10 mL of distilled water after 6 min. The absorbance of the solution was read in a Thermo Scientific Genesys 10 S UV-Vis spectrophotometerat 425 nm. A calibration curve was plotted with quercetinas the standard at a concentration range of 0–8 mg/L. The results were expressed as milligrams of quercetine quivalent (QEQ) per 100 g of honey.

2.4 Radical Scavenging Activity

The antioxidant activity of all honey samples was measured using the scavenging activity for the radical DPPH as developed by Meda et al (2005). The homogenized sample was weighed and dissolved in methanol (150 mg/mL). The honey solution (0.075 mL) was mixed with the DPPH reagent solution (0.025 mg/mL) and left for 15 min at room temperature in the dark condition. The absorbance of the mixture was measured at 517 nm against methanol blank. The RSAwas expressed as % inhibition according to the following equation:

$$\%\ \text{inhibition} = \frac{\text{Blank absorbance} - \text{Control absorbance)}}{\text{Blank absorbance}} \times 100\%$$

2.5 Analysis of Phenolic Compound Profile

2.5.1 Sample Extraction

The homogenized sample (0.5 g) was dissolved in 10 mL of acidified deionized water (pH 3.5) and inserted into the SPE column Bond Elut octadecyl C18 (500 mg) from Agilent Technology (Santa Clara, CA, USA)whichwas previously conditioned using 4mL of methanol and 2mL of deionized water. Subsequently, the column was washed with 6 mL of deionized water (pH 3.5) and the desirable fraction was eluted by passing 2 mL of absolute methanol. Before injecting into the HPLC system, the sample extracts were filtered through a 0.45-µm membrane filter (Millipore) (Gašić et al., 2014; Beretta et al., 2005).

2.5.2 Preparation of Polyphenolic Standards

All standards were prepared using methanol to make 500 mg/L of standard stock solution. Working standards were prepared by diluting the stock solution with methanol to serial concentrations of 0.10, 0.25, 0.50, 0.75, and 1.00 mg/L. A calibration curve was drawn by plotting the analyte peak areas against the serial concentration of the working standard solutions. The calibration curve was considered to have good linearity if the R^2 values were >0.99. The standards of ferulic acid, *p*-coumaric acid, vanillic acid, gallic acid, quercetin, tricine,4-hydroxybenzoic acid, (+)-catechin, syringaldehyde, rutin trihydrate, chrysin, galangin, epigallocatechin, and 3,4-dihydroxy benzaldehydewere purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Methanol was of HPLC grade purchased from Merck. All solutions were filtered and degassed before use.

2.5.3 HPLC Analysis

The honey samples were analyzed using a Shimadzu liquid chromatographic system equipped with binary pumps LC20-A, degasser DGU-20A5, autosampler SIL-20AC, photodiode array detector set to a wavelength of 280 nm, and a Shimadzu Shim pack GIST column (5µm, 4.6 × 15 mm.). The mobile phase consisted of a combination of water–acetic acid (phase A) and methanol–acetic acid (phase B). The system was run with a gradient elution with the following conditions: time 0.02 min, 18.3% phase B; time 10 min, 100% phase B; time 13.1 min, 18.3% phase B. The flow rate was set to1mL/min with an injection volume of 20 µL.

2.6 Statistical Analysis

Statistical analyses were conducted using the computer programs SPSS 24.0 Statistics software (SPSS Inc., 2016), Minitab® 18.1 (State College, Pennsylvania, USA) and Microsoft Excel 2016 (Microsoft Corp.). Data were presented as mean ± S.D. The Pearson correlation was calculated to determine the relationship between the

phenolic acid content and antioxidant properties. The level of significance was set at $p<0.01$.

3. Results and Discussion

3.1 TPC and TFC

The TPC (mg/kg of honey) exhibited significant variations among the various samples of IWH (Table 1), which might be due to the differences in the botanical and regional origin (Tahir, et al 2017; Mamary et al., 2002). The TPC of the IWH samples varied from 188.03(mg/kg GAE) in Kalimantan to 467.84 (mg/kg GAE) inEast Nusa Tenggara (Table 1) with standard linearity ($R^2 = 0.9990$). This value was still higher than previously reported, namely, lime honey (83.7mg/kg), Slovenia honey (44.8 mg/kg), and honey from Burkina Faso (74.38mg/kg) (Moniruzzaman, et al., 2014;Bertoncelj, et al., 2007;Meda, et al 2005).

In this study, the TFC was evaluated using an aluminum chloride reagent, which was specific to only flavones and flavonols, so that the test results would provide a lower estimation of the number of flavonoids because they ignored the flavanone compounds (Meda, et al., 2015). The TFC of the IWH samples ranged from 0.81 mg QEQ/100 gin honey from Kalimantan to 3.09 mg QEQ/100 g in honey from Sumatra (Table 1), made by calibration curve of quercetin with $R^2 = 0.9999$. These results were closely similar to the values of other honey samples in the world such as those from Bangladesh(2.57 mg QEQ/100 g) (Moniruzzaman, et al., 2011), Burkina Faso(2.57 ± 2.09 mg QEQ/100 g), Europe (0.5–2 mg QEQ/100 g) (Amiot et al., 1989; Martos et al., 2000), Czech (1.9–15.74 mg QEQ/100g) (Vit, et al., 2008),andFrance (<1 mg QEQ/100 g).The most significant positive linear correlation was observed between the phenolic and flavonoid parameters ($r = 0.533$, p = 0,000, Table 2). It has been reported that honey samples with a higher polyphenol content produce high levels of flavonoids (Moniruzzaman, 2014, Khalil, 2012).

Table 1. Mean and standard deviation of antioxidant parameters

Antioxidant Parameters		Origin of Indonesia Wild Honey						
		Bangka Belitung	*Sulawesi*	*Kalimantan*	*Sumatra*	*West Nusa Tenggara*	*East Nusa Tenggara*	*Java*
TPC*	mean	254.92±	423.17±	188.03±	422.9528±	467.84±	343.15±	250.96±
	±SD	83.26^{ac}	55.45^{b}	6.74^{ca}	64.95^{d}	45.66^{e}	84.18^{f}	66,46^{g}
TFC**	Mean±SD	1.09±0.38^{a}	2.57±1.35^{bd}	0.81±0.21^{c}	3.09±0.79^{db}	2,22±0.57^{e}	1.46±0.29^{fg}	1,13±0.64^{gf}
% Inhibition	Mean±SD	28.23±6,68^{a}	80.74±19.00^{bd}	49.24±5.15^{ceg}	69.85±9.76^{db}	51.24±10.61^{ecg}	68.65±12.23^{f}	50.42±4.6^{gec}
Number of samples		7	12	10	41	29	30	20

*Total Phenolic Content (mg of gallic acid equivalent (GAE) per kg)

**Total Flavonoid Content (mg quercetin(QEQ)/100 g)

3.2 Radical Scavenging Activity

The results of RSA are summarized in Table 1. The percentage inhibitory activity of IWH ranged from 28.23%to 80.74%. The highest mean value was found in honey collected from Sulawesi, whereas the lowest was observed in honey collected from Bangka Belitung. These results were higher to those of Portuguese honey collected from several vegetation sources such as rosemary (4.5%–59.3%), orange (8.8%–23.2%), thyme (35.8%–47.3%), and eucalyptus (27.7%) (Alves, et al., 2013). Other studies have reported that Lithuania honey has an RSA value of 43.0%–95.7% (Baltrušaityte, et al., 2005),dark honey samples have a DPPH inhibitory value of69.2%, and light honey samples have an inhibitory value of 37% (Estevinho et al.,2008). Similarly, rhododendron honey samples showed inhibitory values between 2.30% and 90.73% (Silici et al., 2010).

A positive correlation was observed among the RSA, TPC, and TFC of the honey samples, with a Pearson coefficient<0.001. The TPC showed a weak correlation with the antioxidant activity, whereas the TFC exhibited a moderate correlation with RSA (Table 2).These results were similar to those of previous studies that reported that the phenolic acid and flavonoid contents and the antioxidant potential of honey were significantly correlated, which was influenced by the origin of the sample (Jaromír, et al 2010; Beretta, et al., 2005). This correlation indicates that the phenolic compounds have an effect on the antioxidant potential.

Table 2. Correlation of Total Phenolic, Total Flavonoid, and % Inhibition

		Total Phenolic	Total Flavonoid	% Inhibition
Total Phenolic	Pearson Correlation	1	.533**	.336**
	Sig. (2-tailed)		.000	.000
	N	145	145	138
Total Flavonoid	Pearson Correlation	.533**	1	.504**
	Sig. (2-tailed)	.000		.000
	N	145	149	142
% Inhibition	Pearson Correlation	.336**	.504**	1
	Sig. (2-tailed)	.000	.000	
	N	138	142	142

**. Correlation is significant at the 0.01 level (2-tailed).

3.3 Profile of Phenolic Compounds

In general, the analysis procedure for determining a single phenolic compound needs the sample preparation step intended for extraction from the honey matrix, followed by separation, identification, and quantification steps. The extraction step generally involves SPE or solvent extraction using various solvents. Separation is generally done by HPLC or CE because of the presence of electroactive phenolic hydroxyl groups with simple oxidation potential. Some authors had conducted SPE procedures to extract phenolic components from honey (Aljadi & Kamarudin, 2004). Quantitation was done on 13 phenolic compounds, but only 10 of them were able to produce linear equations with R^2=0.99 (Table 3). Other flavonoids such as syringaldehyde and rutin, although they were detected, could not produce linear equations that met the requirements for quantification.

Results of the phenolic profile are represented in Table 4 and Figure 1. Gallic acid, (+)-catechin, quercetin, epigallocatechin, pinocembrin, 4-hydroxybenzoic acid, chrysin, galangin, vanillic acid, and ferulic acid were detected in all types of honey samples collected from various regions, whereas3,4-dihydroxy benzaldehyde was detected only in the honey samples collected from Sumatra, East Nusa Tenggara, and Java. Syringaldehyde was detected only in Sumatra and Java honey samples, andrutin was found only in Sumatra and East Nusa Tenggara honey samples. An earlier study reported that pinocembrin, pinobanksin, chrysin, and luteolin represented approximately61% of the flavonoid content of Manuka honey (Chan, et al., 2013). In this study, the levels of each phenolic compound in each region varied significantly, which may be due to variation in the types of vegetation or multiflora. The dominant phenolic compound found in honey collected from Bangka Belitung was galangin (75.43–103.88 mg/kg), which was also detected in honey collected from Sulawesi but at a lower level (14.68–26.52 mg/kg). Epigallocatechin was the most dominant phenolic compound in honey collected from Kalimantan (3.027–34.617 mg/kg), whereas catechin was the dominant phenolic compound found in honey collected from Java. Ferulic acid was the most dominant phenolic compound in honey collected from West Nusa Tenggara (51.64–286.43 mg/kg), whereas the honey samples collected from East Nusa Tenggara and Sumatra showeda lower concentration of this compound.Polyphenolic content in several honey were than their TPC and TFC. This condition allows the exploration of other dominant phenolic compounds,which could not be identified in these experiments. It has been reported that the variation in the composition of phenolic compounds depends on the floral source transfer to the nectar, climate and other environmental factors, bee variety, and the processing technologies, handling, and storage (Kaskonienė and Venskutonis, 2010; Bertoncelj, et al., 2007). This variation in the profile of phenolic compounds was considered to be responsible for their diverse response in protecting against oxidative reactions. This compound could also be used as an indicator in studies analysing the flower and geographic origins of honey (Idris, Mariod, & Hamad, 2011; Alvarez-Suarez, et al., 2009).

Table 3. HPLC data for polyphenol standards at λ280nm

Polyphenols	T_R (min)	Regression Equation	R^2
Gallic acid	2.74	Y= 45617.3*x-10073.4	0.99
(+)-catechin	5.82	Y= 10181.8*x-2078.88	0.99
3,4-dihydroxy benzaldehyde	6.06	Y=55287.5*x-10670.5	0.99
Quercetin	6.41	Y= 29263.2*x-2762.52	0.99
Epigallocatechin	6.74	Y= 26279.1*x-2731.40	0.99
Pinocembrin	7.06	Y=34401.9*x-6223.43	0.99
4-hydroxybenzoic acid	7.46	Y= 31344.3*x-6814.47	0.99
Chrysin	7.78	Y= 132703*x-5297.36	0.99
Galangin	8.02	Y=2500.68*x+2802.56	0.99
Vanillic acid	9.03	Y= 21305.2*x-8083.04	0.99
Ferulic acid	9.93	Y=1138.20*x-239.447	0.99

Table 4. Quantification data of phenolic compounds in Indonesia wild honey

Polyphenols (μg/mL)	Bangka Belitung	Sulawesi	Kalimantan	Sumatra	West Nusa Tenggara	East Nusa Tenggara	Java
Gallic acid	0.25–4.92	0.25–0.78	0.29–1.57	0.41–4.0	0.36–6.09	0.27–4.43	0.27–5.67
(+)-catechin	42.59–69.52	1.69–17.09	0.84–7.14	4.71–29.00	6.28–22.86	0.60–16.40	1.02–27.77
3,4 dihydroxy benzaldehyde	ND	ND	ND	0.19–9.61	Not detected	0.20–0.19	0.21–0.81
Quercetin	6.57–17.10	0.14–23.86	0.78–6.58	0.31–4.76	1.10–7.11	0.41–2.22	0.09–2.02
Epigallocatechin	6.64–65.81	1.25–48.09	3.03–34.62	0.24–5.83	4.69–16.64	0.23–8.62	0.098–3.99
Pinocembrin	2.10–9.36	0.24–4.86	1.15–6.16	1.51–15.20	1.46–22.39	0.48–3.70	0.34–2.42
4-hydroxybenzoic acid	1.56–3.66	0.27–0.57	5.65–8.82	0.25–9.66	1.18–4.75	0.30–4.09	0.25–15.79
Chrysin	8.56–8.75	0.16–2.72	0.37–2.63	1.35–12.19	0.23–2.29	0.57–3.73	0.32–3.57
Galangin	75.43–103.88	14.68–26.53	0.492–23.22	0.64–20.38	0.88–15.54	1.13–51.27	0.45–44.67
Vanillic acid	9.05–33.52	0.44–0.45	0.52–4.89	0.47–1.86	2.02–13.50	0.57–9.77	0.44–18.56
Syringaldehyde	ND	ND	ND	Detected	ND	ND	Detected
Ferulic acid	1.58–2.47	1.59–43.39	0.78–34.77	10.22–41.71	51.64–286.43	1.01–108.73	2.82–11.45
Rutin	ND	ND	ND	Detected	ND	Detected	ND

ND: Not Detected

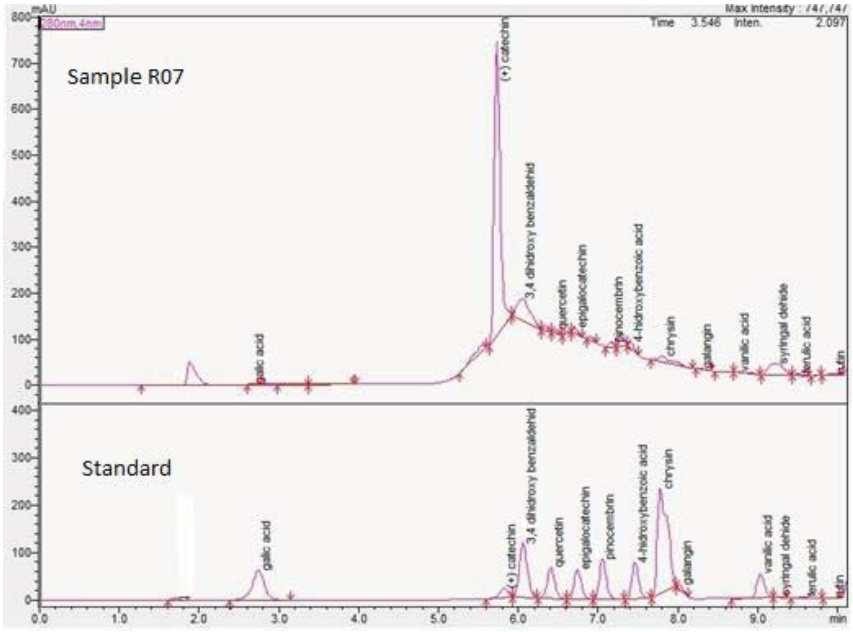


Figure 2.HPLC Chromatogram for Sample from Sumatra region and polyphenolic standard

4. Conclusion

This study demonstrated that a correlation exists among the TPC, TFC, and RSA values of the IWH samples. The phenolic profile varied significantly at both intra- or inter-region levels, which could be due to the variation in the planttype as a source of nectar. The dominant phenolic compounds in the IWH samples were (+)-catechin, ferulic acid, and galangin, whereas some compounds were found only in honey collected from certain regions; for example, 3, 4-dihydroxy benzaldehyde was detected only in honey collected from Sumatra, East Nusa Tenggara, and Java; syringaldehyde was found in honey collected from Sumatra and Java; and rutin was detected in honey samples collected from Sumatra and East Nusa Tenggara.

Acknowledgements

The authors would like to thank National Agency of Drug and Food Control (NADFC) Republic of Indonesia/ Badan Pengawas Obat dan Makanan (BPOM) for their financial support. Hermanto of Indonesia Wild Honey Network (IWHN)/Jaringan Madu Indonesia (JMHI), Budi Aulia and Afri Yondra of WWF Indonesia, Riau Program, Bahrizon of Riau beekeepers, Eman Sulaeman of Koperasi Hanjuang, Banten, Novemris Tefa and Asbanu Nicodemus of Gunung Mutis National Park, Anis Hayong of Rumadu, Flores, Jalisman of Waslit, North Luwu, Tri Astuti Rahmawati from BPOM provincial office in Bangka Belitung, and Kristiorini Dwi Nursanti for supplying honey samples.

References

Aljadi, A. M., & Kamaruddin, M. Y. (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chem., 85*, 513-518. https://doi.org/10.1016/S0308-8146(02)00596-4

Alvarez-Suarez, J., Gasparrini, M., Forbes-Hernández, T., Mazzoni, L., & Giampieri, F. (2014). The Composition and Biological Activity of Honey: A Focus on Manuka Honey. *Foods*, *3*(3), 420-432. https://doi.org/10.3390/foods3030420

Alvarez-Suarez, J. M., Tulipani, S., Romandini, S., Vidal, A., & Battino, M. (2009). Methodological aspects about determination of phenolic compounds and in vitro evaluation of antioxidant capacity in the honey: A review. *Current Analytical Chemistry*, *5*(4), 293-302. https://doi.org/10.2174/157341109789077768

Alves, A., Ramos, A., Gonçalves, M. M., Bernardo, M., & Mendes, B. (2013). Antioxidant activity, quality parameters and mineral content of Portuguese monofloral honeys. *Journal of Food Composition and Analysis*, *30*(2), 130-138. https://doi.org/10.1016/j.jfca.2013.02.009

Amoit, M. J., Aubert S., Gonnet, M., & Tacchini, M. (1989). The phenolic compounds in honeys: preliminary study upon identification and family quantification. *Apidologie, 20*, 115-125. https://doi.org/10.1051/apido:19890202

Anklam, E. (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry, 63*(4), 549-562. https://doi.org/10.1016/S0308-8146(98)00057-0

Anthony, S. M., Rieck, J. R., &Dawson, P. L. (2000). Effect of dry honey on oxidation in turkey breast meat. *Poult. Sci., 79*, 1846-1850. https://doi.org/10.1093/ps/79.12.1846

Ardehali, P., Asadi, F., Javan, A., Jahantigh, M., & Aminzare, M. (2017). Application of honey to reduce oxidation in soybean oil. *Journal of Food Quality and Hazards Control*, *4*(3), 79-84.

Baltrušaityte, V., Venskutonis, P. R., & Čeksteryte, V. (2007). Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chemistry*, *101*(2), 502-514. https://doi.org/10.1016/j.foodchem.2006.02.007

Beretta, G., Granata, P., Ferrero, M., Orioli, M., & Maffei Facino, R. (2005). Standardization of antioxidant properties of honey by combination of spectrophotometric / fluorimetric assays and chemometrics of spectrophotometric / fluorimetric assays and chemometrics. *Analytica Chimica Acta*, *533*, 185-191. https://doi.org/10.1016/j.aca.2004.11.010

Bertoncelj, J., Doberšek, U., Jamnik, M., & Golob, T. (2007). Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chemistry*, *105*(2), 822-828. https://doi.org/10.1016/j.foodchem.2007.01.060

Chan, C. W., Deadman, B. J., Manley-Harris, M., Wilkins, A. L., Alber, D. G., & Harry, E. (2013). Analysis of the flavonoid component of bioactive New Zealand mānuka (Leptospermum scoparium) honey and the isolation, characterisation and synthesis of an unusual pyrrole. *Food Chemistry*, *141*(3), 1772-1781. https://doi.org/10.1016/j.foodchem.2013.04.092

Chayati, I. (2008). Sifat fisikokimia madu monoflora dari daerah istimewa yogyakarta dan jawa tengah. *Agritech*, *28*(1), 9-14.

Chen, L., Mehta, A., Berenbaum, M., Zangerl, A. R., & Engeseth, N. J. (2000). Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. *J. Agric. Food Chem., 48*, 4997-5000. https://doi.org/10.1021/jf000373j

Ciappini, M. C., & Stoppani, F. S. (2014). Determination of antioxidant capacity, flavonoids, and total phenolic content in eucalyptus and clover honeys. *Journal of Apicultural Science*, *58*(1), 103-111. https://doi.org/10.2478/jas-2014-0010

Escriche, I., Kadar, M., Juan-Borrás, M., & Domenech, E. (2014). Suitability of antioxidant capacity, flavonoids and phenolic acids for floral authentication of honey. Impact of industrial thermal treatment. *Food Chemistry*, *142*, 135-143. https://doi.org/10.1016/j.foodchem.2013.07.033

Estevinho, L., Pereira, A. P., Moreira, L., Dias, L. G., & Pereira, E. (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicology*, *46*(12), 3774-3779. https://doi.org/10.1016/j.fct.2008.09.062

Ferreres, F., Tomas-Barberan, F. A., Gil, M. I., & Tomas, F. (1991). HPLC technique for flavonoid analysis in honey. *J. Sci. Food Agric., 56*, 49-56. https://doi.org/10.1002/jsfa.2740560106

Gašic´, U., Dabic, D., Kec, S., Trifkovic, J., Milojkovic, D., Tešic, Z., & Natic, M. (2014). Phenolic profile and antioxidant activity of Serbian polyfloral honeys. *Food Chemistry*, *145*, 599-607. https://doi.org/10.1016/j.foodchem.2013.08.088

Gheldof, N., & Engeseth, N. J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry*, *50*(10), 3050-3055. https://doi.org/10.1021/jf0114637

Gheldof, N., Xiao-Hong, W., & Engeseth, N. (2002). Identification and quantification of antioxidant components of honeys from various floral sources. *J. Agric. Food Chem., 50*, 5870-5877. https://doi.org/10.1021/jf0256135

Gül, A., & Pehlivan, T. (2018). Antioxidant activities of some monofloral honey types produced across Turkey. *Saudi Journal of Biological Sciences, 25*, 1056-1065. https://doi.org/10.1016/j.sjbs.2018.02.011

Haroun, M. I., Poyrazoglu, E. S., Konar, N., & Artik, N. (2012). Phenolic acids and flavonoids profiles ofsome Turkish honeydew and floral honeys. *J Food Technol, 10*, 39-45. https://doi.org/10.3923/jftech.2012.39.45

Kartika, S. R., & Bertoni, R. (2014). Kajian Manfaat Madu Hutan Anggota JMHI terhadap Penyakit Kanker dan Anti Aging. *Jaringan Madu Hutan Indonesia Scientific Report*.

Kaškonien, V., & Venskutonis, P. R., (2010). Floral Markers in Honey of Various Botanical and Geographic Origins : A Review. *Comprehensive Reviews in Food Science and Food Safety*, *9*. https://doi.org/10.1111/j.1541-4337.2010.00130.x

Khalil, M. I. N., Alam, M., Moniruzzaman, S. A., & Gan, S. H. (2011). Phenolic acid composition and antioxidant properties of Malaysian honeys. *Journal of Food Science, 76*(6), C921-C928, 2011. https://doi.org/10.1111/j.1750-3841.2011.02282.x

Kustiawan, P. M., Puthong, S., Arung, E. T., & Chanchao, C. (2014). In vitro cytotoxicity of Indonesian stingless bee products against human cancer cell lines. *Asian Pac. J. Trop. Biomed., 4*, 549-556. https://doi.org/10.12980/APJTB.4.2014APJTB-2013-0039

Kuś, P. M., Jerković, I., Tuberoso, C. I. G., Marijanović, Z., & Congiu, F. (2014). Cornflower (Centaurea cyanus L.) honey quality parameters: Chromatographic fingerprints, chemical biomarkers, antioxidant capacity and others. *Food Chemistry*, *142*, 12-18. https://doi.org/10.1016/j.foodchem.2013.07.050

Mamary, M. A. Al-Meeri, & Al-Habori, M. (2002). Antioxidant activities and total phenolics of different types of honey. *Nutrition Research, 22*(9), 1041-1047. https://doi.org/10.1016/S0271-5317(02)00406-2

Michalkiewicz, A., Biesaga, M., & Pyrzynska, K. (2008). Solid-phase extraction procedure for determination of phenolic acids and some flavonols in honey. *Journal of Chromatography A*, *1187*(1-2), 18-24. https://doi.org/10.1016/j.chroma.2008.02.001

Martos, I., Ferreres, F., Yao, L., D-Arcy, B., Caffin, N., & Tomas-Barberan, F. A. (2000). Flavonoids in

monospecific Eucalyptus honeys from Australia. *J. Agric. Food Chem., 48*, 4744-4748. https://doi.org/10.1021/jf000277i

McKibben, J., & Engeseth, N. J. (2002) Honey as a protective agent against lipid oxidation in ground turkey. *J. Agric. Food Chem., 50*, 592-595. https://doi.org/10.1021/jf010820a

Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*, *91*(3), 571-577. https://doi.org/10.1016/j.foodchem.2004.10.006

Milojkovic, D., & Kec, S. (2013). The determination of phenolic profiles of Serbian unifloral honeys using ultra-high-performance liquid chromatography / high resolution accurate mass spectrometry. *Food Chemistry, 138*, 32-40. https://doi.org/10.1016/j.foodchem.2012.10.025

Moniruzzaman, M., An, C. Y., Rao, P. V., Nurul, M., Hawlader, I., Binti, S. A., & Gan, S. H. (2014). Identification of Phenolic Acids and Flavonoids in Monofloral Honey from Bangladesh by High Performance Liquid Chromatography: Determination of Antioxidant Capacity. *BioMed Research International,* Volume *2014, Article ID 737490*. https://doi.org/10.1155/2014/737490

Nagai, T., Sakai, M., Inoue, R., Inoue, H., & Suzuki, N. (2001). Antioxidative activities of some commercially honeys, royal jelly, and propolis. *Food Chemistry, 75*, 237-240. https://doi.org/10.1016/S0308-8146(01)00193-5

Oelschlaegel, S., Gruner, M., Wang, P. N., Boettcher, A., Koelling-Speer, I., & Speer, K. (2012). Classification and characterization of manuka honeys based on phenolic compounds and methylglyoxal. *Journal of Agricultural and Food Chemistry*, *60*(29), 7229-7237. https://doi.org/10.1021/jf300888q

Osés, S. M., Pascual-Maté, A., de la Fuente, D., de Pablo, A., Fernández-Muiño, M. A., & Sancho, M. T. (2016). Comparison of methods to determine antibacterial activity of honeys against Staphylococcus aureus. *NJAS - Wageningen Journal of Life Sciences*, *78*, 29-33.Https://doi.org/10.1016/j.njas.2015.12.005

Silici, S., Sagdic, O., & Ekici, L. (2010). Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys. *Food Chemistry*, *121*(1), 238-243. https://doi.org/10.1016/j.foodchem.2009.11.078

Soria, A. C., Sanz, J., & Martínez-Castro, I. (2009). SPME followed by GC-MS: A powerful technique for qualitative analysis of honey volatiles. *European Food Research and Technology*, *228*(4), 579-590. https://doi.org/10.1007/s00217-008-0966-z

Spilioti, E., Jaakkola, M., Tolonen, T., Lipponen, M., Virtanen, V., Chinou, I., & Moutsatsou, P. (2014). Phenolic acid composition, antiatherogenic and anticancer potential of honeys derived from various regions in Greece. *PLoS ONE*, *9*(4), 1-10. https://doi.org/10.1371/journal.pone.0094860

Sumarlin, L. O., Muawanah, A., & Wardhani, P. (2014). Aktivitas Antikanker dan Antioksidan Madu di Pasaran Lokal Indonesia (Anticancer and Antioxidant Activity of Honey in the Market Local Indonesia). *Jurnal Ilmu Pertanian Indonesi (JIPI)*, *19*(3), 136-144.

Tahir, H. E., Xiaobo, Z., Zhihua, L., Jiyong, S., & Zhai, X. (2017). Rapid prediction of phenolic compounds and antioxidant activity of Sudanese honey using Raman and Fourier transform infrared (FT-IR) spectroscopy. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2017.01.024

Tomas-Barberán, F. A., Martos, I., Ferreres, F., Radovic, B. S., & Anklam, E. (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *J. Sci. Food Agric., 81*, 485-496. https://doi.org/10.1002/jsfa.836

Tomas-Barberán, F. A., Martos, I., Ferreres, F., Radovic, B. S., & Anklam, E. (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *J. Sci Food Agric, 81*. https://doi.org/10.1002/jsfa.836

Tomas-Barberan, F. A., Ferreres, F., Garcia-Viguera, C., & Tomas-Lorente, F (1993). Flavonoids in honey of different geographical origin. *Z. Lebensm. Unters. Forsch. 196*, 38-44. https://doi.org/10.1007/BF01192982

Yaoa, L., Jiang, Y., Singanusong, R., Datta, N., & Raymont, K. (2005). Phenolic acids in Australian Melaleuca, Guioa, Laphostemon, Banksia and Helianthus honeys and their potential for floral authentication. *Food Res. Internat., 38*, 651-658. https://doi.org/10.1016/j.foodres.2005.01.002

Pyrzynska, K., & Biesaga, M. (2009). Analysis of phenolic acids and flavonoids in honey. *TrAC - Trends in Analytical Chemistry*, *28*(7), 893-902. https://doi.org/10.1016/j.trac.2009.03.015

Tomaand́s-Barberaand́n, F. A., Martos, I., Ferreres, F., Radovic, B. S., & Anklam, E. (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture*, *81*(5), 485-496. https://doi.org/10.1002/jsfa.836

Yao, L., Jiang, Y., D'Arcy, B., Singanusong, R., Datta, N., Caffin, N., & Raymont, K. (2004). Quantitative High-Performance Liquid Chromatography Analyses of Flavonoids in Australian Eucalyptus Honeys. *Journal of Agricultural and Food Chemistry*, *52*(2), 210-214. https://doi.org/10.1021/jf034990u

Zhao, J., Du, X., Cheng, N., Chen, L., Xue, X., Zhao, J., Wu, L., & Cao, W. (2016). Identification of monofloral honeys using HPLC-ECD and chemometrics. *Food Chemistry, 194*, 167-174. https://doi.org/10.1016/j.foodchem.2015.08.010

Zhishen, J., Mengcheng, T., & Jianming, W. (1990). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*(4), 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Chia (*Salvia hispanica* L.) – A Potential Crop for Food and Nutrition Security in Africa

Pauline Ikumi[1], Monica Mburu[1] & Daniel Njoroge[1]

[1]Institute of Food Bioresources Technology, Dedan Kimathi University of Technology, Private Bag. Dedan Kimathi, Nyeri, Kenya

Correspondence: Monica Mburu, Institute of Food Bioresources Technology, Dedan Kimathi University of Technology, Private Bag. Dedan Kimathi, Nyeri, Kenya. E-mail: monimbu8@gmail.com

Received: October 1, 2019 Accepted: October 19, 2019 Online Published: October 30, 2019

doi:10.5539/jfr.v8n6p104 URL: https://doi.org/10.5539/jfr.v8n6p104

Abstract

There has been an increasing demand for functional foods with numerous health benefits due to intensified consumer awareness leading to a major shift in the consumption patterns of food. This has largely been attributed to increased lifestyle diseases across different populations. The use of food with nutraceutical and functional properties for management of lifestyle diseases like diabetes, obesity, and cardiovascular problems is now gaining momentum among the public. Consequently, the consumption of chia seeds (*Salvia hispanica* L.) has increased in recent years particularly due to its high content of omega-3 fatty acids and dietary fiber. Chia seeds also contain high quality proteins which offer all the essential amino acids and several vital minerals. In addition, chia seeds are a potential source of antioxidants and polyphenolic compounds such as chlorogenic acid, caffeic acid, myricetin, quercetin, and kaempferol with the major phenolic acid being rosmarinic. Owing to the rich nutritional profile, chia seeds provide numerous health benefits such as; cardiac protective and hepatic protective effects, anti-aging and anti-carcinogenic properties. The high amounts of dietary fibers present in the seeds also confer benefits by preserving good glycemic control thus helps in controlling diabetes mellitus. In addition to the numerous health benefits accrued to consumption of chia seeds, they have great potential in application in the food industry for; development of various baked products, production of biodegradable edible films, use as emulsifiers and stabilizers among other uses. This review provides an in-depth insight into the potential of chia crop in promoting nutrition and food security by providing comprehensive information pertaining to; its origin, cultivation and distribution, physical properties, nutritional characteristics, health benefits, functional properties and its potential use in the food industry. It evident that chia seeds contain superior nutritional components as compared to other seeds and also confers many health benefits such as antioxidative, anti-hypertensive and hypoglycemic properties among others. It possesses physical and functional properties that make it an excellent crop for use in food applications for the development of functional foods. Moreover, its application not only limited to food but can also be used for feed. Chia therefore demonstrates great potential as a crop that can be utilized for improved food and nutrition security.

Keywords: chia, food security, functional, nutritional

1. Introduction

Chia (*Salvia hispanica* L.) is classified as a herbaceous plant that belongs to the order- Lamiales, family -Lamiaceae, subfamily -Nepetoideae, and genus -*Salvia* (Cahill & Provance, 2002; Artcos, 2018). Chia is reported to have originated from the low latitudes of Mexico and Guatemala, along with amaranthus, quinoa and maize and it was one of the four staple foods of Mayas and Aztech populations (Munoz, Cobos, Diaz, & Aguilera, 2013). It is majorly cultivated for its seeds and yields white or purple blossoms. Chia can grow up to 1m tall and has inversely orchestrated leaves, its flowers are little (3-4mm) with little corollas and combined bloom parts that add to a high self-pollination rate. The seed shading and color differs from dark, grey, and dark-spotted to white, and the shape of the chia seed is semi-oval, with a length somewhere in the range of 1 and 2 mm, a diameter somewhere in the range of 0.8 and 1.3 and a width somewhere in the range of 0.8 and 1.4 mm (Munoz, Cobos, Diaz, & Aguilera, 2012; Cahill & Provance, 2002).

Chia is a word that was obtained from a Spanish word meaning oily, thus, it is referred to as an oily seed packing a power house of omega 3 fatty acids. It is also rich in predominant quality protein, high degree of dietary fiber,

minerals, vitamins, and a variety of polyphenolic antioxidants which work to protect the seed from microbial and chemical degradation (Cahill, 2003). The value of chia arises from is nutritional properties and medicinal use. The contents of chia seeds include polyunsaturated fatty acids, dietary fiber, vitamins, calcium, protein-including all essential amino acids and other vital minerals (Munoz *et al.*, 2013; Ullah *et al*., 2016). After a long oblivion in recent years this crop has been rediscovered, nowadays it is cultivated as a seed crop and commercialized as a functional food and feed. Chia seeds are perhaps the most advantageous nourishment on earth, stuffed with supplements and medical advantages for human body and mind. Health benefits include nourishing the digestive system, promoting healthier skin, strong muscles and bones, lowers the risks of cardiovascular diseases, diabetes and aging signs (Ullah *et al*., 2016; Ali *et al*., 2012). Mature chia seeds contain mucilage in their epidermal cells thus, when the seed is placed in water, it raptures the primary cell layer that obtrudes from the epidermal cells developing a coating around the seed which surges in size and forms a gel-like appearance on chia (Muñoz *et al.*, 2012). This gel characteristic being a natural phenomenon of a chia seed has great potential in the development of functional food products such as stabilizers, emulsifiers and thickeners (Coorey, Tjoe & Jayasena, 2014). Moreover, over the years' special attention has been drawn to the use of chia as feed, for instance dairy industries have explored to improve the nutraceutical profile of milk by increasing the content of polyunsaturated fatty acids (PUFA) omega-3 FA (Dewhurst, Fisher & Wilkins, 2006). The lipid profile in milk can be sharply modified by feeding animals with forages rich in omega-3 (Dewhurst, Shingfield, Lee & Scollan, 2003) as well as by supplementing oilseeds or marine oils (Chilliard *et al.*, 2001). Chia, therefore offers massive nutritional and therapeutic potential with a diverse future perspective for food, feed, pharmaceutical and nutraceutical sectors. Owing to its superior nutritional, functional and health benefits. The contents of this paper will provide a detailed analysis of chia as a crop that promotes nutrition and food security by accounting comprehensively on its origin, cultivation and distribution, physical properties, nutritional characteristics, health benefits, functional properties and its potential use in the food industry.

2. Origin, Cultivation and Distribution of Chia

Chia originated at the low latitudes of Mexico and Guatemala. Along with other crops such as amaranthus, quinoa and maize it was one of the four staple foods of Mayas and Aztech populations (Munoz *et al.*, 2013). Surpassed only by maize and beans, the pre-Colombian society practiced chia cultivation as a main crop (Craig, 2004). Around, 3500 BC chia seeds were used as human food, over the years from 1500BC to 900 BC, Central Mexico adopted chia as a staple food BC (Cahill, 2003; Ayerza & Coates, 2005). The seeds were used in the preparation of a variety of food, medicine etc. by the Mayas and Aztecs tribes. Nonetheless, there is evidence that suggests in 1531 there was cultivation of chia in the Southside of Sinaloa (Dubernard, 1991). There was however a decline of chia as a staple food in Mexico (1550-1810) after the Spanish colonization and during this period when its cultivation was prohibited the crop faced extinction. Following this duration where chia was at the verge of extinction, renaissance of chia as food in Mexico occurred between 1810 to 1990 after which it was integrated as a modern food in the world (1990-2010) (Anacleto *et al*., 2016). The incorporation of chia as a modern food was facilitated by the various studies on chia as a vegetal and crop source of PUFAs ω3, proteins and fiber (Weber, Gentry, Kolhepp, & McCrohan, 1991) with chia currently being produced in over fourteen countries around the world (Ayerza & Coates, 2011; Coates & Ayerza, 1996). From a nutritional point of view, while there exist several food sources to cover the requirement of PUFAs ω3 such as menhaden fish, salmon, algae and flax (Sapio, Bueno, Busilacchi & Severin, 2008; Castro, 2002), none of them is safer, more sustainable and traceable than chia due to the fact that it is a unique source that can be directly consumed.

Literature suggests that a daily consumption ranging from 25-50 grams of seed per day is enough to cover the PUFAs ω3 requirements for adults (Souza, Souza, Santo & Rosa, 2015; Vuksan *et al*., 2007). Over the last six years, the worldwide demand for chia has risen linearly and in 2014 Paraguay, Argentina, Mexico and Bolivia were identified as the four major producers of this oilseed (Peperkamp, 2015). Presently, the demand for chia as a food crop is growing with countries such as USA, Chile, Argentina and Italy where for their climatic conditions it has proven difficult to cultivate chia hence they are assessing various agronomic practices to acclimate it to their agricultural sectors (Kaiser & Ernst, 2016; Amato *et al*., 2015; Bochicchio *et al*., 2015; Tello, 2014).

3. Nutritional Properties of Chia

Chia seed commercialization in Europe, United States and Canada has famed recent interests due to its nutritional properties. The European Council and the European Parliament has recently approved the use of chia seeds as a novel food ingredient in amounts not exceeding 10% in breakfast cereals, baked products, nut and seed mixes and fruits (Commission, 2013; Commission, 2009). The recommendation of chia seeds globally has been attributed to their high protein degree, dietary fiber, antioxidants, minerals and vitamins. However, their oil

content having the largest fraction of α-linolenic acid (ω-3) likened to other natural sources known to date is their nutritional breakthrough (Guiotto, Ixtaina, Tomás, & Nolasco, 2013). The other parts of chia plant such as leaves and stems have shown to contain nutritive value especially the leaves which contain PUFA and other essential oils thus a good ruminant nutrition source (Peiretti & Gai, 2009; Ahmed, Ting & Scora, 1994). The seed composition has shown to vary from region to region depending on the conditions of where it grows. Although chia seeds have shown to thrive in tropical and subtropical areas, their cultivation can also be done in trivial climates (Ayerza, 2010). As a botanical source, chia seeds composition is expected to vary as a result of different growing location, years within a growing location due to environmental and genetic factors (Ayerza & Coates, 2004). In spite of this, chia has been reported to be of great nutritional value and auspicious bioactive compounds for human health (Table 1).

Table 1. Nutritional composition of chia seeds from different regions

Component	Brazilian [a] chia seeds	Mexican [b] chia seeds	Indian [c] chia seeds
Dietary fiber (g/100g)	33.37± 0.26	41.41 ± 0.2	18.51 ± 0.04
Lipids (g/100g)	32.16 ± 0.29	35.13 ± 0.04	28.1 ± 0.5
18:2 (g/100g)	5.69 ± 0.42	2.43 ± 0.03	6.4± 0.2
18:3 (g/100g)	20.37 ± 1.38	68.52 ± 0.02	64.0± 2.0
Proteins (g/100g)	18.18 ± 1.20	24.11± 0.43	20.76 ± 0.05
Moisture (g/100g)	7.14 ± 0.26	6.82 ± 0.13	8.95 ± 0.06
Carbohydrates (g/100g)	4.59 ± 0.34	1.51 ± 0.08	16.88 ± 0.63
TPC (mg GAE)	0.97 − 0.99	0.757	nd

[a] Adapted from Silva *et al*. (2017).

[b] Adapted from (Segura-campos, Salazar-vega, Chel-guerrero & Betancur-ancona, 2013) and (Reyes-Caudillo, Tecante & Valdivia-Lo´pez, 2008)

n.d: not determined.

[c] Adapted from Ramzi *et al*. (2016)

TPC: Total phenolic content

GAE: Gallic acid extract

According to a report USDA, (2004) chia seeds contain approximately 34.40% total dietary fiber (TDF), 30.74% total lipids, 42.12% total carbohydrates, 5.80% moisture, high values (335–860 mg/100 g) of calcium, phosphorus and to a smaller extent sodium, iron, and zinc (4.58–16 mg/100 g). To add on that, niacin, vitamin C and A can be found substantially in chia seeds.

3.1 Lipids

Chia seeds comprise about 25–38% oil by weight, which makes them the richest botanical source of omega-3 α-linolenic acid (C18:3, ALA, up to 68%) of any identified vegetable source (Ayerza & Coates 2011). It possesses high amounts of alpha-linolenic (omega-3 or n-3) (19.5%) and alpha-linoleic acids (omega-6 or n-6) (5.2%), all essential nutrients since they cannot be synthesized by the human body. As Ayerza, (1995) writes, fatty acids in chia oil are extremely unsaturated specifically linoleic (17–26%) and linolenic (50–57%) acids. The oil content and the amount of oleic, linoleic, and linolenic acids is influenced by the region of cultivation. For example, in terms of quality, variation in temperature levels has been ascribed as the key factor that affects oil composition (Velasco & Fernandez-Martinez, 2002). Ayerza & Coates, (2011) reported that oil content in relation to the weight of the chia seed disclosed no significant ($P < 0.05$) differences among different locations. However, oil composition, measured as fatty acid percentages, were significantly ($P < 0.05$) affected by location (Table 2)

Table 2. Mean values of protein content, oil content and fatty acid composition of chia grown in three ecosystems.

Origin	Protein[a] (%)[b,d]	Lipids (%)[b]	16:0 (%)[c]	18:0 (%)[c]	18:1 (%)[c]	18:2 (%)[c]	18:3 (%)[c]	SAT (%)[c]	PUFA (%)[c]	-6:-3 (ratio)	PUFA: SAT
Argentina	16.45b	33.5a	6.89b	2.36a	6.73b	22.5a	60.35b	9.26b	82.85a	0.37a	9.01a
Bolivia	26.03a	29.98a	7.72a	3.59a	9.12a	21.93a	56.93c	11.32a	78.87b	0.39a	6.97b
Ecuador	15.95b	31.47a	6.39c	3.74a	6.59b	16.99b	64.75a	10.14b	81.74a	0.26b	8.12a
LSD[e]	5.673	5.743	0.636	1.643	1.166	2.172	3.658	1.755	2.363	0.049	1.287

[a] No replication.

[b] % of seed weight.

[c] % of total fatty acids.

[d] Means in a column within a group with the same letter are not statistically different ($P < 0.05$).

[e] Least significant difference for $P < 0.05$.

Adapted from (Ayerza & Coates, 2011)

3.2 Proteins

When it comes to protein content, chia seeds have dominated the world of oilseeds and most cereal seeds with protein levels ranging from 19-23 %. According to Ayerza & Coates, (2005) some of the functional characteristics of chia seeds are attributed as a result of the 17-20% protein content found in the seeds. Protein fraction in a recent characterization review of flour of seed has shown a relatively good equilibrium of essential amino acids especially with regards to cysteine and methionine (Ixtaina, Nolasco & Tomàs, 2008 ; Sandoval-Oliveros & Paredes-López, 2013). According to a report by Olivos-Lugo, Valdivia-Lo´pez & Tecante, (2010) the chemical store, amino acid profile and the in vitro digestibility tests of chia revealed that high amounts of glutamic acid (123 g/kg of raw protein), arginine (80.6 g/kg of raw protein), and aspartic acid (61.3 g/kg of raw protein) were present in the chia protein. To the contrary, the profiling essential amino acids revealed certain deficiencies as opposed to the World Health Organization standards for young children (FAO/WHO/UNU, 1985). Consequently, chia is not recommended as the only source of proteins thus, supplements which are lysine-rich would be vital since it was the deficient amino acid. On analyzing the protein content of chis seeds, reports according to Ayerza & Coates, (2011) showed that they varied between regions of growth. The protein content seemed to decrease as the altitude increased and vice versa (Ayerza & Coates, 2011). Even though chia is found to have limiting amino acid factors when consumed by preschool children and it's not commercially cultivated, Weber points out that its amino acid profile has no effect on adult (Weber *et al*., 1991). Moreover, when opposed to other traditional cereals such as wheat, rice, oats, barley, and corn its protein content proved superior in both quantity and quality (Ayerza & Coates, 2005). Further studies and research have also confirmed that chia can be variegated with other cereals and grains to maintain a more balanced and healthy protein source (Fernandez *et al*., 2005; Pallaro *et al*., 2004).

3.3 Dietary Fiber

Dietary fiber is defined as a mixture of complexes made up of plant carbohydrate polymers, both oligosaccharides and polysaccharides, e.g., cellulose, hemicelluloses, pectic substances, and gums that may be linked with lignin and other non-carbohydrate constituents, e.g., polyphenols, waxes, saponins, cutin, phytates, and resistant protein (Elleuch *et al*., 2011). Dietary fiber constitutes more than 30% of the total weight of the seed (Spotorno *et al*., 2011). Chia seed is a source of dietary fiber at 35% (Silva *et al*., 2017), in greater levels as compared to other seeds like amaranth (7.3%), quinoa (7.0%), and corn (8.3%) (Srichuwong *et al*., 2017).

The concentration of total dietary fiber (TDF) in chia (33.04%) was found to be higher than in other cereals and oilseeds such as linseed (22.30%), soy (15.00%), corn (13.40%), wheat (12.60%), and sesame (7.79%). Out of the total dietary fiber content found in chia, insoluble fiber represented 92% (30.37 ± 1.02 g/100 g chia) and soluble fiber 8% (2.67 ± 0.26 g/100 g) (Dhingra, Michael, Rajput & Patil, 2012). In a study by Reyes-Caudillo *et al*. (2008a), Mexican chia revealed a higher amount of fiber with seeds from the states of Jalisco and Sinaloa being rich in total dietary fiber (TDF) at 39.94% and 36.97%, respectively. However, a study by Ramzi et al. (2016) reported a lesser value for dietary fiber at 18.15% as compared to other findings that state dietary fiber ranges between 30-35%. This discrepancy can be explained by the fact that the nutritional composition of chia is influenced by different environmental factors such as; temperature, light and soil composition (Suri, Passi & Goyat, 2016a). The levels of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) for Jalisco seeds were 6.84 and 34.9 g/100 g, respectively, while for Sinaloa seeds, the contents were 6.16 and 32.87 g/100 g, respectively. Insoluble fiber is chiefly composed of lignin, cellulose, and hemicellulose, while mucilage is the main type of soluble fiber of the seed (Reyes-Caudillo *et al*., 2008). This mucilage has high ability for water uptake and can absorb about 27 times its own weight (Mu˜noz *et al*., 2012). The main element of IDF is Klason lignin (KL), which makes up to 39–41% of TDF; KL allegedly protects unsaturated fats in chia seeds by building a strong and resistant structure and also by the antioxidant compounds present. The amount of neutral sugars in IDF (NSIDF) ranges between 13.79% to 14.97%, which indicates the presence of cellulose and hemicellulose in disparity with a lower content of uronic acids (UAIDF), 3.05–3.60%, that represent the amount of glucuronic acid residues associated with insoluble hemicellulose (Valdivia-lópez & Tecante, 2015). As a result of its capacity to absorb bile acids, lignin is responsible for the hypocholesterolemic effect associated with fiber intake (Valdivia-lópez & Tecante, 2015). The SDF represents about 6% of chia seeds (Reyes-Caudillo *et al*., 2008).

SDF is predominantly composed of neutral sugars (NSSDF), which shows the presence of various carbohydrates that form the structure of the mucilage. The little quantity of uronic acids (UASDF) suggests that there is no pectin associated with the mucilage, and that the total of UASDF comes from the uronic acid residues found in the polysaccharide chains of the mucilage (Valdivia-lópez & Tecante, 2015).

3.4 Antioxidants

Antioxidants are also an important constituent of chia consisting of majorly phenolic compounds and flavonoids. The phenolic nature of antioxidants present in chia can either be in sugars by glycosidic linkages or in a free form. These glycosidic linkages are responsible for the increased solubility of chia in water (Valdivia-lópez & Tecante, 2015). According to Ayerza & Coates, (2011), the most important phenolic compounds are flavanols myricetin, quercetin, kaempferol, and chlorogenic and caffeic acids. Caffeic and rosmarinic acids (Reyes-Caudillo *et al*., 2008; Capitani *et al*., 2013; Martinez-Cruz &Paredes-Lopez, 2014) are some of the identified phenolic compounds in chia products which act majorly in the management and prevention of various neurological conditions such as epilepsy (Coelho *et al*., 2015). Some of the advantages of caffeic acid found in chia are memory protective effect and hypoglemic activity. In salvia species, rosmarinic acid is reported to be predominant and widely known for its immunorugulatory activities including but not limited to; anti-inflammatory and antioxidant activities, anti-diabetic effect and antimicrobial effect (Jayanthy & Subramanian, 2014). Moreover, it is associated with the ability to inhibit inflammatory process that is related to ischemia reperfusion (Rocha *et al*., 2015). In chia seed antioxidant composition, rosmarinic acid possesses the greatest percentage amounting to (0.927 mg/g), seconded by protocatechuic acid (0.747 mg/g), caffeic acid (0.027 mg/g), and gallic acid (0.012 mg/g). In addition, ferulic acid, glycitin, genistin, glycitein, and genistein were also detected in chia. (Mart´ınez-Cruz & Paredes-L´opez, 2014). In a study carried out by, Marineli *et al*., (2014), chia seed extract revealed the existence of chlorogenic acid (353.09 mg/g), quercetin (301.04 mg/g), myricetin (317.04 mg/g) and decarboxymethyl elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) (377.13 mg/g) but caffeic acid and kaempferol were absent in the tested samples. The differences identified may be attributed to a set of variable such as extraction techniques, identification methods, and losses during analysis, region of cultivation of seeds and equipment used. All these factors have a significant contribution to the diverse content of bioactive compounds (Ayerza & Coates, 2011). Flavonoids have also been found to be in high quantities as flavones and flavanones. Phytates and tannins traces can be found in relatively smaller quantities but antioxidant compounds such as vitamin E and carotenoids are found in greater quantities (Silva *et al*., 2017; Oliveira-Alves *et al*., 2017).

3.5 Minerals and Vitamins

Chia is an outstanding source of minerals such as potassium, zinc, calcium, phosphorus and copper (Ayerza, 2001). The most significant minerals in chia include; calcium (631 mg.100 $g^{-}1$), potassium (407 mg.100 $g^{-}1$), magnesium (335 mg.100 $g^{-}1$), iron (7.72 mg.100 $g^{-}1$) and zinc (4.58 mg.100 $g^{-}1$) (USDA. 2015). Studies by Ullah *et al.* (2016), reported that macronutrients in chia seeds are composed of; phosphorus 860, calcium 631, potassium 407, and magnesium 335 mg/100 gm, and microelements; selenium 55.2 µg/100 g, sodium 16 µg/100 g, iron 7.72 µg/100 g, manganese 2.72 µg/100 g, zinc 4.58 µg/100 g, copper 0.924 µg/100 g and molybdenum 0.2 µg/100 g. The phosphorous, calcium and potassium content of chia seed is greater than that of other crops such as wheat, rice, oats and corn (Beltran-Orozco & Romero, 2003). Furthermore, chia also contains vitamins such as niacin (8.83 mg/100 g), thiamine (0.62 mg/100 g) and riboflavin (0.17 mg/100 g), with higher levels as compared to different seeds (Mu˜noz *et al*., 2012).

4. Physical Properties of Chia

The physical properties of chia seeds dictate the importance of establishing and developing necessary machines for transportation, handling, storage, drying and additional industrial activities such as extraction of oil. To understand the complexity of chia seed cultivation, distribution, adequate design for cleaning equipment, grading and separation technology, moisture content analysis to effect the drying process, understanding on the morphology and size distribution is essential (Kachru, Gupta & Alam, 1994). In a study conducted by Ixtaina *et al*., (2008), the moisture content was observed to be 7.2 and 6.6% for dark and white chia seeds, correspondingly. In addition, Longitudinal measurement (L) ranged from 1.73 to 2.63mm. The bulk of chia seeds (59% of dark seed and 62% of white seed by number) were medium-sized ($2.00 \leq L \leq 2.25$ mm). The average seed width and thickness were 1.32–0.81mm and 1.40–0.83mm for dark and white seeds, in that order. These findings were in the same range as those reported by Rulfo, (1937) for chia seeds. These dimensions of chia seeds were found to lie within the same range to quinoa and rapeseed seeds but were found to be higher than amaranth seeds. Also, these dimensions were found to be lower than cumin, pearl millet, sesame, safflower, flaxseed and coriander

(Vilche, Gely & Santalla, 2003; Cahsir, Glu, ¨Og˘u¨, & O. ztu, 2005; Abalone , Cassinera & Lara, 2004; Coskuner & Karababa, 2007).

The bulk density ranged from 0.667 to 0.722 g cm^{-3} for white and dark seeds respectively, with the bulk density of white chia seed (0.667 g cm^{-3}) being ascribed to its bigger size. The true density and porosity was observed to be between 0.931 and 1.075 g cm−3, and 22.9 and 35.9% for white and dark chia seeds respectively (Ixtaina *et al*., 2008). As compared to sunflower, safflower and coriander the true density was found to be higher (Gupta & Das, 1997; Bau¨mler, Cuniberti, Nolasco, & Riccobene, 2006 ; Coskuner & Karababa, 2007). However, it was lesser than amaranth, sesame, soybeans and millet (Abalone *et al*., 2004; Tunde-Akintunde & Akintunde, 2004). Further, Ixtaina *et al*., (2008) recounted that the equivalent diameter and sphericity of dark and white chia seeds were in the range of 1.32 to 1.39mm, and 62.2 to 66.0%, geometric mean diameter ranged from 1.10 to 1.54mm and surface area of each seed was 5.42±0.15mm^2 and 5.79±0.12mm^2 for the dark and white seeds. Guiotto *et al*., (2011), also reported that the average of the specific surface area (*S*) for both white and black chia seeds was 5.20 mm^2.

The mean values of volume were 1.21 and 1.12 mm^3 for dark and white seeds as reported by (Guiotto *et al*., 2011), while according to (Ixtaina *et al*., 2008) the volume of single chia grain (*V*)

was in the range of 1.19 to 1.42mm^3. The aspect ratio of chia seed was 62.7±1.5% and 65.3±1.3% for dark and white seeds. In view of the low aspect ratio (seeds width to length) and sphericity, it may be presumed that chia seeds would slide on their flat surfaces other than roll. This propensity to either roll or slide is very important in the design of hoppers, since most flat seeds slide more easily than spherical seeds, which tend to roll on structural surfaces (Ixtaina *et al*., 2008).

5. Functional Properties of Chia Seeds

Products that are rich in protein often show different functional characteristics that are often predominant as compared to characteristics of the source flour (Rodrı´guez, Rez & Dufour, 2011) The preference of the products is established when simplifying the extraction process or in case of existence of other components, which as a result pose the threat of complicating the separation process or lead to the loss of essential components. This can be evident in cases of wet extraction (Bergthaller, Dijkink, Langelaan & Vereijken, 2001). Food proteins get their function characteristics from their charge distribution, three dimensional structures and their molecular sizes. The behavior of proteins when they interact with themselves and other components in a complex food system is determined by their structure-function relationship (Joshi, Adhikari, Aldred, Panozzo & Kasapis, 2011). The most essential functional properties of protein in food include water- and fat-binding, hydration, rheological behaviors, foaming and emulsifying. Environmental factors such as climate and other processing conditions greatly affect these properties (Shevkani, Singh, Kaur & Rana, 2015).

The water and oil holding capacity of foods rich in protein is influenced by inherent factors like protein conformation, amino acid structure and surface polarity or hydrophobicity of the food (Haripriya & Aparna, 2018). For example, the water holding capacity WHC (the ability of a moist material to keep water when imperiled to an external centrifugal gravity force or compression (Alfredo, Gabriel, Luis & David, 2009), of flour is carefully linked to both amount of amino acids in various flours and accessibility of proteins functional groups within the flour (Kouakou *et al*., 2013). In a study by (Taylor, Vázquez-ovando, Betancur-ancona & Chel-guerrero. (2012), chia fibrous rich fraction displayed a water holding capacity of 15.4 times of its weight. This value is greater than that reported for fibrous fractions obtained from soy, wheat and maize hulls (Cruz-Salazar, 2002). The mucilaginous fraction obtained from chia may contribute to WHC because mucilages are known to have exceptional water-holding features. Fiber arrangement may also enhance WHC (Pez *et al*., 1997) and the high quantities of hemicellulose and lignin (both have a certain amount of WHC) in the chia FRF may have improved this property. Haripriya and Aparna, (2018), observed that water absorption levels of untreated and roasted chia seeds were 5.2±0.25 ml/g, and 2.7 ± 0.30 ml/g respectively while chia defatted meals demonstrated a WHC of 9.2 to 10.13 g/g. Alfredo *et al*. (2009), described that the fiber structure and high amounts of hemicellulose and lignin may contribute to the high WHC values acquired for chia seeds and their derivatives.

Oil holding capacity (OHC) on the other hand is an essential property to develop novel food products and store them for a long period (Haripriya & Aparna, 2018). Chia mucilage showed higher OHC (5.85 g oil/ g sample) than both whole seeds and powder (3.5, 2.5 g oil/ g sample) respectively (Darwish, Khalifa & Sohaimy, 2018). These results could suggest that chia mucilage can be used as a stabilizer and emulsifier which previously mentioned by (Salgado-Cruz *et al*., 2013; Suri *et al*., 2016). The oil absorption capacity of untreated and roasted chia seeds as reported by Haripriya & Aparna, (2018) were 0.9±0.26 ml/g, & 0.8±0.32 ml/g respectively. Alfredo

et al. (2009) also informed that the fiber-rich chia fraction revealed a low oil-holding capacity (2.2 g/g). Furthermore, Vázquez-ovando *et al*. (2013) described that the chia fibrous rich fraction (FRF) had a low oil-holding capacity (OHC) of 2.02 g oil/g sample. The particle size may affect the OHC in that smaller particles have comparatively more contact surface and therefore would theoretically hold more oil (Lo´ pez *et al*., 1997). As a result of its low OHC, Vázquez-ovando *et al*. (2013) reported that chia fibrous rich fraction can be utilized as a possible ingredient in fried products since it would offer a non-greasy perception.

The capability of chia flour to form gels is reliant on the swelling capacity and solubility of chia seeds. These two factors are linked to the interaction of the chia flour components with the water molecules (Munoz *et al*., 2012). The swelling capacity is the volume of expansion of molecule due to the consumption of water up to a level where the colloidal suspension is complete (Ayernor & Ocloo, 2002). The swelling capacity of untreated and roasted chia seed powder were recorded as, 8.5±1.74% and 6.3±0.53% respectively (Haripriya and Aparna, 2018). Chia mucilage is highly soluble in water at 50g/ml hence it has a potential industrial use, as it is reflected that gums and/or mucilages with higher solubility possess better quality (Mhinzi & Mrosso, 1995). According to Capitani *et al*. (2013), the water adsorption capacity (WAbC) of chia meals with and without mucilage was higher 5.25 ± 0.39 g/g and 10.64 ± 0.60 g/g than that witnessed for canola and soybean meals (3.90 g/g and 3.28 g/g, correspondingly) and alike to that of linseed meal (6.03 g/g) (Khattab & Arntfield, 2009). Capitani *et al*. (2013), also observed that chia seeds meals with and without mucilage did not reveal any differences in the emulsifying activity although the stability of the emulsion prepared with chia seed meal with mucilage was statistically higher than the chia meal without mucilage. This effect can be linked with the ability of mucilage to act as a thickening agent owing to its ability to raise the viscosity of the aqueous phase in an O/W emulsion, hence preventing the movement of the oil droplets of the distributed phase (McClements, 1999). This feature is similar in the linseed mucilage, which has a robust thickening capacity, favorably influencing the water-holding capacity and the emulsifying features of defatted linseed flour (Dev & Quensel, 1986).

6. Health Benefits of Chia

There is current evidence presenting the health benefits linked with the consumption of foods predominant in omega-3 (Vannice & Rasmussen, 2014). Chia seeds constitute a vital plant source of n-3 PUFA to be exploited in diverse research models for human health and the prevention of diseases. This is owing to its rich nutritional profile consisting of proteins, minerals, fiber, polyphenols, and polyunsaturated fatty acids (PUFAs) and is presently recognized as one of the best plant sources of the omega-3 (n-3) fatty acid, α- linolenic acid (ALA) (Mohd *et al*., 2012). Certain rodent experiments have demonstrated that chia may reduce serum cholesterol, LDL (low density lipoproteins), and triglycerides while raising HDL (high density lipoproteins) (Ayerza & Coates, 2007). Alpha linolenic acid (ALA) from chia may also increase adiposity, blood lipid parameters, and insulin resistance in dyslipidemic rats (Chicco, D'Alessandro, Hein, Oliva, & Lombardo, 2009) Moreover, chia has been established to display anti-tumor activity in murine mammary gland adenocarcinoma (Espada, Berra, Martinez, Eynard, A & Pasqualini, 2007). A study by Vuksan *et al*. (2016), recommended that chia was more effective than flax in affecting postprandial outcomes such as postprandial glycemia and satiety. Three previous studies have assessed the effects of chia in respect to blood pressure (BP). Vuksan *et al*. (2007) established a reduction in systolic BP (− 6.3±4 mmHg) in diabetic patients who consumed 37 g / day of chia seeds for 12 weeks. Nevertheless, Nieman *et al*. (2009) observed that there were no changes in BP in overweight or obese adults who ingested 50 g / day of chia seeds for 12 weeks. A comparable result was stated by Nieman *et al*. (2012) who also established no changes in BP in overweight women who consumed 25 g / day of chia seeds for 10 weeks. Toscano and Surama, (2014), determined the effect of 12 week duration of chia flour supplementation using clinical and 24-h ambulatory measurements. They also identified whether oxidative stress, inflammation and endothelial functions are linked with the potential lowering of BP in pharmacologically untreated hypertensive persons and in those formerly treated with drug therapy. They concluded that the intake of chia flour is steadily able to decrease the BP in hypertensive individuals and in patients previously treated with medicine in a manner comparable to the patients not under any medication. Moreover, substitute antihypertensive drugs are being researched on due to the high cost, side effects, and safety issues of current drugs. Indications suggests that palatable foods such as milk, egg, plants, and grains have peptides involved in the prevention and lowering of high blood pressure (Herna´ndez-Ledesma, Contreras, del & Recio, 2011 ; Montoya-Rodrı´guez, Go´mez-Favela, Reyes-Moreno, Mila´n-Carrillo & Mejı´a, 2015; Rosales-Mendoza, Paz-Maldonado, Govea-Alonso & Korban, 2013). A study by Orona-Tamayo, Valverde & Pez. (2016), accounted that the ACE-inhibitory peptides resultant from explicit protein fractions of chia seeds can be a natural remedy for hypertensive persons when they ingest chia as a supplement. In a study to define whether 6-month dietary inclusion of chia would prompt a substantial weight reduction likened to an oat bran-based control; when consumed in combination with a calorie-restricted

diet, and added on top of conventional treatment in overweight and obese individuals with type 2 diabetes (T2DM), Vuksan *et al*., (2017) reported that there is an advantageous role of Salba-chia seeds in promoting weight loss and improvements of obesity related risk factors, while preserving good glycemic control. Supplementation of Salba-chia may be a valuable dietary addition to conventional therapy in the management of obesity in diabetes. While examining the effects of dietary chia seed and oil on plasma and liver oxidative status in diet-induced obese rats, no differences in plasma and liver superoxide dismutase activity between chia diets and high-fat and high-fructose diet (HFF) group. Chia (seed and oil) consumption did not change liver lipid peroxidation, but was able to decrease plasma thiobarbituric acid reactive substances (TBARS) and 8-isoprostane amount increased by HFF group. Plasma and hepatic antioxidant capacity values were raised in chia seed and oil groups at 35% and 47%, correspondingly, compared to HFF group. Dietary chia seed and oil lowered oxidative stress *in vivo*, as it enhanced antioxidant status and lowered lipid peroxidation in diet-induced obese rats (Marineli *et al*., 2015). However, they added that consideration is necessary before inferring these findings to humans and proposed that the quantity of chia tested in the current study should be further tested in clinical studies for chia oil diet replacement or introduction of chia seed in normal human nutrition. The amino acids present in chia seeds are vital for numerous human metabolic activities. Precisely, glutamic acid has the capability to stimulate the central nervous system, is involved in its immunologic performance and increases athletic stamina (Brosnan & Brosnan, 2013 and Paredes Lo´pez, 1991). Aspartic acid on the other hand stimulates hormonal regulation for the appropriate functionality of the nervous system (Brosnan & Brosnan, 2013). Other amino acids like arginine apparently guard against cardiovascular diseases (Bo¨ger, 2007) and sulfur amino acids may be involved in the functionality of the tertiary and also quaternary structures of the proteins (Sandoval-Oliveros & Paredes-Lo´pez, 2013). The above annotations propose that the quality of chia proteins and their amino acids is similar or higher than other vital cereals and oilseeds. The occurrence of these biomolecules in chia seeds epitomizes an essential nutraceutical impact to the daily diet (Sandoval-Oliveros & Paredes-Lo´pez, 2013).

7. Utilization of Chia in the Food Industry

The superior nutritional quality of chia seeds potentiates the use of chia in application in various foods as a functional food (Munoz *et al*., 2012). Chia is used as whole seeds, chia flour or ground chia, soaked in water or in dry form. Based on the use, only mucilage or oil undergoes farther processing (Zettel & Hitzmann, 2018).

Chia seed gel possesses abilities that allow its use in numerous products in the food industry (Ali *et al*., 2012) such as thickener, gel former and chelator (Capitani *et al*., 2012). Moreover, it can act as a fat replacer since it has the ability to hydrate, improve viscosity and conserve freshness, principally in baked products (Vázquez-ovando, Betancur-ancona, Chel-guerrero, Betancur-ancona & Chel-guerrero, 2013). This potential property is used in developing products with reduced fat and that can be used as functional foods due to the fiber content. According to Fernandes & Salas-mellado, (2017), breads and chocolate cakes prepared with chia mucilage can substitute up to 50% of fat without influencing the technological and physical features. Consequently, chia mucilage is a new alternative for substituting fat in food products, conserving the quality characteristics and ensuring healthier foods. Also, chia mucilage could be used in the food industry as a foam stabilizer, a suspending agent, emulsifier, adhesive or binder, due to its water holding capacity, and viscosity (Salgado-Cruz *et al*., 2013). The mucilage acquired from chia seeds is a novel source of polysaccharides and could potentially generate remarkable polymer blends for edible films and coatings (Mu˜noz *et al*., 2012).

Dick *et al*. (2015), produced novel, biodegradable edible films using only chia mucilage as the principal raw material and reported that edible films plasticized with glycerol can be prepared successfully. Additionally, it was observed that chia mucilage films exhibited high solubility in water, good thermal resistance, transparency, and UV light barrier properties, which could provide increased protection to packaged food. This study revealed that chia mucilage films have potential as edible film or coating, with the health benefits of chia mucilage soluble dietary fiber.

Studies have also revealed that chia protein hydroslates when used in white bread and carrot cream increased product biological potential without remarkably changing product quality (Segura-campos *et al*., 2013). Additional studies have also exposed that chia gel can substitute as much as 25% of oil or eggs in cakes while giving a more nutritious product with acceptable sensory parameters (Borneo, Aguirre & León, 2010). These authors indicated that replacing eggs or oil in the cake formulation with chia gel up to a level of 25% retained the functional and sensory properties of the product. However, they recommended that more research is needed to optimize functional and sensory characteristics of cakes with chia gel. The assimilation of chia oil into functional oil in water (O/W) emulsions was also studied by Magdalena *et al*. (2015), who reported that O/W emulsions are a possible alternative system to enhance chia oil stability against lipid oxidation thus chia oil can be integrated

into oil-in-water (O/W) emulsions as ω-3 fatty acid delivery systems in food matrices. Chia flour has also been used to develop bread with improved nutritional value and healthy features that can be used not only by persons with celiac disease but also sustain health status of people with various diseases. It was observed that chia flour contains no gluten and can be used by patients with celiac disease (Costantini *et al*., 2014). The incorporation of chia into dairy products such as yoghurt and ice-cream has also increased (Chavan, Gadhe, Dipak & Hingade, 2017; (Chavan *et al*., 2017; Campos, Ruivo, Scapim, Madrona & Bergamasco, 2016) tested the use of chia as a thickener in the manufacture of ice-cream while Ayaz *et al*. (2017) also included chia seeds in yoghurt which gave the feeling of short-term satiety.

In other studies, an assessment of the possibility to increase *B. infantis* ATCC 15679 and *L. plantarum* ATCC 8014 survival after spray drying using mucilage and soluble protein fractions from chia seeds or flaxseed as encapsulating materials was carried out. Findings exhibited that the mucilage and soluble protein fractions obtained from chia seed and flaxseed enhanced probiotic (*B. infantis* and *L. plantarum*) survival during spray drying and viability during storage at 4 °C when used as constituents of the encapsulation solution with maltodextrin. Furthermore, cells encapsulated with the seed fractions improved resistance against simulated gastric conditions. These results revealed that chia seed and flaxseed are exceptional sources of probiotic encapsulating agents (Bustamante, Oomah, Rubilarb& Shene, 2016).

8. Environmental Impact

The chia plant can be utilized as a whole in the following ways: chia seeds to obtain oil for food applications and cosmetology, cake meals for animal feeds and the leaves and whole plants for ensiling. Consequently, the promotion of chia as a potential crop for food and nutrition security in Africa does not pose any adverse effects to the environment.

9. Conclusion

In the recent years there has been an increased demand for functional food products that possess numerous health benefits as well as provide basic nutrients due to the emerging trend towards a healthy lifestyle. This review shows that chia seeds contain superior nutritional components as compared to other seeds and also confers many health benefits such as antioxidative, anti-hypertensive and hypoglycemic properties among others. Furthermore, its physical and functional properties make it an excellent crop to be used in food applications in the development of functional foods and its application is not only limited to food but can also be used in feed. However, most of the chia based research has been conducted in America and hence new investigations that focus on chia cultivated in Africa are necessary so as to demonstrate its potential as a crop for food and nutrition security.

References

Abalone, R., Cassinera, A., On, A. G., & Lara, M. (2004). Some physical properties of Amaranth seeds. *Biosyst. Eng*, *89*, 109-117. https://doi.org/10.1016/j.biosystemseng.2004.06.012Homa Mahmoodzadeh

Ahmed, M., Ting, I., & Scora, R. (1994). Leaf oil composition of Salvia hispanica L. from three geographical areas. *J Essent Oil Res*, *6*, 223-228. https://doi.org/10.1080/10412905.1994.9698368

Alfredo, V., Gabriel, R., Luis, C., & David, B. (2009). Physico chemical properties of fibrous fraction from chia (Salvia hispanica L.). *LWT-Food Science and Technology*, *42*, 168-173. https://doi.org/10.1016/j.lwt.2008.05.012

Ali, N. M., Yeap, S. K., Ho, W. Y., Beh, B. K., Tan, S. W., & Tan, S. G. (2012). *The Promising Future of Chia , Salvia hispanica L . Journal of Biomedical Biotechnology*, *2012*, 1-9. https://doi.org/10.1155/2012/828139

Amato, M., Caruso, M. C., Guzzo, F., Galgano, F., Commisso, M., Bochicchio, R., … Favati, F. (2015). Nutritional quality of seeds and leaf metabolites of Chia (Salvia hispanica L.) from Southern Italy. *European Food Research and Technology*. https://doi.org/10.1007/s00217-015-2488-9

Anacleto, S., Guadalupe, R., Jat, R., Gerardo, G., Heather, W., Maneesh, S., … Torre, R. de la. (2016). Chia Crop (Salvia hispanica L.): its History and Importance as a Source of Polyunsaturated Fatty Acids Omega-3 Around the World: a Review. *Journal of Crop Research and Fertilizers*, *1*, 1-9. https://doi.org/10.17303/jcrf.2016.104

Artcos, S. D. (2018). Collaborative collection management solution. *Arctos Plants*. Retrieved from http://arctos.database.museum/name/Salvia hispanica#

Ayaz, A., Akyol, A., Inan-Eroglu, E., Cetin, A. K., Samur, G., & Akbiyik, F. (2017). Chia seed (salvia hispanica

l.) added yogurt reduces short term food intake and increases satiety: randomised controlled trial. *Nutrition Research and Practice*, *11*(5), 412-418. https://doi.org/10.4162/nrp.2017.11.5.412

Ayernor, G., & Ocloo, F. C. (2002). Physiochemical changes and diastatic activity associated with germinating paddy rice (PSB.RC 34). *African Journal of Food Science*, *1*, 37-42.

Ayerza, R. (1995). Oil content and fatty acid composition of chia (Salvia hispanica L.) from five northwestern locations in Argentina. *Journal of the American Oil Chemists' Society*, *72*(9), 1079-1081. https://doi.org/10.1007/BF02660727

Ayerza, R. (2001). Wax-ester composition of ten jojoba clones growing in two arid ecosystems of South America. *Journal of Tropical Science*, *41*, 168-171.

Ayerza, R. (2010). Effects of seed color and growing locations on fatty acid content and composition of two chia (Salvia hispanica L.) genotypes. *Journal of the American Oil Chemists Society*, *87*(10), 1161-1165. https://doi.org/10.1007/s11746-010-1597-7

Ayerza, R., & Coates, W. (2007). Effect of dietary alpha-linolenic fatty acid derived from chia when fed as ground seed, whole seed and oil on lipid content and fatty acid composition of rat plasma. *Ann Nutr Metab*, *51*(1), 27-34. https://doi.org/10.1159/000100818

Ayerza, R., & Coates, W. (2005). *Chia Rediscovering a Forgotten Crop of the Aztecs*. Tucson, AZ, USA.

Ayerza, R, & Coates, W. (2004). *Composition of chia (Salvia hispanica) grown in six tropical and subtropical ecosystems of South America*. (October 2003), 131-135. https://doi.org/10.1002/ts.154

Ayerza, R., & Coates, W. (2011). Protein content , oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia (Salvia hispanica L .). *Journal of Industrial Crops and Products*, *34*, 1366-1371. https://doi.org/10.1016/j.indcrop.2010.12.007

Bau¨mler, E., Cuniberti, A., Nolasco, S., & Riccobene, I. (2006). Moisture dependent physical and compression properties of safflower seed. *Journal of Agricultural Engineering*, *72*, 134-140. https://doi.org/10.1016/j.jfoodeng.2004.11.029

Beltran-Orozco, M., & Romero, M. (2003). *La chia, alimento milenario*. Mexico.

Bergthaller, W., Dijkink, B., Langelaan, H., & Vereijken, J. (2001). Protein from pea mutants as a co-product in starch separation. Isolates from wet and dry separation: Yield, composition and solubility. *Nahrung-Food*, *45*, 390-392. https://doi.org/10.1002/1521-3803(20011001)45:6<390::AID-FOOD390>3.0.CO;2-#

Bo¨ger, R. (2007). The pharmacodynamics of L-arginine. *Journal of Nutrition*, *137*, 1650S_1655S. https://doi.org/10.1093/jn/137.6.1650S

Bochicchio, R., Rossi, R., Labella, R., Bitella, G., & Perniola, M. (2015). Effect of sowing density and nitrogen top-dress fertilization on growthand yield of chia (Salvia hispanica L.) in a Mediterranean environment: first results. *Italian Journal of Agronomy*, *10*, 163-166. https://doi.org/10.4081/ija.2015.640

Borneo, R., Aguirre, A., & León, A. E. (2010). Chia (Salvia hispanica L) Gel Can Be Used as Egg or Oil Replacer in Cake Formulations. *YJADA*, *110*(6), 946-949. https://doi.org/10.1016/j.jada.2010.03.011

Bustamante, M., Oomah, B. D., Rubilar, M., & Shene, C. (2016). Effective Lactobacillus plantarum and Bifidobacterium infantis encapsulation with chia seed (Salvia hispanica L.) and flaxseed (Linum usitatissimum L.) mucilage and soluble protein by spray drying. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2016.08.019

Cahill, J. (2003). No Title. *Economic Botany*, *57*, 604-618. https://doi.org/10.1663/0013-0001(2003)057[0604:EOCSHL]2.0.CO;2

Cahill, J. P., & Provance, M. C. (2002). Genetics of qualitative traits in domesticated chia (Salvia hispanica L.). *Journal of Heredity*, *93*(1), 52-55. https://doi.org/10.1093/jhered/93.1.52

Cahsir, S., Glu, T. M.,¨Og˘u¨, T. H., & Rk, O. O. Z. (2005). Physical properties of rapeseed (Brassica napus oleifera L.). *Journal of Food Enngineering*, *69*, 61-66. https://doi.org/10.1016/j.jfoodeng.2004.07.010

Campos, B., Ruivo, T. D., Scapim, M. da S., Madrona, G., & Bergamasco, R. de C. (2016). Optimization of the mucilage extraction process from chia seeds and application in ice cream as a stabilizer and emulsifier. *LWT - Food Science and Technology*, *65*, 874-883. https://doi.org/10.1016/j.lwt.2015.09.021

Capitani, M. I., Nolasco, S. M., & Tomás, M. C. (2013). Effect of Mucilage Extraction on the Functional Properties of Chia Meals. *Food Industry*, 422-437.

Castro, G. M. (2002). Ácidos grasos Omega 3: beneficios y Fuentes. *Interciencía*, *27*, 128-136.

Chavan, V., Gadhe, K., Dipak, S., & Hingade, S. (2017). Studies on extraction and utilization of chia seed gel in ice cream as a stabilizer. *Journal of Pharmacognosy and Phytochemistry*, *6*(5), 1367-1370.

Chicco, A., D'Alessandro, M., Hein, G., Oliva, M., & Lombardo, Y. (2009). Dietary chia seed (Salvia hispanica L.) rich in alpha-linolenic acid improves adiposity and normalizes hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. *Br J Nutr*, *101*(1), 41-50. https://doi.org/10.1017/S000711450899053X

Chilliard, Y., Ferlay, A., & Doreau, M. (2001). Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Journal of Livestock Production Science*, *70*, 31-48. https://doi.org/10.1016/S0301-6226(01)00196-8

Coates, W., & Ayerza, R. (1996). Production potential of chia northwestern Argentina. *Journal of Industrial Crop and Products*, *5*, 229-233. https://doi.org/10.1016/0926-6690(96)89454-4

Coelho, V., Vieira, C. G., Souza, L., Moyses, F., Basso, C., Papke, D. K., … Pereira, P. (2015). Antiepileptogenic, antioxidant and genotoxic evaluation of rosmarinic acid and its metabolite caffeic acid in mice. *Journal of Life Sciences*, *121*, 65-71. https://doi.org/10.1016/j.lfs.2014.11.009

Commission, E. (2009). *Commission decision authorizing the placing on the market of Chia seed (Salvia hispanica) as novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council*.

Commission, E. (2013). *Commission implementing decision authorizing an extension of use of Chia (Salvia hispanica) seed as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council*. Off J Eur Union L21/34.

Coorey, R., Tjoe, A., & Jayasena, V. (2014). Gelling Properties of Chia Seed and Flour. *Journal of Food Science*, *79*(5), 859-866. https://doi.org/10.1111/1750-3841.12444

Coskuner, Y., & Karababa, E. (2007). Physical properties of coriander seeds (Coriandrum sativum L.). *Journal of Food Enngineering*, *80*, 408-416. https://doi.org/10.1016/j.jfoodeng.2006.02.042

Costantini, L., Lukšič, L., Molinari, R., Kreft, I., Bonafaccia, G., Manzi, L., & Merendino, N. (2014). Development of gluten-free bread using tartary buckwheat and chia flour rich in omega-3 fatty acids and flavonoids as ingredients. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2014.05.095

Craig, R. (2004). *Application for approval of whole chia (Salvia hispanica L.) seed and ground whole seed as novel food ingredient* (Company Re; N. I. (2004). Mr D Amstrong, Ed.). Northern Ireland.

Cruz-Salazar, M. (2002). *Caracterizacio´n del residuo fibroso obtenido de la ca´ scara de maracuya´ Passiflora edulis Flavicarpa Dagener*. de Maestrı´a. Universidad Auto´noma de Yucata´ n, Me´ xico.

Darwish, A. M. G., Khalifa, R. E., & Sohaimy, S. A. El. (2018). *Functional Properties of Chia Seed Mucilage Supplemented In Low Fat Yoghurt*. https://doi.org/10.21608/asejaiqjsae.2018.13882

Dev, D., & Quensel, E. (1986). Functional and microestructural characteristics of linseed (Linum usitatissimum L.) flour and a protein isolate. *Lebensmittel Wissenschaft and Technology - Food Science Technology*, *19*, 331-337.

Dewhurst, R. J., Fisher, W. J., Tweed, J. K. S., & Wilkins, R. J. (2003). Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. *Journal of Dairy Science*, *86*, 2598-2611. https://doi.org/10.3168/jds.S0022-0302(03)73855-7

Dewhurst, R., Shingfield, K., Lee, M., & Scollan, N. (2006). Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Journal of Animal Feed Science*, *131*, 168-206. https://doi.org/10.1016/j.anifeedsci.2006.04.016

Dhingra, D., Michael, M., Rajput, H., & Patil, R. (2012). Dietary fibre in foods: a review. *Journal of Food Science and Technology*, *49*, 255-266. https://doi.org/10.1007/s13197-011-0365-5

Dick, M., Maria, T., Costa, H., Gomaa, A., Subirade, M., Oliveira, A. De, & Hickmann, S. (2015). Edible film production from chia seed mucilage : Effect of glycerol concentration on its physicochemical and mechanical properties. *Carbohydrate Polymers*, *130*, 198-205. https://doi.org/10.1016/j.carbpol.2015.05.040

Dubernard, C. (1991). Códices de Cuernavaca y unos títu¬los de sus pueblos. *Porrúa.*, 396.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Journal of Food Chemistry*, *124*, 411-421. https://doi.org/10.1016/j.foodchem.2010.06.077

Espada, C., Berra, M., Martinez, M., Eynard, A., & Pasqualini, M. (2007). Effect of Chia oil (Salvia hispanica) rich in omega-3 fatty acids on the eicosanoid release, apoptosis and T-lymphocyte tumor infiltration in a murine mammary gland adenocarcinoma. Prostaglandins Leukot. *Journal of Essentual Fatty Acids*, *77*(1), 1-8. https://doi.org/10.1016/j.plefa.2007.05.005

FAO/WHO/UNU. (1985). *Energy and protein requirements- Report of a joint FAO/ WHO/UNU expert consultation: Technical report series*. Geneva: WHO.

Fernandes, S. S., & Salas-mellado, M. D. M. (2017). Addition of chia seed mucilage for reduction of fat content in bread and cakes. *Food Chemistry*, *227*, 237-244. https://doi.org/10.1016/j.foodchem.2017.01.075

Fernandez, I., Vidueiros, S., Feliu, M., Angellini, E., Ayerza, R., Coates, W., … Pallaro, A. (2005). Calidad proteica de nuevos viejos cultivos característicos de Mesoamérica y Sudamérica: quinoa y chia. VIII. *Congreso Nacional Bioquímico de La República Argentina*, 76. San Juan, Argentina,: Libro de Resúmenes.

Guiotto, E. N., Ixtaina, V. Y., C.Tomás, M., & Nolasco, S. M. (2011). Moisture dependent physical propertie of Chia. *American Society of Agricultural and Biological Engineers*, *54*(2), 527-533. https://doi.org/10.13031/2013.36455

Guiotto, E. N., Ixtaina, V. Y., Tom´as, M. C., & Nolasco, S. M. (2013). Moisture-dependent engineering properties of chia (Salvia hisp´anica L.) seeds. *Food Chemistry*, 381-397.

Gupta, R., & Das, S. (1997). Physical properties of Sunflower seeds. *Journal of Agricultural Engineering*, *66*, 1-8. https://doi.org/10.1006/jaer.1996.0111

Haripriya, A., & Aparna, N. (2018). Effect of roasting on selected nutrient profile and functional properties of chia seeds (Salvia hispanica) and optimization of chia seed based instant soup mix. *International Journal of Food Science and Nutrition*, *3*(2), 200-206.

Herna´ndez-Ledesma, B., Contreras, M. del M., & Recio, I. (2011). Antihypertensive peptides: Production, bioavailability and incorporation into foods. *Advances in Colloid and Interface Science*, *165*, 23-35. https://doi.org/10.1016/j.cis.2010.11.001

Ixtaina, V.Y, Nolasco, S., & Tomàs, M. (2008). Physical properties of Chia (Salvia hispanica L.) seeds. *Journal of Industrial Crops and Products*, *28*, 286-293. https://doi.org/10.1016/j.indcrop.2008.03.009

Jayanthy, G., & Subramanian, S. (2014). Rosmarinic acid, a polyphenol, ameliorates hyperglycemia by regulating the key enzymes of carbohydrate metabolism in high fat diet - STZ induced experimental diabetes mellitus. *Journal of Biomedicine and Preventive Nutrition*, *4*(3), 431-437. https://doi.org/10.1016/j.bionut.2014.03.006

Joshi, M., Adhikari, B., Aldred, P., Panozzo, J., & Kasapis, S. (2011). Physicochemical and functional properties of lentil protein isolates prepared by different drying methods. *Journal of Food Chemistry*, *129*(4), 1513-1522. https://doi.org/10.1016/j.foodchem.2011.05.131

Kachru, R., Gupta, R., & Alam, A. (1994). *Physico-chemical Constituents and Engineering Properties*. Jodhpur, India.: Scientific Publishers.

Kaiser, C., & Ernst, M. (2016). *Chia. Center for Diversification Crop Profile*. KY, USA.

Khattab, R., & Arntfield, S. (2009). Functional properties of raw and processed canola meal. *LWT - Food Science and Technology*, *42*, 1119-1124. https://doi.org/10.1016/j.lwt.2009.02.009

Kouakou, B., Marie, N., Halbin, K., Tagro, G., Florent, N., & Dago, G. (2013). Biochemical Characterization and Functional Properties of Weaning Food Made from Cereals (Millet, Maize) and Legumes (Beans, Soybeans. *J Food Chem Nutr*, *1*(1), 22-32.

Magdalena, L., Yanet, V., Alejandra, M., Maria, R., Sánchez, T., Ricardo, J., … Cristina, M. (2015). Chia seed oil-in-water emulsions as potential delivery systems of x -3 fatty acids. *JOURNAL OF FOOD ENGINEERING*, *162*, 48-55. https://doi.org/10.1016/j.jfoodeng.2015.04.005

Marineli, R. da S., Lenquiste, S. A., Moraes, E. A., & Jr., M. R. M. (2015). Antioxidant potential of dietary chia seed and oil (Salvia hispanica L.) in diet-induced obese rats. *Food Research International*. https://doi.org/10.1016/j.foodres.2015.07.039

Marineli, S., Aguiar, É., Alves, S., Teixeira, A., Nogueira, M., Roberto, M., & Jr, M. (2014). LWT - Food Science and Technology Chemical characterization and antioxidant potential of Chilean chia seeds and oil (Salvia hispanica L .). *LWT - Food Science and Technology*, *59*(2), 1304-1310. https://doi.org/10.1016/j.lwt.2014.04.014

McClements, D. (1999). *Food emulsions: Principles, practice, and techniques* (B. Raton & FL, Eds.). CRC Press.

Mhinzi, G., & Mrosso, H. D. (1995). Studies on Tanzania Acacia gum exudates from the series Vulgares and Gummiferae. *Food Chemistry*, *54*, 261-264. https://doi.org/10.1016/0308-8146(95)00038-K

Montoya-Rodrı´guez, A., Go´mez-Favela, M., Reyes-Moreno, C., Mila´n-Carrillo, J., & Mejı´a, E. G. de. (2015). Identification of bioactive peptide sequences from amaranth (Amaranthus hypochondriacus) seed proteins and their potential role in the prevention of chronic diseases. *Comprehensive Reviews in Food Science and Food Safety*, *14*, 139-158. https://doi.org/10.1111/1541-4337.12125

Munoz, L., Cobos, A., Diaz, O., & Aguilera, J. (2013). Chia seed (Salvia hispanica): an ancient grain and a new functional food. *Food Rev. Int*, *29*, 394-408. https://doi.org/10.1080/87559129.2013.818014

Muñoz, L. A., Cobos, A., Diaz, O., & Aguilera, J. M. (2012). *Chia seeds : Microstructure , mucilage extraction and hydration*. *108*, 216-224. https://doi.org/10.1016/j.jfoodeng.2011.06.037

Nieman, D.C, Gillitti, N., Jin, F., Henson, D., Kennerly, K., Shanely, R., … Schwartz, S. (2012). Chia seed supplementation and disease risk factors in overweight women: a metabolomics investigation. . *J Altern Complement Med*, *18*, 700-708. https://doi.org/10.1089/acm.2011.0443

Nieman, David C, Cayea, E. J., Austin, M. D., Henson, D. A., Mcanulty, S. R., & Jin, F. (2009). Chia seed does not promote weight loss or alter disease risk factors in overweight adults. *Nutrition Research*, *29*(6), 414-418. https://doi.org/10.1016/j.nutres.2009.05.011

Oliveira-Alves, C., Vendramini-Costa, D., Cazarin, C. B., J´unior, M. M., Ferreira, J. B., Silva, A., & Bronze, M. (2017). Characterization of phenolic compounds in chia (Salvia hispanica L.) seeds, fiber flour and oil. *Journal of Food Chemistry*, *232*, 295-305. https://doi.org/10.1016/j.foodchem.2017.04.002

Olivos-Lugo, B., Valdivia-Lo´pez, M., & Tecante, A. (2010). Thermal and physicochemical properties and nutritional value of the protein fraction of Mexican chia seed (Salvia hispanica L. *Food Science and Technology International*, *16*(1), 89-96. https://doi.org/10.1177/1082013209353087

Orona-Tamayo, D., Valverde, M. E., & Pez, O. P.-L. (2016). *Chia—The New Golden Seed for the 21st Century: Nutraceutical Properties and Technological Uses*. https://doi.org/10.1016/B978-0-12-802778-3.00017-2

Pallaro, A., Feliu, M., Vidueiros, S., Slobodiank, N., Ayerza, R., Coates, W., & Fernandez, I. (2004). *Estudio de la calidad proteica de una fuente no tradicional. Resumenes del Congreso Internacional de Ciencia y Tecnologia de los Alimentos*. Cordoba, Argentina.

Peiretti, P., & Gai, F. (2009). Fatty acid and nutritive quality of Chia (Salvia hispanica L.) seeds and plant during growth. *Journal of Aanimal Feed Science Technology*, *148*, 267-275. https://doi.org/10.1016/j.anifeedsci.2008.04.006

Peperkamp, M. (2015). *CBI Tailored Intelligence: chia from Bolivia ´a modern super seed in a classic pork cycle*. The Hague, Netherlands.

Pez, G. L., Ros, G., N, F. R., Periago, M., Martı´nez, M., & J. Ortun˜ o. (1997). Propiedades funcionales de la fibra diete´ tica. Mecanismo de accio´n en el tracto gastrointestinal. *Archivos Latinoamericanos de Nutricio´n*, *47*(3), 203-207.

Reyes-Caudillo, E., Tecante, A., & Valdivia-Lo´pez, M. (2008a). Dietary fiber and antioxidant activity of phenolic compounds present in Mexican Chia (Salvia hispanica L.) seeds. *Journal of Food Chemistry*, *107*, 656-663. https://doi.org/10.1016/j.foodchem.2007.08.062

Reyes-Caudillo, E., Tecante, A., & Valdivia-Lo´pez, M. A. (2008b). Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (Salvia hispanica L .) seeds. *Food Chemistry*, *107*, 656-663. https://doi.org/10.1016/j.foodchem.2007.08.062

Rocha, J., Eduardo-Figueira, M., Andreia, B., Adelaide, F., Dora, B., Rosario, B., … Bruno, S. (2015). Anti‐inflammatory Effect of Rosmarinic Acid and an Extract of Rosmarinus officinalis in Rat Models of Local and Systemic Inflammation. *Basic & Clinical Pharmacology & Toxicology*, *116*, 398-413. https://doi.org/10.1111/bcpt.12335

Rodrı´guez, P., Pérez, E., Romel, G.,& Dufour, D. (2011). Characterization of the proteins fractions extracted from leaves of Amaranthus dubius (Amaranthus spp.). *African Journal of Food Science*, *5*, 417-424.

Rosales-Mendoza, S., Paz-Maldonado, L. M., Govea-Alonso, D., & Korban, S. (2013). Engineering production of antihypertensive peptides in plants. *Plant Cell, Tissue and Organ Culture*, *112*, 159-169. https://doi.org/10.1007/s11240-012-0231-9

Rulfo, J. (1937). La chia. *Agricultura*, *1*, 28-37.

Salgado-Cruz, M., Calderón-Domínguez, G., Chanona-Pérez, J., Farrera-Rebollo, R., Méndez-Méndez, J., & Díaz-Ramíreza, M. (2013). Chia (Salvia hispanica L.) seed mucilage release characterisation. A microstructural and image analysis study. *Journal of Industrial Crops Products*, *51*, 453-462. https://doi.org/10.1016/j.indcrop.2013.09.036

Sandoval-Oliveros, M., & Paredes-López, O. (2013). Isolation and characterization of proteins from Chia seeds (Salvia hispanica L.). *Journal of Agriculture and Food Chemistry*, *61*, 193-201. https://doi.org/10.1021/jf3034978

Sapio, O. Di, Bueno, M., Busilacchi, H., & Severin, C. (2008). Chía: importante antioxidante vegetal. *Agromensajes de La Facultad*, *56*, 11-13.

Segura-campos, M. R., Salazar-vega, I. M., Chel-guerrero, L. A., & Betancur-ancona, D. A. (2013). LWT - Food Science and Technology Biological potential of chia (Salvia hispanica L .) protein hydrolysates and their incorporation into functional foods. *LWT - Food Science and Technology*, *50*(2), 723-731. https://doi.org/10.1016/j.lwt.2012.07.017

Shevkani, K., Singh, N., Kaur, A., & Rana, J. (2015). Structural and functional characterization of kidney bean and field pea protein isolates: a comparative study. *Food Hydrocolloids Journal*, *43*, 679-689. https://doi.org/10.1016/j.foodhyd.2014.07.024

Silva, B. da, Anunciac¸˜ao, P. C., Matyelka, J., C. M. da. S., Lucia,C. M. D.,Martino, H. S. D., Pinheiro-Sant'Ana, H. M. (2017). Chemical composition of Brazilian chia seeds grown in different places. *Journal of Food Chemistry*, *221*, 1709-1716. https://doi.org/10.1016/j.foodchem.2016.10.115

Souza, F. De, Souza, F. L. de, Santo, S. E. G., & Rosa, G. (2015). Effect of chia seed (Salvia hispanica L.) consumption on cardiovascular. *Journal of Nutrición Hospitalaria*, *32*, 1909-1918.

Spotorno, V., Mateo, C. M., Ixtaina, V. Y., Martı, M. L., Diehl, B. W. K., Nolasco, S. M., & Toma, M. C. (2011). *Journal of Food Composition and Analysis Characterization of chia seed oils obtained by pressing and solvent extraction*. *24*, 166-174. https://doi.org/10.1016/j.jfca.2010.08.006

Srichuwong, S., Curti, D., Austin, S., King, R., Lamothe, L., & Gloria-Hernandez, H. (2017). Physicochemical properties and starch digestibility of whole grain sorghums, millet, quinoa and amaranth flours, as affected by starch and non-starch constituents. *Journal of Food Chemistry*, *233*, 1-10. https://doi.org/10.1016/j.foodchem.2017.04.019

Suri, S., Passi, S., & Goyat, J. (2016). Chia Seed (Salvia Hispanica L.) - a New Age Functional Food. *4th International Conference on Recent Innovations in Science Engineering and Management*, 286-299. New Delhi: India International Centre.

Taylor, P., Vázquez-ovando, A., Betancur-ancona, D., & Chel-guerrero, L. (2012). *Physicochemical and functional properties of a protein-rich fraction produced by dry fractionation of chia seeds (Salvia hispanica L.)*. (November 2013), 37-41. https://doi.org/10.1080/19476337.2012.692123

Tello, E. N. (2014). *Efecto de la fecha de siembra sobre el crecimiento y rendimiento de chía blanca (Salvia hispanica L.) establecida en la localidad de las cruces provincia de San Antonio*. Universidad de Chile. Santiago de Chile.

Toscano, L. T., & Surama, C. (2014). *Chia Flour Supplementation Reduces Blood Pressure in Hypertensive Subjects*. 392-398. https://doi.org/10.1007/s11130-014-0452-7

Tunde-Akintunde, T., & Akintunde, B. (2004). Some physical properties of Sesame seed. *Biosyst. Eng*, *88*, 127-129. https://doi.org/10.1016/j.biosystemseng.2004.01.009

Ullah, R., Nadeem, M., Khalique, A., Imran, M., Mehmood, S., & Javid, A. (2016). Nutritional and therapeutic perspectives of Chia (Salvia hispanica L.): a review. *Journal of Food Science and Technology*, *53*(April), 1750-1758. https://doi.org/10.1007/s13197-015-1967-0

USDA. (2004). *Seeds, chia seeds, dried. Nutrient Database for Standard Reference*.

Valdivia-lópez, M. Á., & Tecante, A. (2015). Chia (Salvia hispanica): A Review of Native Mexican Seed and its Nutritional and Functional Properties. In *Advances in Food and Nutrition Research* (1st ed., Vol. 75). https://doi.org/10.1016/bs.afnr.2015.06.002

Vannice, G., & Rasmussen, H. (2014). Position of the academy of nutrition and dietetics: Dietary fatty acids for healthy adults. *J. Acad. Nutr. Diet*, *114*, 136-153. https://doi.org/10.1016/j.jand.2013.11.001

Vázquez-ovando, A., Betancur-ancona, D., Chel-guerrero, L., Betancur-ancona, D., & Chel-guerrero, L. (2013). *Physicochemical and functional properties of a protein-rich fraction produced by dry fractionation of chia seeds (Salvia hispanica L .)*. *6337*. https://doi.org/10.1080/19476337.2012.692123

Velasco, L., & Fernandez-Martinez, J. (2002). Breeding oilseed crops for improved oil quality. *Journal of Crop Production*, *5*(1-2), 309-344. https://doi.org/10.1300/J144v05n01_13

Vilche, C., Gely, M., & Santalla, E. (2003). Physical properties of quinoa seeds. *Biosyst. Eng*, *86*, 59-65. https://doi.org/10.1016/S1537-5110(03)00114-4

Vuksan, V., Choleva, L., Jovanovski, E., Jenkins, A. L., Dias, A. G., Ho, H. V. T., … Duvnjak, L. (2016). *Comparison of fl ax (Linum usitatissimum) and Salba-chia (Salvia hispanica L .) seeds on postprandial glycemia and satiety in healthy individuals : a randomized , controlled , crossover study*. (May), 1-5. https://doi.org/10.1038/ejcn.2016.148

Vuksan, V., Jenkins, A. L., Brissette, C., Choleva, L., & Jovanovski, E. (2017). *Nutrition , Metabolism & Cardiovascular Diseases Salba-chia (Salvia hispanica L .) in the treatment of overweight and obese patients with type 2 diabetes : A double-blind randomized controlled trial*. 138-146. https://doi.org/10.1016/j.numecd.2016.11.124

Vuksan, V., Whitham, D., Sievenpiper, J., Jenkins, A., Rogovik, A., Bazinet, R., … Hanna, A. (2007). Supplementation of conventional therapy with the novel grain salba (Salvia hispanica L.) improves major and emerging cardiovascular risk factors in type 2 diabetes. *Diabetes Care*, *30*, 2804-2810. https://doi.org/10.2337/dc07-1144

Weber, W., Gentry, S., Kolhepp, A., & McCrohan, R. (1991). The nutritional and chemical evaluation of chia seeds. *Journal of Ecology of Food Nutrition*, *26*, 119-125. https://doi.org/10.1080/03670244.1991.9991195

Zettel, V., & Hitzmann, B. (2018). Applications of chia (Salvia hispanica L.) in food products. *Trends in Food Science & Technology*, 1-48. https://doi.org/10.1016/j.tifs.2018.07.011

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Mould Characterization and Mycotoxin Quantification of Chia Seeds (*Salvia hispanica L.*) Grown in Kenya

Veronicah Njeri[1], Monica Mburu[1], & Kipkorir Koskei[1]

[1]Institute of Food Bioresources Technology, Dedan Kimathi University of Technology, Nyeri, Kenya

Correspondence: Veronicah Njeri, Dedan Kimathi University of Technology, P.O Box Private bag, Nyeri, Kenya. Tel: 254-718-458-186. E-mail: njeri.veroh87@gmail.com

Received: October 1, 2019 Accepted: October 17, 2019 Online Published: October 30, 2019

doi:10.5539/jfr.v8n6p119 URL: https://doi.org/10.5539/jfr.v8n6p119

Abstract

Chia seeds are functional food that have been considered highly nutritious. They have high levels of polyunsaturated fatty acid content therefore counteract lifestyle disorder such as cardiovascular diseases. This study sought to determine the level of mould contamination in chia seeds; enumeration and characterization of the types of molds and quantification of mycotoxin level, for chia seeds grown and sold in Kenya. A complete randomized block design with triplicates was used in the study. Samples were collected at random from farmers and distributors in Nyeri, Nakuru, Busia and Trans Nzoia counties. Moulds species were isolated from PDA and MEA Medias and morphological characteristics was determined under X 40 magnification power. Mould counts were found to be between 1.33 X 10^3cfu/ml to 2.67 X 10^3cfu/ml. Mould characterization done by microscopic and macroscopic technique showed evidence of *Rhizopus spp*, *Trichoderma* spp and *Fusarium* spp. Amongst the three genera found, *Rhizopus spp* was the predominantly occurring mould. The percentage moisture content of chia seeds samples ranged from 6.49±1.26 and 9.16±0.43. Significant variations on moisture content ($p< 0.05$) were observed among chia samples from different farmers. Aflatoxin was not detected in all chia samples. It can therefore be concluded that the chia samples were not contaminated with aflatoxin although different species of mold were present. Farmers need to be trained on proper postharvest handling methods of chia seeds, as well as proper storage and an objective method of analyzing the moisture content of the chia seeds need to be developed.

Keywords: chia seeds, mould characterization, mycotoxins

Practical Application

This research will provide information on the levels of moulds and mycotoxin contamination in chia seeds which will be used for extension services geared towards training of farmers and chia seeds handlers on proper postharvest handling techniques by researchers and relevant bodies in achieving food security, improved nutrition, promotion of sustainable agriculture and addressing factors related to chia seeds contamination.

1. Introduction

Chia plant is a hardy annual herbecoues species that grows 1- 1.5 meters high. Taxonomically, it belongs to the Kingdom *Plantae*, order *Lamiales*, *Labiatae* family and Genus *Salvia*. Its name is derived from the Latin word '*Salare*' which means to save; referring to its curative abilities, being a source of energy and stamina thus the vital reason why it was used by the Aztec and Mayan warriors of old Mexico during Pre-Columbian times (Ayerza and Coates, 2009). In the plant native habitat of South West America, it has been highly valued as a staple food for hundreds of years. Chia is currently grown in Bolivia, Mexico, Peru, Argentina with emphasis on the Salta, Jujuy, Tucuman and Catamarca (Busilacchi *et. al*, 2013). In Kenya, Chia growing has gained momentum because of its nutrition benefits thus it is grown in most counties such as Busia, Nakuru, Trans Nzoia, Machakos and Nyeri counties. Its wide applicationis because of the hardy properties in that chia seeds can withstand different kinds of climatic conditions.

The growing concern in the modern society to have a healthy diet has contributed to the increase in demand for foods that are rich in nutrients, containing beneficial substance to humans.Chia seeds are edible and rich source of essential nutrients, including proteins, antioxidant, fibre, polyunsaturated fatty acids like alpha-linolenic and linoleic acids (Menga *et. al*, 2017). They offer five times more calcium than milk and the enzymes in the seed aid in digestion.(Yao *et.al*, 2012) Polyunsaturated fatty acid are of great interest because it supports immune

functions, inhibit the proliferation of inflammatory lymphocytes and cytokines, act to prevent the incidence of cardio vascular diseases and maintain the integrity of cell membrane and neurotransmitters (Ludwig *et. al*, 2013). They are considered safe as they do not have gluten and toxic compounds thus making the seeds a safe ingredient for gluten free diets (Menga *et. al*, 2017). Studies done by Ayerza in 2013, shows that for the chia seed types commercilaized today, the coat colour is either black or white and there is no chemical composition difference.

A major challenge with agricultural crops is contamination with moulds due to poor handling and storage. Moulds growth lead to production of mycotoxins such as *aflatoxins, Ochratoxins A, fumonisins, deoxynivalenol, T-2 toxin and zearalenone* (Proctor *et. al*, 2009). These mycotoxins have been associated with a wide range of adverse and toxic effects in humans like liver cancer, thus classified as foodborne hazards. Regulations for major mycotoxins across different food and feed and their maximum tolerated levels are documented. For instance, importation of chia seeds to the European countries require stringent maximum tolerable limits so that it is allowed in their market. Similar chia studies done in Argentina have shown that chia seeds can be contaminated with aflatoxins (JEFCA, 2008).

2. Materials and Methods

2.1 Treatments and Experimental Design

A complete randomized block design with three replicates was used in sample collection from randomly selected farmers from Nyeri, Nakuru, Busia and Trans Nzoia counties. From each county three farmers were selected randomly and for every farmer, samples weighing 250 grams was collected from different locations in their stores and replicated three times. Samples were also collected from a major supermarket who operate as the main local distributor of chia seeds in Nyeri county.

The samples collected were put in sterile plastic bags, labelled and refrigerated at 8 ℃. The sample origin, date of harvesting, weight and sampling date were recorded.

2.2 Study Site

The samples were randomly drawn from different counties namely Trans Nzoia, Nakuru, Nyeri and Busia, which are known for chia seeds cultivation in Kenya and have different climatic conditions that affect the post-harvest handling methods and storage. A preliminary study was carried out to know how the different farmers and supermarkets handle chia seeds after harvesting.

2.3 Preliminary Study

2.3.1 Determination of Post-harvest Handling Techniques

Purposive sampling was used to administer survey questionnaire for the preliminary study to ascertain whether post-harvest handling techniques influenced chia mould contamination. Structured and open-ended questionnaires was administered to farmers and the main distributor to study the nature of post-harvest handling techniques employed. The study involved qualitative analysis of data where data cleaning was done to determine inaccurate, missing data and inconsistencies. Thematic and content analysis was employed in the analysis of this data taking on descriptive and analytical explanations.

2.3.2 Determination of Moisture Content

Moisture content of the chia seeds was determined according to AOAC (2000) method. Moisture dishes were washed and placed in an oven to dry at 105^0C for one hour. The dishes were then cooled in a desiccator to room temperature. The initial weight of the dishes was taken using analytical balance and recorded as (W_1). Ground chia seeds sample of 5grams was weighed in moisture dishes and weight recorded (W_2). The moisture dishes were then placed into an oven maintained at 105^0C for two hours after which they were cooled in a desiccator to room temperature and weight taken. The dishes were returned to the oven for thirty minutes and weight taken. This was repeated until a constant weight is obtained (W_3). This was done in triplicates.

$$\text{Moisture content (\%)} = (W_3 - W_1)/ (W_2 - W_1) * 100$$

2.4 Mould Enumeration and Identification

From each chia sample, 5 g of the seeds was taken. Isolation was performed by direct plating technique (Perrone *et. al*, 2007) where the seeds were applied on the surface of solidified Potato Dextrose Agar medium. The plates were then incubated at 25°C for five days. The colonies were then counted using a colony counter and expressed as CFU/ml After incubation the plates were examined for colony growth visually. The suspected colonies of *Aspergillus* species were isolated by streaking in Malt Extract Agar (MEA) and incubated at 25°C for seven

days.

Morphological analysis of *Aspergillus* was done by microscopic and macroscopic technique. *Aspergillus ssp*. was identified according to Samson *et al.*, (2014) where the colony characters such as color and texture of the mycelium, characteristics of conidiophores such as shape of conidial heads, color of stipes were examined. Moulds were identified and characterized according to their unique morphological features.

2.5 Screening for Mycotoxin (aflatoxin) Production

2.5.1 Aflatoxin Standards Solution Preparation and Analysis

Stock standards of two milligrams Aflatoxins B_1 was dissolved in 100ml methanol to make 20 ppm B_1 or 20000 ppb. Stock standards of one milligram of Aflatoxins B_2 and G_2 were dissolved in 100 ml methanol to make 20 ppm B_1 or 10000 ppb. From stock above, five milliliters of each standard were pipetted to make 15mls combined standards which were evaporated under nitrogen. The resultant was reconstituted with twenty-five milliliter of acetonitrile. The resulting concentration was calculated as per (AOAC, 2000).

2.5.2 Standard Derivatization

Each standard solution was dried under a stream of nitrogen and 200 µl hexane was added to dissolve aflatoxins in hexane. One hundred microliter of trifluoroacetic acid was added, mixed for five minutes. Two milliliters of Acetonitrile and water in the ratio of (ACN: DH_2O 10:90) was added and the aqueous layer filtered; and using 0.45 µm inject into high performance liquid chromatography system.

2.5.3 Sample Preparation According to LS Bates (with modifications)

Fifty grams of the ground sample was weighed in triplicate and transferred into 250ml flask then added with 200 ml 80% methanol. The mixture was ultrasonicated for 2 minutes, filtered and pipetted where 100mls was transferred into 250 ml conical flask. Eighty milliliters of 20% Ammonium sulphate was added and then more forty milliliters of hexane added to the mixture; then ultrasonicated for two minutes. This was then drained, and the lower layer saved and transferred into a 200ml separating funnel. Six milliliter of methylene chloride was added and shook vigorously, then collected. Four milliliter of methylene chloride (chloroform) was added and shook vigorously to combine the two layers and a five milliliter of the solution was pipetted for clean-up and a further five milliliter preserved in the deep freeze at 8 ℃.

2.5.4 Column Preparation

Eight grams of sodium sulphate (Na_2SO_4) was added to a 22 X 400mm chromatographic tube to give base for silica gel. Chloroform was then added up to ¾ of the column. Ten grams of the activated gel was added.

Activating silica gel was prepared by weighing about 200 grams silica gel of size 0.04. The silica gel was then put in oven for one hour set at 105^{o}c. Two milliliters of distilled water were then added and shaken thoroughly to mix. Silica gel was then stored in an airtight container for more than fifteen hours. After addition of activated silica gel the column was left to settle for thirty minutes. Chloroform was then drawn until it was just above silica gel. Fifteen grams of the silica gel was added to the column and five milliliters of filtrate was added to column. Fifty milliliter of *n* hexane was added to the column and drawn to just above anhydrous sodium sulphate in order to remove pigments. Twenty milliliter of diethyl ether was added and drawn for the level to be just above that of anhydrous sulphate to remove fats and oils. Twenty milliliters of methanol: chloroform (3:97) mixture was then added in order to elute the toxins.

2.5.5 Sample Derivatization

Each of the sample was dried under a stream of nitrogen and two hundred microliters of hexane added to dissolve aflatoxins. One hundred microliter of trifluoroacetic acid was added to the mixture which settled for five minutes. Two milliliters of Acetonitrile and distilled water (10:90) was added to the mixture and the aqueous layer filtered; and from the mixture 0.45 µm was injected into the reverse phase high performance liquid chromatography system.

2.5.6 Spiking

Fifty grams of the ground sample was weighed in triplicate and transferred into a 250 ml conical flask. In the flask, 200 ml of 80% methanol and 600µl aflatoxin B_1 of 24 ppm and 800 µl 16 ppm B_2 and G_2 were added in the sequence as stated. The mixture was ultrasonicated for two minutes, then one hundred milliliters was filtered and pipetted and transferred to a 250ml conical flask where 80 ml 20% Ammonium sulphate was added followed by addition of 40 mls hexane and ultrasonication for two minutes. It was then drained, and the lower layer was saved and transferred into a 200ml separating funnel 6ml of methylene chloride was added. This was then

shaken vigorously and the lower layer of Methylene Chloride (Chloroform) was collected. The two layers were then combined and shaken vigorously, and a five milliliter was pipetted for clean-up whereas another five milliliters was preserved in the deep freezer.

2.6 Data Analysis

SPSS Version 23 with Duncan test program software was used for statistical analysis and results was presented as mean ±SD. Comparison of the mean difference was done by one-way analysis of variance (ANOVA) and significance differences between the means was established at $p<0.05$. Data was presented using graphs and tables.

3. Results and Discussion

3.1 Preliminary Study

3.1.1 Post-harvest Handling Techniques and Moisture Content (%)

The results for the moisture content for the samples from different farmers are presented in Table 1. The percentage moisture content for the chia samples ranged between 6.49±1.26 and 9.16±0.43. This was within the range reported by authors who did similar studies on flax seeds and chia seeds which had a range of 6- 8 (FAO, 2015) There were significant variations in the moisture content of the samples obtained from the different farmers. This could be attributed to lack of a standard method which farmers can use in the determination of moisture content level. From the preliminary study done, most of the chia farmers use traditional methods where they either chewed, touched or looking to determine if the chia seeds are dry. The counties where samples were collected have different climatic conditions and how they dry the chia seeds and the amount of exposure time to the sun during drying could also lead to the difference in moisture content between the samples from different counties.

Table 1. Moisture content analysis of means with Duncan test of samples from different counties

Sample Area	Sample description	N	% Moisture content	Standard Error	95% Confidence Interval for Mean		Minimum Level	Maximum Level
					Lower Bound	Upper Bound		
1.Naivas	100A1	3	9.06±0.41[a]	0.03	8.69	9.43	9.04	9.09
Supermarket	100B1	3	9.16±0.43[b]	0.24	9.05	9.27	9.12	9.20
	100C1	3	9.04±0.02[a]	0.01	9.00	9.07	9.02	9.05
	Total	9	9.09±0.07[a]	0.02	9.03	9.15	9.02	9.30
2.Nyeri	101A1	3	8.02±0.01[a]	0.01	7.92	8.12	8.01	8.03
	101B1	3	7.92±0.02[a]	0.01	7.90	7.94	7.91	7.93
	101C1	3	7.81±0.06[a]	0.04	7.66	7.97	7.76	7.88
	Total	9	7.91±0.09[a]	0.03	7.83	7.98	7.76	8.03
	102A1	3	7.88±0.02[a]	0.02	7.69	8.07	7.87	7.90
3. Nakuru	102B1	3	6.49±1.26[c]	0.73	3.35	9.63	5.10	7.57
	102C1	3	7.91±0.03[a]	0.02	7.83	7.99	7.88	7.94
	Total	9	7.36±0.99[c]	0.35	6.54	8.20	5.10	7.94
4.Trans Nzoia	103A1	3	8.36±0.00[a]	0.00	8.35	8.37	8.36	8.36
	103B1	3	8.37±0.02[a]	0.01	8.32	8.43	8.35	8.39
	100C1	3	8.33±0.01[a]	0.01	8.31	8.36	8.32	8.34
	Total	9	8.36±0.02[a]	0.01	8.33	8.38	8.32	8.39
5. Busia	104A1	3	9.11±0.08[a]	0.03	8.69	9.33	9.04	9.09
	104B1	3	9.15±0.23[b]	0.14	9.02	9.27	9.12	9.21
	104C1	3	9.08±0.0[a]	0.01	9.00	9.17	9.02	9.05
	Total	9	9.11±0.04[a]	0.01	9.05	9.16	9.02	9.20

All values are mean values of triplicates ± standard deviations. Values in the same column with different letters are significantly different ($p<0.05$) by Duncan test.

It is important to get the timing right for harvesting as in the preliminary studies it was observed that farmers have different ways of noting that the time for harvesting is ready. The farmers look at the change of flower colors which was like studies done on ground nuts where the color change f petals denoted that the plant was ready for harvesting. If the seeds are harvested too early, then the seeds will shrink and may lower the oil content

and quality of the seed. If the seeds are delayed at the farm, then chia seeds may drop on the ground leading to yield losses and poor-quality seeds due to mould infection

3.2 Mould Characterization and Enumeration

The results for moulds levels in the samples from different counties are presented in Table 2. In the analysis, different types of moulds were identified which includes; *Fusaruim* spp, *Rhizopus* spp, *Alternaria* spp and *Trichoderma* spp. From the results, it was observed that the county that had the most mould contamination of chia seeds is Nyeri county with the highest levels of *Fusarium* and *Rhizopus* species. The county with the lowest level of mould contamination is From the results, the county with the lowest level of mold conatmination is Busia county. This could be deduced from the fact that the climatic conditions in Nyeri county are very humid and the mold growth is accelerated in such conditions while in Busia has dry and hot climatic conditions that do not favour mould growth and mould contamination.. The most predominant mould species according to the result was observed to be *Rhizopus* species. This is similar to the studies done by Rifai (1969) which indicated that *Rhizopus spp* are very common in soil and air. The acceleration of mould growth is favoured by the humid conditions, nutrients for the fungi and also poor post harvest handling techniques (USAID, 2012). From the preliminary studies indicated in the appendix, it is observed that farmers in the sampled counties spread the chia seeds on a gunny bag to dry on the sun. There are many types of fungi that are found in soil and air that could contaminate the chia seeds. The accelerating factor is that the climatic conditions in Nyeri county favoured even more fungi to be present in the chia seeds.

Table 2. Mould enumeration in the sampled chia seeds from different counties

Mould Type	Busia	Nyeri	Naivas	Nakuru	Trans Nzoia
Fusarium	333.33	1333.33	333.33	1000	1000
Alternaria	0	333.33	333.33	333.33	333.33
Trichoderma	0	666.67	0	0	333.33
Rhizopus	1333.33	2666.67	1333.33	1333.33	2000

Upon observing the petri dishes macroscopically, chia sprouts were observed in a number of the petri dishes confirmimg that the chia seeds were not dry enough. Further moulds observation macroscopically and microscopically, it was evident that colonies of predominant fungal species of *Rhizopus* were identified positively. The county with the most fungal growth was observed to be Nyeri county and the least was observed to be Busia county. This can be deduced from the fact that the humid climatic conditions in Nyeri favoured the mold growth unlike the hot and dry conditions in Busia county. Across all counties, *Rhizopus spp* was evidently present than any other species. *Trichoderma* spp was less predominant and this could be because they are not commonly occuring moulds.

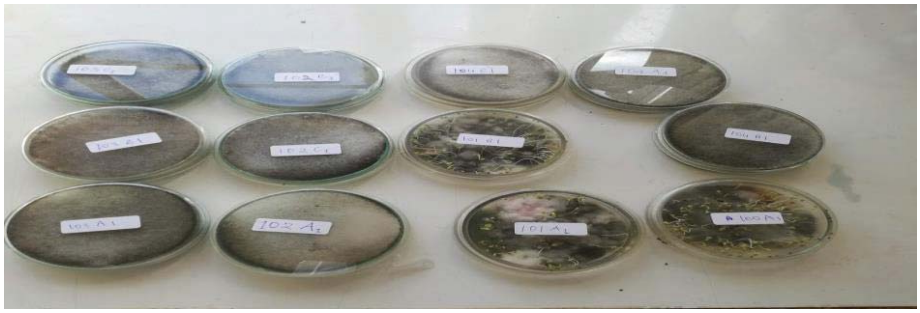


Figure 1. Picture of macroscopical characters of the fungal growth observed on potato dextrose agar media

Chia sprouts could be growing in diferent petri plates as an indication that the chia seeds contained higher moisture content.This is evident in the preliminary study as the farmers do not have an objective way of determining whether the chia seeds are dry enough. The results were similar to the study done in Uganda for post harvest losses in grains that denoted that farmers in Uganda did not have an objective way of determinging dryness of grains and so when they processed, it resulted to yield losses (USAID, 2012).

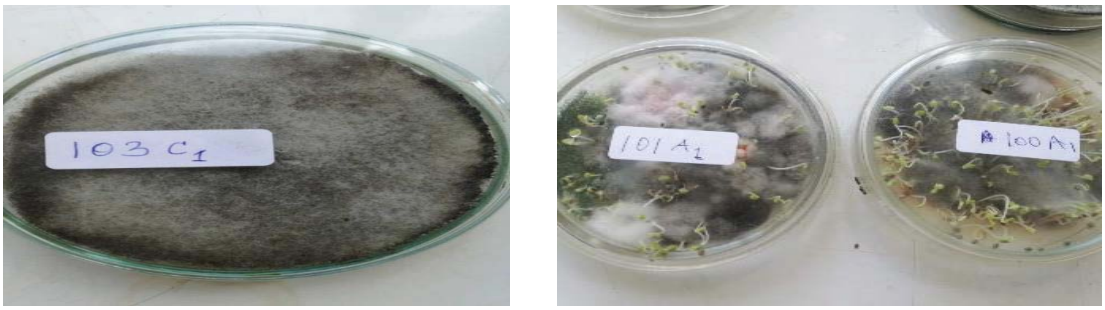
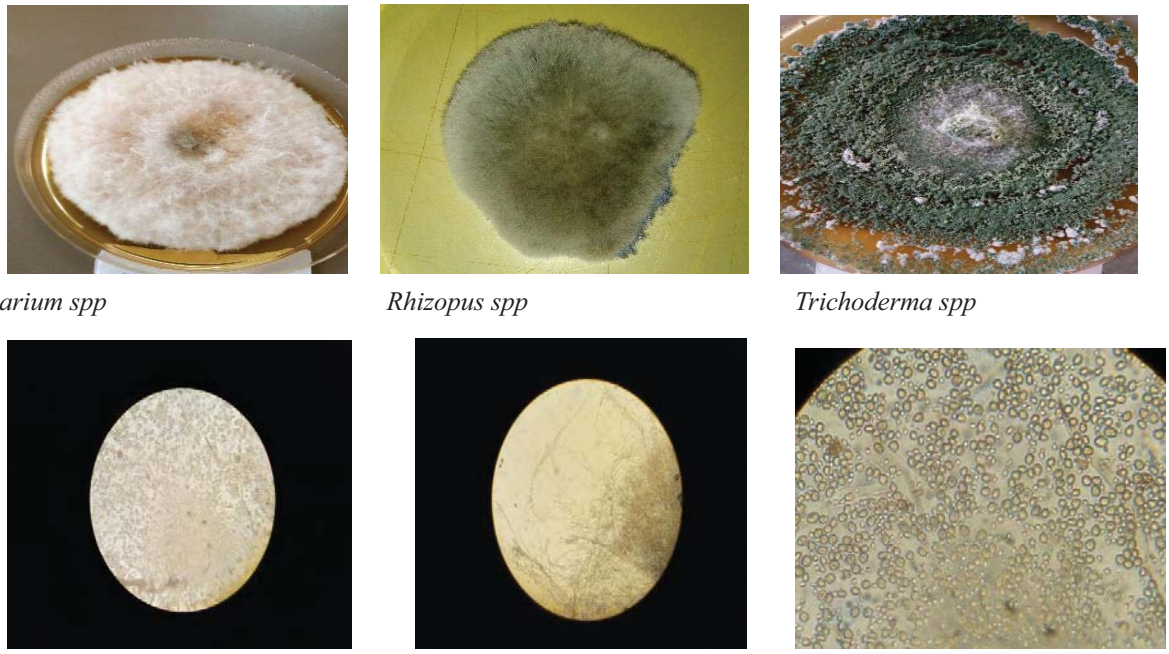


Figure 2. Colonies of *Rhizopus spp* and chia sprouts observed macroscopically

3.2.1 Morphological Description of Identified Moulds Species

Morphological analysis was done by microscopic and macroscopic technique according to Samson *et. al*, (2014) where the colony characters such as color and texture of the mycelium, characteristics of conidiophores such as shape of conidial heads, color of stipes were examined. Moulds were identified and characterized according to their unique morphological features where a drop of methylene blue was added to the slide and cover slip put on top where observation was done under a light microscope at magnification X40.

From the results, different molds types were observed macroscopically as shown in the photos of the petri plates above. Upon transferring the molds to MEA, the different mould species were noted and macroscopically identified.



Fusarium spp *Rhizopus spp* *Trichoderma spp*

Figure 3. Picture of microscopical characters of the different type of molds as seen under a light microscope of X40 magnification

3.3 Aflatoxin Analysis Quantification

The results for aflatoxin quantification are presented in Table 3. From the analysis, there were no aflatoxins detected in the samples from all the counties. The stock solution of the prepared standards Aflatoxin B_1, G_1 and G_2, were used in the analysis and the results for all the samples did not match any of this aflatoxin standards.

Table 3. Aflatoxin quantification analysis of the chia seeds samples of farmers from different counties

Counties	Samples	Aflatoxin B1	Aflatoxin G1 and G2
Busia	Farmer 1	Not Detected	Not Detected
	Farmer 2	Not Detected	Not Detected
	Farmer 3	Not Detected	Not Detected
Nyeri	Farmer 1	Not Detected	Not Detected
	Farmer 2	Not Detected	Not Detected
	Farmer 3	Not Detected	Not Detected
Naivas	Farmer 1	Not Detected	Not Detected
	Farmer 2	Not Detected	Not Detected
	Farmer 3	Not Detected	Not Detected
Nakuru	Farmer 1	Not Detected	Not Detected
	Farmer 2	Not Detected	Not Detected
	Farmer 3	Not Detected	Not Detected
Trans Nzoia	Farmer 1	Not Detected	Not Detected
	Farmer 2	Not Detected	Not Detected
	Farmer 3	Not Detected	Not Detected

Upon subjecting the samples to the high-performance liquid chromatography for mycotoxin quantification, the following chromatograms were observed. The aflatoxin standard was derived from the stock solution and prepared for reverse phase high-performance liquid chromatography where upon injection, the chromatogram 1A below was observed.

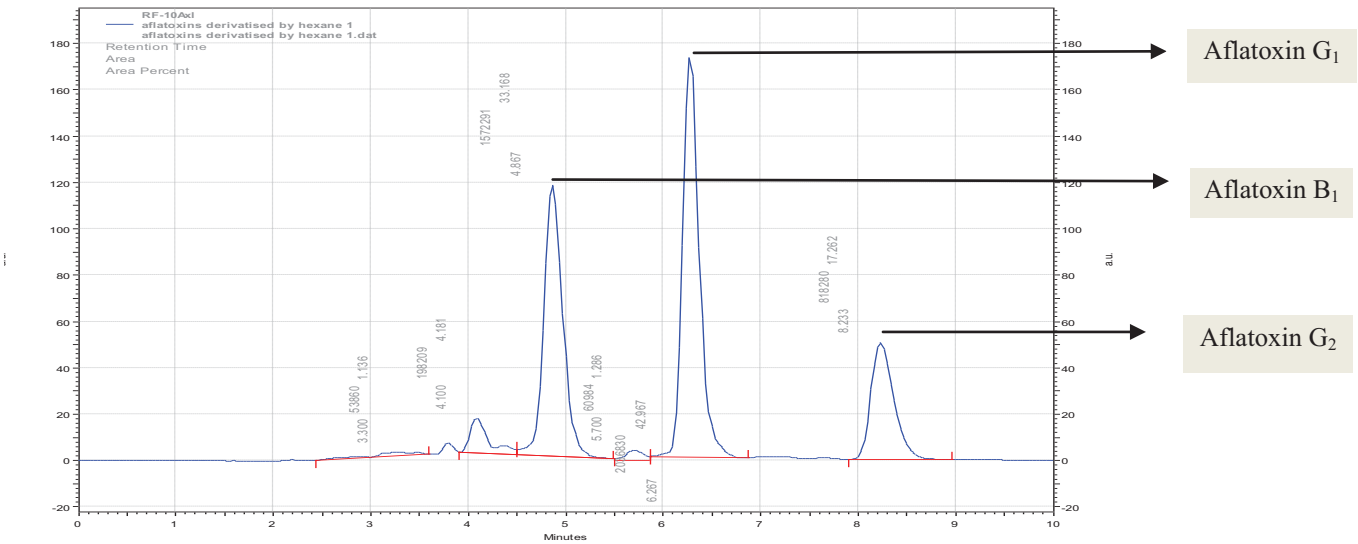


Figure 4. Chromatogram for the standards aflatoxin solution upon injecting into the high-performance liquid chromatography

The chromatogram below depicts the standard solutions chromatogram where they were overlaid as shown in the below chromatogram to indicate the mycotoxins of interest for ease of identification.

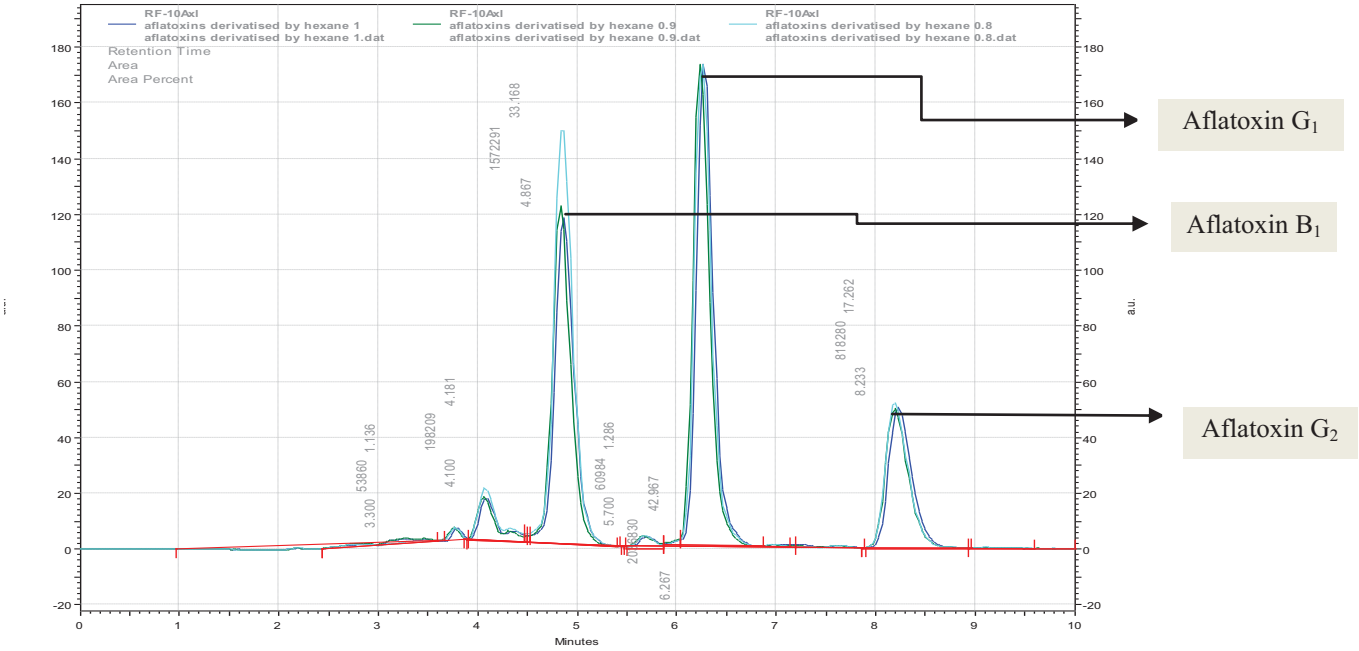


Figure 5. Chromatogram of overlaid standards

The chromatogram in figure 6 indicates that no mycotoxin of interest was present, and this was counterchecked by spiking the sample to check for accuracy. Quality assurance for the data was also done to ensure that the R^{2-} was correct**.** The chromatogram attached in the appendix also shows that no aflatoxin of interest was observed in the chia seed sampled from the farmers in the different counties. Spiking was done to countercheck the methodology was correct and that the findings observed were true and correct.

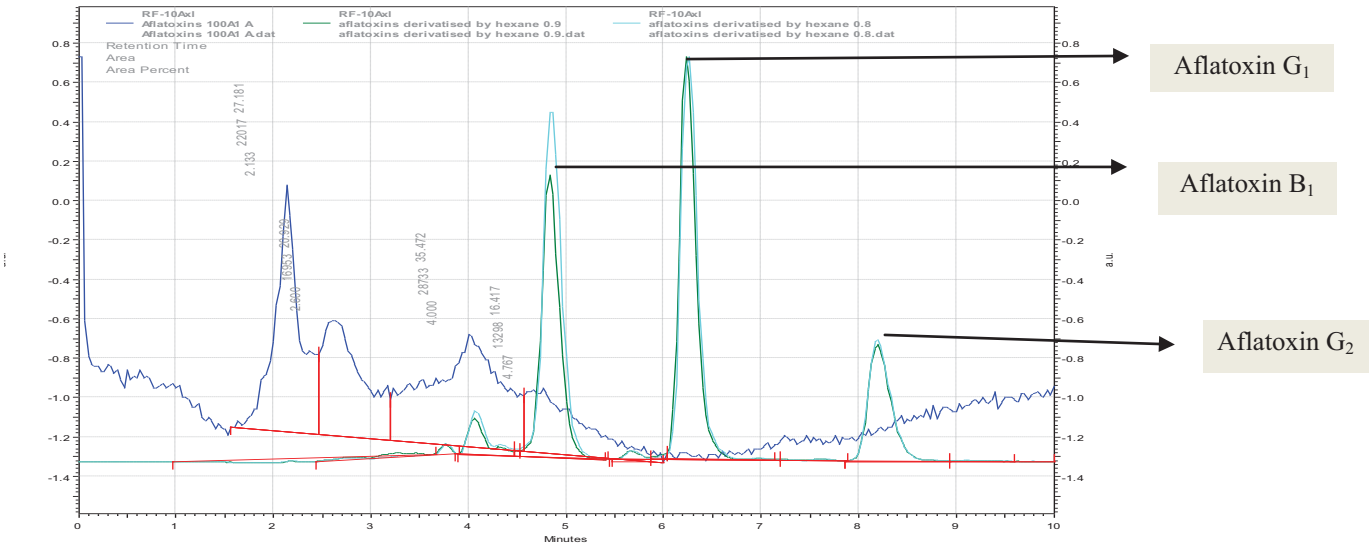


Figure 6. Sample chromatogram of the chia seeds aflatoxin analysis

4. Conclusion

From the preliminary study, as much as the different farmers have been trained on proper post-harvest handling techniques, modern technology needs to be employed in handling chia seeds post-harvest to reduce losses. This will reduce the manual handling and gut feeling from different farmers when determining the dryness of the chia seed to employ better shelling and winnowing technologies and separation of the chia seeds from the trash. The chia seeds purity level, moisture content, lack of damaged or diseased seeds play a great role in determining the

grade of the chia seeds and thus its price. The better the grade, the better the price and more revenue to the farmers.

The findings indicate that chia seeds from different farmers have different moisture content levels and thus an objective method of determining dryness of the seed should be developed and communicated to all chia seed farmers for standardization purposes. This will ensure that the moisture content is optimum and will not favor mould contamination no matter the climatic conditions. Grading of chia seeds should also be objectively done for standardization purposes through ascertaining scientifically the level of purity, moisture level and absence of diseases and aflatoxins.

Sampled chia seeds from different farmers did not have aflatoxin contamination although mould contamination was noted. Just as the European union has set a guideline on the thresholds of Aflatoxin maximum allowable limits, the government needs to put in policy for the maximum permissible threshold for mould contamination because most of these mould cause allergic reaction and mycotoxicosis in humans upon exposure or ingestion.

Acknowledgments

This study was carried out at the Institute of Food Bioresources Technology, Dedan Kimathi University of Technology, supported by the National Research Fund, Kenya.

Conflicts of Interest

The authors declare no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

References

Abarca, M. L., Bragulat, M. R., Castell, G., & Kabanes, F. J. (2006). Mycoflora and aflatoxins producing strains in chia seeds. *Journal of Food Protection, 57*(3), 256-258. https://doi.org/10.4315/0362-028X-57.3.256

Ali, M. A., Soad, A., & Hosny, A. L. (2005). Quantitative, qualitative and toxigenic evaluations of mold *in Salvia hispanica*. *Journal of Food Safety, 25*(1), 9-18. https://doi.org/10.1111/j.0149-6085.2005.25546.x

Anand, P., Kunnumakkara, A. B., Sundaram, C., Tharakan, S. T., Lai, O. S., Sung, B., & Aggarwal, B. B., (2008). Cancer is a preventable disease that requires lifestyle changes. *Pharm Rev.,* 25(9), 20-25. https://doi.org/10.1007/s11095-008-9690-4

AOAC Official method 994:08 (2000). *Aflatoxin in corn, almonds and pistachio nuts, multifunctional column (Mycosep) method*. Natural toxins chapter 4 (page 26-27). Official methods of analysis of AOAC international, 17th edition, volume II, AOAC international, Maryland, USA.

Arun, S., Desai, P. S. R., & Nadkarni, G. B. (2007). A new method for aflatoxin free storage of agricultural commodities. *Journal of Food Science, 52*(2), 497. https://doi.org/10.1111/j.1365-2621.1987.tb06652.x

Ayerza, R. (2009). The seeds protein and oil content, fatty acid composition and growing cycle length of a single genotype of Chia (*Salvia hispanica L.*) as affected by environmental factors. *Journal of Oleo science, 58*(7). https://doi.org/10.5650/jos.58.347

Ayerza, R. (2013). Seed composition of two chia (*Salvia hispania L.*) genotypes which differs in seed color. *Emir Journal of Food Agriculture, 25*, 495-550. https://doi.org/10.9755/ejfa.v25i7.13569

Bochicchio, R., Rossi, R., Labelle, R., Bitella, G., Perniola, M., & Amato, M. (2015). Effect of sowing density and nitrogen top dress fertilization on growth and yield of chia (*Salvia hispanica L.*) in a Mediterranean Environment: First results. *Italian journal of Agronomics, 10*(3), 163-166. https://doi.org/10.4081/ija.2015.640

Bullerman, L. (2006). Mycotoxins and food safety. *Food Technology*, 59-66.

Bukola, C. A., Abiodun, A. O., & Ukpe, G. P. (2008). Microflora of Chia seeds Sold in Uyo, Eastern Nigeria. *World Journal of Agricultural Sciences, 4*(3), 346350.

Busillachi, H., Quiroga, M., Bueno, M., Di Sapio, O., Flores, V., & Severin, C. (2013) Evaluation of *Salvia Hispanica* cultivated in Santa Fe (Republic of Argentina). *Cultivated in Tropics, 34*(4), 55-59.

Brunding, G. J. (2008). *Biology and Cultivation of Chia Seeds*. Academic Press, New York.

Bobek P., Ozdin, O., & Mikus, M. (1995). Dietary of Chia seeds accelerates cholesterol turnover in hypercholesterolemia rats. *Physiological Research Journal, 44*(5), 287-291

Cahill, J. P., & Provance, M. C. (2004). Genetics of qualitative traits in domesticated chia (*Salvia hispanica L.*). *Journal of Heredity, 93*(1), 52-55. https://doi.org/10.1093/jhered/93.1.52

CDC, (2015). Aflatoxicoses outbreak in Kenya -2015: Report to the Ministry of Health, Kenya. Center for Disease Control.

Centers for Disease Control and Prevention (2004). Outbreak of aflatoxin poisoning-eastern and central provinces, Kenya, January-July 2004. *MMWR Mortal weekly Rep., 53*, 790-793.

Coates, W., & Ayerza, R. (2006). Production potential of chia in North Western Argentina. *Journal of crops production, 5*, 229-233. https://doi.org/10.1016/0926-6690(96)89454-4

FAO. (2004). Worldwide regulation for mycotoxins in food and feed in 2003.FAO Food and Nutrition paper 81. Rome: Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/docrep/007/y5499e/y5499e00.htm

JEFCA (Joint FAO/WHO Expert committee on food additives) (2008). In sixty-eight meeting, 19-28 June, Geneva

Ludwig, T., Worsch, S., Heikenwalder, M., Daniel, H., Hauner, H., & Bader, B. L. (2013). Metabolic and immunomodulatory effects on n-3 fatty acids are different in mesenteric epididymal adipose tissue of diet-induced obese mice. *American journal of physiology in endocrinal metabolism, 304*(11), 1140-1156. https://doi.org/10.1152/ajpendo.00171.2012

Menga, V., Amato, M., Phillips, T. D., Angelino, D., Morreale, F., & Fares, C. (2017). Gluten-free pasta incorporating chia (*Salvia hispanica L.*) as thickening agent: An approach to naturally improve the nutritional profile and the in vitro carbohydrate digestibility. *Journal of Chemistry, 221*, 1954-1961. https://doi.org/10.1016/j.foodchem.2016.11.151

Perrone, G., Susca, A., Cozz, G., Ehrlich, K., Varga, J., Frisvad, J. C., … Samson, R. A., (2007). Biodiversity of *Aspergillus* species in some important agricultural products. *Study of Mycology, 59*, 53-66. https://doi.org/10.3114/sim.2007.59.07

Proctor, R. H. (2009). Evidence that a secondary metabolic biosynthetic gene cluster has grown by gene relocation during evolution of the filamentous fungus *Fusarium*. *Molecular Microbiology. Malden, 74*(5), 1128-1142. https://doi.org/10.1111/j.1365-2958.2009.06927.x

Rifai, M. A. (1969). A revision of the Genus Trichoderma. *Mycology Paper, 116*, 1-56.

Samson, R. A., Visagie, C. M., Houbraken, J., Hong S. B., Hubka, V., Klaassen, C. H. W., Perrone, G. … Frisvad, J. C. (2014). Phylogeny, identification and nomenclature of the genus Aspergillus. *Studies in Mycology, 78*, 141-173. https://doi.org/10.1016/j.simyco.2014.07.004

Yao, W., Li, J., Wang, J. J., Zhou, W., Wang, Q., Zhu, R., Wang, F., & Thacker, P. (2012). Effects of dietary ratio of n-6 to n-3 polyunsaturated fatty acid on immunoglobulins, cytokines, fatty acids compositions and performance of lactating sows and suckling piglet. *Journal of animal science and biotechnology, 3*(1), 43-50. https://doi.org/10.1186/2049-1891-3-43

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Modeling the Influence of Temperature-dependent Thermal Properties on the Freezing Front

Victor M. Chavarria[1]

[1]Food Research Consultant, La Paz, Bolivia
Correspondence: Victor M. Chavarria, La Paz, Bolivia. Tel: 591-22752177. E-mail: victor.chavarria@yahoo.com

Received: September 25, 2019 Accepted: October 17, 2019 Online Published: October 30, 2019

doi:10.5539/jfr.v8n6p129 URL: https://doi.org/10.5539/jfr.v8n6p129

Abstract

Although numerical methods enable comprehensive analyses of food freezing, a thorough quantification is lacking the effects on the process introduced by uncertainties in variable thermal properties. Analytical models are, however, more suitable tools to perform such calculations. We aim to quantify these effects by developing a solution to the freezing front (FF) problem subject to temperature-dependent thermal properties and one-dimensional convective cooling. The heat integral balance method, Kirchhoff's transformation, and Plank's cooled-surface temperature equation (as a seed function) enabled us to obtain an approximate solution to the FF penetration time (τ). To optimize model accuracy, two adjustable parameters were correlated with the inputs via nonlinear regression referenced to numerical simulation FF data. The mapped (τ) sensitivities, generated by perturbations in the temperature-dependent thermal conductivity and effective heat capacity (σ_κ and σ_λ, respectively), undergo rapid nonlinear changes for Biot numbers ≤ 6. Above this level, these sensitivities stabilize depending on the cooling medium temperature and a thermal conductivity parameter. The median σ_κ is 0.348 and its interquartile range (IQR) is 0.220 to 0.425, whereas the median σ_λ is 0.967 (IQR: 0.877 to 0.985). Statistical error measures and a ten-split *K*-fold validation support the model accuracy and reliability of the parameter estimates. Together, the model allows for gaining insights into the nonlinear behavior and magnitude of the influence of variable properties on the FF for a wide range of conditions. Nonlinear methods and prior information enable practical modeling of transport phenomena in foods.

Keywords: freezing front, moving boundary, mathematical modeling, perturbation analysis, temperature-dependent thermal properties

1. Introduction

The exact mathematical analysis of phase change in foods is largely limited by nonlinearities introduced a set of assumptions comprising temperature-dependent material properties, convective cooling (heating) and finite domains. Because of their multi-component system, structure and stability properties, thermal properties are difficult to measure and model accurately (Heldman & Singh, 1981). Consequently, it is relevant to understand the impact that uncertainties in physical properties have on product design to energy consumption aspects of industrial freezing operations (Schwartzberg, 1976; Pham & Willix, 1990).

Invoking the moving boundary (MB) concept reduces the complexity of the phase change problem by formulating one or more material regions separated by well-defined interfaces and subject to simplified physical assumptions. For example, pseudo-steady state and constant thermal property assumptions were central to Plank's analysis of freezing times of food materials (López-Leiva & Hallström, 2003). Analytic solutions of the freezing front (FF) with variable thermal properties are available only for limited cases (Kucera, 1985; Lunardini, 1988; Ramos, Cerrato & Gutierrez, 1994; Voller, Svenson & Paola, 2004; Singh, Gupta & Ral, 2011; Rajeev, 2014; Kumar & Singh, 2018). Specifically, the problem becomes analytically intractable when formulated with convective cooling, finite geometry, gradual heat release (absorption), and temperature-dependent thermal property models with meaningful physical parameters (Özisik, 1980; Delgado & Sun, 2001; Schwartzberg, Singh & Sarkar, 2007).

Although numerical models are suitable to solve complex phase change problems, analytic models are more efficient and compact to understand the specific roles that variable thermal properties play in the process (Özisik, 1980). The central objective of this exploratory research is to quantify and characterize the effects of variable thermal properties on the freezing front. To accomplish this aim, we apply nonlinear methods to analyze the FF problem subject to the

aforementioned formulation. Our modeling strategy includes (1) solution to the FF problem using nonlinear methods and prior information characterizing the convective surface temperature, (2) FF data generation via numerical simulation, (3) nonlinear-regression estimation of two adjustable parameters to optimize model accuracy, (4) implementation of the *K*-fold model validation, and (5) quantification and mapping of the effects of temperature-dependent thermal properties on the FF.

2. Literature Review

The MB formulation has been applied to processes like food freezing, thawing, frying, ablation, bubble growth, sedimentation, and shoreline movement (Kucera, 1985; Farid, 2002; Voller et al., 2004; Rajeev, 2014). This partial review identifies analytic methods used in heat conduction problems with moving boundaries characterized by the Stefan condition.

Throughout this paper, we refer to the following assumptions:

- A1, constant thermal properties for each phase;
- A2, Dirichlet, fixed cooling medium temperature, boundary condition (BC);
- A3, Robin BC with constant medium temperature;
- A4, Neumann BC with constant surface heat flux;
- A5, semi-infinite domain;
- A6, one-dimensional finite geometry;
- A7, initial temperature at the initial freezing point;
- A8, no sensible heat release;
- A9, total latent heat release at a unique temperature;
- A10, pseudo-steady state (PSS), for Stefan values smaller than 1; and
- A11, temperature-dependent thermal properties and gradual heat release (absorption).

Although we review some relevant solutions to illustrate their functional expressions, we focus on the potential analytical methods to solve the MB problem with conditions A3, A6, A7, and A11. An in-depth theoretical treatment of the Stefan problem can be found elsewhere (Goodman, 1958; Solomon, Wilson & Alexiades, 1981; Kucera, 1985; Lunardini, 1988; Mitchell & Myers, 2012).

Neumann applied similarity transformation to a two-phase freezing problem with assumptions A1, A2, A5, and A9 (Solomon et al., 1981). His exact solution describes the moving boundary ($X(t)$) being proportional to $\sqrt{t}$. This square-root of time relation is a well-known result of MB physics. By further imposing assumption A10, the similarity solution becomes $X(t) = \sqrt{2\,\mathrm{St}\,\alpha_f\,t}$. If thermal properties and gradual latent heat release are temperature dependent, the FF motion may depart from the $\sqrt{t}$ law (Bazant, 2018). The set of assumptions behind Plank's freezing time Eq. (1) includes A1, A3, A6 to A10 (Hu & Argyropoulos, 1996). In addition to small Stefan numbers, assumption A10 also requires negligible diffusion in the freezing region (Kucera, 1985; Lunardini, 1988). Several modifications of Eq. (1) have been developed to compensate for Plank's simplified set of assumptions (Pham, 1986; Mittal, Hanenian & Mellikarjunan, 1993; Delgado & Sun, 2001; Fricke & Becker, 2001). Plank's freezing time equation is given by:

$$\tau_{Pk} = \frac{s(\tau)}{\mathrm{Bi}\cdot\mathrm{St}}\,(0.5\,\mathrm{Bi}\,s(\tau) + 1), \tag{1}$$

where $s(\tau)$ is the dimensionless FF, τ the dimensionless time, Bi the Biot number, and St the Stefan number; all based on the frozen-state material properties.

Considering that St is less than 1 for most foodstuffs (Schwartzberg, 1976), its effect is to slow down the diffusion process. Kucera (1985) applied boundary fixing transformation and iterative series methods to the Stefan (solidification) problem invoking assumptions A1, A4, A5, A7, A9, and A10. The upper-bound FF penetration time expression given by Kucera is:

$$\tau_{\mathrm{Kc}} = \tau_{\mathrm{Pk}} + s(\tau)^2\,\frac{(3+\mathrm{Bi}\,s(\tau))}{6(1+\mathrm{Bi}\,s(\tau))}, \tag{2}$$

where τ_{Pk} is estimated from Eq. (1), and valid for long times $\tau \geq \mathcal{O}[\mathrm{St}^{-1}]$.

Goodman's (1958) melting analysis implemented the heat balance integral (HBI) method with assumptions A1, A4, A5, A7, and A9. Several HBI investigations have addressed the Stefan problem with assumption A1, A3 or A4, A6, and A9 using polynomial, exponential or trigonometric temperature profiles (Özisik, 1980; Wood, 2001; Mitchell & Myers, 2012). The latter authors recommended a cubic polynomial for HBI analysis of melting problems as optimum modeling strategy. This HBI volume-averaging method allows removing the temperature variable from the energy partial differential equation (PDE) and reducing it to an ordinary differential equation (ODE). Özisik (1980, p. 419) applied similarity variable transformation to the frozen phase and HBI to the freezing phase using assumptions A1, A2, A6, and A7. His solution requires, however, solving a transcendental equation and specifying a temperature-polynomial degree. Baudouy (2002) implemented Kirchhoff's transformation and HBI methods for heat transfer in Helium II with assumptions A2 and A5. It has been proven that for $c_p \approx 0$, the solution to the Stefan problem with assumptions A1, A3, and A6 to A9 approaches the PSS solution (Solomon et al., 1981). Together, the aforementioned methods, however effective in obtaining approximate FF solutions, did not solve the phase change problem considering assumptions A3, A6, A7, and A11.

Variational iteration (Singh et al., 2011) and homotopy perturbation (Rajeev, 2014) methods were applied to space-variable physical properties and latent heat in Stefan problems. Ramos et al. (1994) implemented the group transformation theory to solve for transient temperatures during freezing (thawing). Their assumptions A3, A6, and A7 also included a variable effective heat capacity (albeit difficult to interpret) and thermal conductivity described, however, by empirical power-law models.

Cho and Sunderland (1974) used a modified error function to solve the Neumann problem considering a linearly temperature-dependent thermal diffusivity. The effect on the moving boundary was significant only for thermal diffusivity with marked temperature dependence and for large Stefan numbers ($St \gg 1$). Kumar and Singh (2018) found comparable results by applying similarity variable transformation and Chebyshev polynomials. Their assumptions comprised A2, A5, specific heat and thermal conductivity properties that followed assumed empirical power-law functions of temperature.

Heldman and Singh (1981) described the influence variable thermal properties on temperature fields applying numerical and experimental methods but without systematic analysis of errors in thermal property data. This type of analysis has been addressed relying on (semi) empirical freezing-time correlations and yielding only mean effects for a narrow range of input physical parameters (Mittal et al. 1993; Fricke & Becker, 2001; Rao, Rizvi & Datta, 2005). Whereas the reported sensitivities due to perturbations in frozen thermal conductivity (σ_κ) vary from ± 0.01 to ± 0.15, the sensitivity driven by latent heat perturbations (σ_λ) is approximately 1.16. Pham and Willix (1990) conducted an error analysis of process times due to Biot number deviations, but not thermal properties. Mittal et al. (1993) evaluated the influence of errors associated with thermal properties on the freezing process considering twelve well-known freezing-time (semi) empirical correlations. The latter are essentially modifications of Plank's Eq. (1).

Collectively, a specific understanding of the role of variable temperature-dependent thermal properties on the freezing front is not thorough and has thus motivated this investigation. An analytical and accurate FF model based on realistic assumptions would enable such quantification. The advantages of such a model over numerical solutions include no computer programming, no numerical convergence or stability problems, and straightforward calculations of the effects of variable thermal properties on the MB. Additionally, the model could be used as a convenient educational tool to describe the FF in food materials.

3. Methods

Our analytical strategy comprised HBI and Kirchhoff's transformation methods and a simple convective surface-temperature expression as a seed function. Ideally, the analysis would yield an FF solution that leads to an improved temperature equation. The iterative process is repeated until it converges. If the functional complexity of the updated surface-temperature expression curtails the iteration process, the accuracy of the first solution is optimized by nonlinear regression of adjustable parameters introduced in the seed function.

3.1 Numerical Models

Strategy steps (1) to (3) relied on Wolfram Mathematica's 11 symbolic, *NDSolve* (Method of Lines), and *NonlinearModelFit* functions for analysis, numerical simulation, and nonlinear regression calculations, respectively. Mathematica's least-squares minimization uses the Levenberg-Marquardt algorithm. A numerical model implemented the finite difference Crank-Nicolson time-stepping scheme (CNFD) and the Thomas algorithm in MS Excel 10 to solve the phase change problem (Carnahan, Luther & Wilkes, 1969). This model allowed for generating one-dimensional transient temperatures and FF position-time data. We validated the

CNFD model against the exact series solution of the heat conduction assuming constant thermal properties (Özisik, 1980). CNFD-model freezing predictions were also compared to Mathematica's numerical temperature solution and to Pham and Willix's (1990) experimental freezing times for tylose substance. The latter validation yielded a Pearson correlation coefficient of 0.998 ($p* < 0.01$).

3.2 Statistical Error Analysis and Validation

Error analysis of nonlinear regression in step (3), fit quality evaluation and selection of correlation models followed published methodologies (Beck & Arnold, 1977; Archontoulis & Miguez, 2015). Mathematica's Generalized Linear Model (GLM) enabled quantifying the significance of thermal property effects on FF penetration-time sensitivities. To validate the final parameterized model in step (4), we implemented a ten split *K*-fold method with a 90:10 data partition. The model training and error validation sets comprised 1 350 and 150 data points, respectively. The final model accuracy is an average of ten validation *K*-fold error estimates (Arboretti-Giancristofaro & Salmaso, 2003; Jung & Hu, 2015).

3.3 Thermal Property Models

To quantify the influence of variable thermal properties on the FF in step (5), we adopted Schwartzberg's (1976) thermal conductivity ($\hat{k}$) and effective heat capacity ($\hat{c}_p$) models. Below the initial freezing point, the normalized properties are given by $\hat{k}(\theta) = 1 - \kappa / (1 - \theta)$ and $\hat{c}_p(\theta) = 1 + \lambda / (1- \theta)^2$. Each model depends on a single thermal-property parameter that controls their temperature dependence. Estimation of κ and λ is straightforward using published thermal property data. These models provide reliable estimates of $\hat{k}$ and $\hat{c}_p$ applicable to a practical range of food types and industrial freezing conditions (Schwartzberg, 1977). Further, the models have monotonic and differentiable properties over the entire freezing temperature range (Figure 1).

3.4 Freezing Front Calculation

Rather than an infinitesimal thin interface, the FF that develops in multi-component foods occurs as a progressive, yet brief, decrease in temperature away from the initial freezing point (Farid, 2002). See the curved end of the plateau (top shadow) of the temperature surface in Figure 2. The FF location was computed as a time-heat event from simulated transient-temperature fields. When the normalized and accumulated heat release (relative to the initial material heat content) changes by a minute but detectable amount or threshold, it signals the front's transition. This detection limit was set equal to 0.334%, considering the total temperature measurement error, which comprises thermocouple probe, instrumentation, and calibration errors (Cottrell, 2006).

3.5 Freezing Front Penetration-time Sensitivity

To map the effects of variable $\hat{k}$ and $\hat{c}_p$, we generated perturbations in the single property parameter (κ or λ) that controls their temperature dependence. By introducing a relative error (uncertainty) in κ ($\hat{\epsilon}_\kappa = \pm 3\%$), keeping λ constant, the effect on FF penetration time was calculated as a sensitivity measure (σ_κ) equal to $\hat{\epsilon}_\tau / \hat{\epsilon}_\kappa$. The same procedure applied to perturbations in λ to calculate σ_λ as $\hat{\epsilon}_\tau / \hat{\epsilon}_\lambda$. The simultaneous effect of κ and λ perturbations on FF ($\sigma_{\kappa\lambda}$) was computed using Monte Carlo simulation with 5 000 iterations and assuming an SD of ±3% of the property parameter means. Under the law of propagation of uncorrelated and random errors, the joint sensitivity becomes (Tellingheusen, 2001):

$$\sigma_{\kappa\lambda} = \sqrt{\frac{(\sigma_\kappa \hat{\varepsilon}_\kappa)^2 + (\sigma_\lambda \hat{\varepsilon}_\lambda)^2}{\hat{\varepsilon}_\kappa^{\,2} + \hat{\varepsilon}_\lambda^2}}, \tag{3}$$

where $\sigma_{\kappa\lambda}$ is $\hat{\varepsilon}_\tau / \sqrt{\hat{\varepsilon}_\kappa^{\,2} + \hat{\varepsilon}_\lambda^{\,2}}$.

3.6 Numerical Test Design

Test grids in Table 1 were designed to control the following input factors: Bi, λ, ψ, κ, and *s* in the numerical computations (Chavarria, 2019). Whereas grid T1.1 was used for parameters (*n*) and (*m*) calculations, grid T1.2 applied to computation of σ_λ and σ_κ penetration time sensitivities. Test grid T1.2 was defined as full factorial. In turn, test grid T1.1 was designed considering $1/Bi^2$ and St effects on FF. The sample size (*N*) for grid T1.1 was estimated following Bellera and Hanley (2007). The sample points were defined according to the following cumulative (percentage) distribution with respect to Bi· St *levels*:

{{< 0.015, 55.1%}, {0.45, 69.6%}, {0.8, 73.5%}, {1.5, 80.2%}, {2.55. 88.5%}, {3.95, 92.5%}, {6.05, 94.9%}, {8.15, 96.7%}, {10.25, 99.0%}, {11,3, 100%}}.

Table 1. Numerical experimentation test design

Analysis	Factor	Levels							
Grid T1.1	λ			95*	105*	115*			
n and *m*	ψ		-40	-32	-24	-16	-8		
parameter	κ			0.4*	0.5*	0.6*			
estimation,	*s*	0	0.4	0.5	0.5	0.8	0.9	1	
$N = 1\ 500$	Bi	0.06	0.061	0.062	0.0634	0.64	0.65	0.0667	0.07
		0.0734	0.0767	0.080	0.834	0.090	0.093	0.967	0.30
		0.40	0.60	1	3	6	10	25	
Grid T1.2	λ			90	100	110	120		
σ_κ, σ_λ	ψ		-40	-32	-24	-16	-8		
$\sigma_{\kappa\lambda}$	κ			0.4	0.5	0.6			
calculations	Bi	0.06	0.07	0.08	0.09	0.1	0.14	0.18	
$N = 1\ 200$		0.22	0.27	0.35	0.45	0.60	1.5	3	
		6	10	13	17	21	25		
Grid T1.3	λ				105				
$\sigma_{\kappa\lambda}$	ψ		-40	-32	-24	-16	-8		
mapping	κ				0.5				
$N = 45$	Bi	0.06	0.09	0.667	1.25	3	6	12	
		18	25						

Test grids for the computation of the final model parameters and sensitivities generated by thermal property perturbations. Levels with an asterisk indicate additional values in the factor neighborhood

4. Analysis

4.1 Freezing Front Derivation

The one-dimensional MB assumes a virtual interface or surface separating the frozen phase from the freezing region across the slab thickness and moving at an unknown velocity towards the thermal center (Farid, 2002). This interface is defined by the Stefan condition that (a) links both temperature fields, (b) requires the rate of latent heat removal to balance the heat flux into the freezing region, and (c) forces the temperature continuity across the boundary (Kucera, 1988). The analytical task is to solve for both the transient thermal fields in each region and the FF location.

In the integral method, all the heat phenomena take place within a thermal layer that grows from the cooled surface towards the slab center. This dynamic depth ends where the heat flux is zero and the temperature remains at the initial phase change point. Once the slab center is reached, the thermal layer concept vanishes (Özisik, 1980).

The one-dimensional heat conduction PDE, which accounts for temperature-dependent thermal conductivity and effective heat capacity, but no density change, is given by (Heldman & Singh, 1981; Schwartzberg et al., 2007):

$$\hat{c}_p\big(\theta(r,\tau)\big)\frac{\partial\theta(r,\tau)}{\partial\tau} = \frac{\partial}{\partial r}\Big[\hat{k}\big(\theta(r,\tau)\big)\frac{\partial\theta(r,\tau)}{\partial r}\Big], \tag{4}$$

where the dimensionless spatial coordinate is $r = x/L$. The initial condition is $\theta\,(r, 0) = 0$, whereas at very long times $\theta\,(r, \tau \to \infty) = \psi$.

The BCs are:

$$\partial_r\theta\,(r,\ \tau)\ =\ 0 \quad \text{at}\ r\ =\ 1, \tag{5}$$

$$-\hat{k}(\theta_w(r,\tau))\,\partial_r\theta\,(r,\tau)\ =\ \mathrm{Bi}\,(\psi - \theta_w(\tau)) \quad \text{at}\ r\ =\ 0, \tag{6}$$

where $\theta_w(\tau) = \theta\,(0, \tau)$.

Applying Kirchhoff's transformation to the $\hat{c}_p\,(\theta)$ model, yields the new temperature u (Özisik, 1980):

$$u = \int_0^\theta \hat{c}_p(\theta)d\theta = \theta\ + \theta\,\lambda/(1-\theta). \tag{7}$$

If Eq. (7) is plugged into (4), we obtain:

$$\frac{\partial u(r,\tau)}{\partial \tau} = \frac{\partial}{\partial r}\left[\widehat{\alpha}\big(\theta(r,\tau)\big)\frac{\partial u(r,\tau)}{\partial r}\right], \tag{8}$$

where $\widehat{\alpha}(\theta) = \hat{k}(\theta)/\hat{c}_p(\theta)$ becomes an apparent diffusivity term that captures the nonlinear temperature effects. The r domain in the thermal layer ranges now from 0 to $s(\tau)$. Whereas the initial condition is $u(r, 0) = 0$, the BCs are:

$$u(r,\tau) = 0 \text{ and } \partial_r u(r,\tau) = 0 \qquad \text{at } r = s(\tau), \tag{9}$$

$$-\hat{\alpha}(\theta_w(\tau))\partial_r u(r,\tau) = \mathrm{Bi}(\psi - \theta_w(\tau)) \qquad \text{at } r = 0. \tag{10}$$

The RHS of Eq, (10) is a *prescribed function* of the convective heat flux in terms of s (short notation for $s(\tau)$) and is only a function of time. Therefore, it requires no Kirchhoff's transformation (Özisik, 1980). If Eq. (8) is integrated over r, from 0 to s, the HBI equation becomes:

$$\int_0^s \partial_\tau u(r,\tau)dr = \widehat{\alpha}(\theta)\,\partial_r u(s,\tau) - \widehat{\alpha}(\theta)\,\partial_r u(0,\tau), \tag{11}$$

where the first term on the RHS vanishes with the zero heat flux at $r = s(\tau)$. Applying Leibniz' integration rule together with $u(s, \tau) = 0$ to the LHS and Eq. (10) to the RHS of Eq. (11) yields:

$$\partial_\tau \int_0^s u(r,\tau)dr = \mathrm{Bi}\,(\psi - \theta_w(\tau)). \tag{12}$$

To eliminate θ_w in Eq. (12), we invoke the following convective-surface temperature function:

$$\theta_w(s) = \mathrm{Bi}\,m\,s\,\psi/(1 + \mathrm{Bi}\,m\,s), \tag{13}$$

which can be readily derived from Eq. (1) setting $m = 1$.

Even though Eq. (13) is a simple rational expression, its introduction as a seed function generates a solution with greater functional properties. Goodman (1958, p. 339) obtained a similar equation for St = 0; but even for 0 < St < 1, it can be shown that his equation still holds with a relative error of 1.2%. For food freezing processes, St values vary from 0.04 to 0.45 (Schwartzberg, 1977). Parameter m was introduced into Eq. (13) to account for Bi and St effects considering Goodman's analysis.

A temperature polynomial of n-th degree that satisfies Eq. (9) is given by:

$$u(r,\tau) = u_w(\tau)(1-r)^n. \tag{14}$$

Evaluating the u integral on the LHS of Eq. (12) with Eq. (14) yields:

$$\int_0^s u(r,\tau)dr = \frac{s\,u_w(s)}{1+n}. \tag{15}$$

If Equations (13) and (15) are plugged into (12), the HBI equation becomes:

$$\frac{\partial}{\partial \tau}\left[\frac{s\,u_w(s)}{1+n}\right] = \frac{\mathrm{Bi}\,\psi}{1+\mathrm{Bi}\,m\,s}. \tag{16}$$

Combining Equations (13) and (14) with (10), recasts the energy balance at the cooled surface as:

$$\frac{-n\,\widehat{\alpha}\,(\theta_w(\tau))\,u_w(s)}{s} = \frac{\mathrm{Bi}\,\psi}{1+\mathrm{Bi}\,m\,s}. \tag{17}$$

Using the expressions of $\hat{k}(\theta)$ and $\hat{c}_p(\theta)$ in $\widehat{\alpha}(\theta)$, we solve for u_w from Eq. (17):

$$u_w(s) = \frac{\mathrm{Bi}\,s\,\psi}{g_1(s)}[(\mathrm{Bi}\,m\,s)^2(\lambda + (\psi-1))^2) + 2\,\mathrm{Bi}\,m\,\mathrm{s}\,(\lambda - \psi + 1) + \lambda + 1], \tag{18}$$

where $g_1(s)$ is:

$$g_1(s) = n\,(\mathrm{Bi}\,m\,s + 1)(1 - \mathrm{Bi}\,m\,s\,(\psi - 1))\,(\mathrm{Bi}\,m\,s\,(\kappa + \psi - 1) + (\kappa - 1)).$$

Plugging Eq. (18) into (16) and applying the chain rule of differentiation results in:

$$\int_0^{\tau} d\tau = \int_0^{s} \frac{s}{g_2(s)}\left[-2\,(\kappa-1)\,(\lambda+1) + (\mathrm{Bi}\,m\,s)^5(\psi-1)\,\psi_1(\lambda+(\psi-1)^2)\right.$$

$$+2(\mathrm{Bi}\,m\,s)^4[\kappa(2\psi-3)+3\text{-}4\psi+\psi^2)\,(\lambda+(\psi-1)^2] + \mathrm{Bi}\,m\,s\,[\kappa\,(\lambda\,(\psi-9)+7\psi-9)+\lambda\,(9-2\psi)-8\psi+9]$$

$$+(\mathrm{Bi}\,m\,s)^3\,[-2\,(\psi-1)^2(4\psi-7)+\lambda\,(14-12\psi+\psi^2)+\kappa\,(2\,\lambda\,(3\,\psi-7)+3\,\psi^3)-19\psi^2)+30\,\psi-14)]$$

$$\left.+4\,(\mathrm{Bi}\,m\,s)^2(-2\,\lambda\,(\psi-2)+3\psi^2-7\psi+4)+\kappa\,(\lambda\,(\psi-4)-2(\psi^2-3\psi+2))\right]ds, \quad (19)$$

where $g_2\,(s)$ is given by:

$$g_2\,(s) = (n+1)\,(1+\mathrm{Bi}\,m\,s)\,(1-\mathrm{Bi}\,m\,s\,(\psi-1))^2\,(\kappa-1+\mathrm{Bi}\,m\,s\,\psi_1)^2,$$

and $\psi_1 = \psi - 1 + \kappa$. Integrating Eq. (19) yields the FF penetration time (τ) as a function of (s) in Eq. (20):

$$\tau = \frac{1}{n(1+n)\mathrm{Bi}^2\kappa\,m^2}\left(\frac{(-1+\kappa)(\kappa^2+\lambda)}{\psi_1^3}\left(\frac{\mathrm{Bi}\,m\,s\,\psi\,\psi_1}{\psi_1\mathrm{Bi}\,m\,s+\kappa-1}+Ln\left[1+\frac{\psi_1\mathrm{Bi}\,m\,s}{(-1+\kappa)}\right]\right)+\frac{\kappa\,\mathrm{Bi}\,m\,s\,(\lambda+(-1+\psi)^2)}{2(-1+\psi)\,\psi_1}(2+\mathrm{Bi}\,m\,s)\right.$$

$$\left.-\kappa\,Ln[1+\mathrm{Bi}\,m\,s]+\frac{\lambda}{(-1+\psi)^3}\left(\frac{\mathrm{Bi}\,m\,s\,\psi(-1+\psi)}{(-1+\mathrm{Bi}\,m\,s(-1+\psi))}-Ln[1-\mathrm{Bi}\,m\,s\,(-1+\psi)]\right)\right). \quad (20)$$

Equation (20) does not explicitly cast the FF (s) in terms of τ, which precludes an updated surface-temperature to be obtained together with further iterations. Goodman's solution (1980, p. 338) is also an implicit expression in s. Such functional relation mirrors temperature measurements in food freezing experiments. Sensing probes cannot directly detect the FF. Instead, the time is measured when the temperature barely deviates from the initial freezing point at specific and known slab depths.

The domain of the FF Eq. (20) is $0 \le s \le 1$, gives $s\,(0) = 0$, and has a monotonically decreasing derivative ($ds/d\tau > 0$) as is characteristic of one-dimensional MB solutions (Özisik, 1980; Kucera, 1985; Ramos et al. 1994). Whereas $1/\mathrm{Bi}^2$, ψ, and $1/m^2$ significantly influence τ by changing its order of magnitude, λ and κ have only a weak to moderate scaling effect. The limit of s as Bi approaches infinity is $\sqrt{2n(n+1)\,\tau}$. This is consistent with the classic HBI solution considering assumptions A1, A2, A5 and A9 (Mitchell & Myers, 2012). As the parameter (m) approaches zero, the FF time profile shifts from a power law to linear time dependence. The temperature-polynomial power (n) has a linear scaling effect on τ, but it does not impact the freezing-front time profile. All rational, linear, and logarithmic terms in Eq. (20) are significant. Noteworthy is the change of the s vs. τ curvature at about $0.3 < s < 0.5$, where it slightly straightens for ($-10 < \psi < -8$) and ($0.06 < \mathrm{Bi} < 0.1$). This subtle FF behavior affects the value of τ at $s = 1$ to some degree, but more so the sensitivity (σ_λ or σ_κ) values, and is addressed in the Discussion.

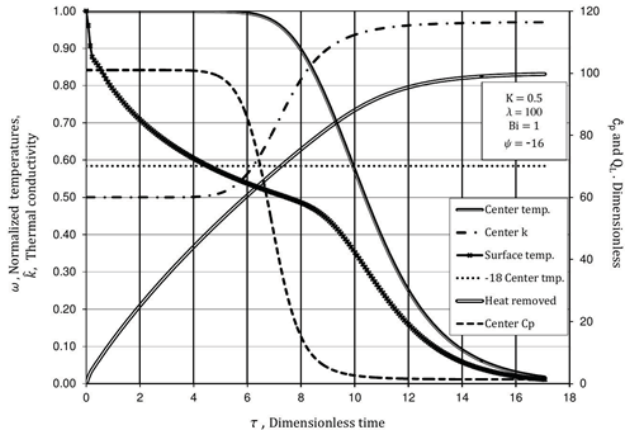


Figure 1. Example of a finite-difference model simulation of normalized slab temperatures, thermal properties, and accumulated heat release below the initial freezing point

5. Results

A slab freezing-simulation case, solved for the normalized temperature ($\hat{v}$) using Mathematica's *NDSolve* function and the CNFD-Excel model, is presented in Figures 1 and 2, respectively. The latter graph also shows the normalized thermal properties ($\hat{k}$ and $\hat{c}_p$) and the accumulated heat released (Q_L.) Note that, for

this specific case, Q_L is approximately 62% when the FF reaches the slab center at $\tau \approx 6$. The accumulated heat release is, though, always incomplete for the conditions in grid T1.1. As a consequence, with everything else being equal except the gradual heat release, the FF will reach the center faster than the Plank-predicted FF penetration time (Figure 5).

5.1 Model Optimization by Parameter Estimation: Fit Quality

Tables 2 and 3 summarize regression results for fitting Eq. (20) with constant ($\hat{n}$) and ($\hat{m}$) values. Notice the relative standard errors are less than 2.8%. The maximum intrinsic (0.01720) and maximum parameter-effects (0.03384) curvatures are less than 6% of the 95% confidence region curvature (0.57718). The fit response (lower plot in Figure 3) shows a regression quality higher for slow cooling rates ($\tau > 7$) than for fast rates ($\tau < 7$). This fit inconsistency is reflected in the high relative mean and median errors (Table 3).

To improve model accuracy, parameters (n) and (m) are developed as correlations in terms of input Bi, St, and κ. Because the term ($1/(n\ (1 + n)\ m^2)$ strongly affects Eq. (20) as one scaling factor, the following models are proposed to minimize dependence between the sensitivity coefficients of sub-parameters n_1, n_2 and m_1:

$$n = n_1(1 + \kappa\ \mathrm{Bi}^{n_2}) \tag{21}$$

$$m = n_2^2/(\mathrm{Bi}^{n_2}(1 + (1 + St)\kappa\ \mathrm{Bi})^{m_1}) \tag{22}$$

5.2 Objective Function Minimization and Parameter Uniqueness

To verify the least-squares global minimum, we evaluated the SSE using eight levels of each sub-parameter ($w_1 \times$ sub-parameter), where w_1 is: {0.1, 0.2, 0.4, 1.11, 1, 2.5, 5, 10}. Results support the SSE values corresponding to the global minimum least-squares criterion (Table 3). Insofar as parameter uniqueness, regressions were tested with six different initial guesses of each sub-parameter ($w_2 \times$ sub-parameter), where w_2 is: {0.01, 0.08, 0.64, 1, 4, 10}. Consistent convergence to the reported estimates and errors in Tables 2 and 3 were obtained as long as the initial values met the following conditions: $1 < n_1 < 10$, $n_2 \leq 4$, and $m_1 < 0.6$. Outside these limits, the initial guesses led to SSE values greater than the minimized regression error value; to suboptimal SSE values or meaningless negative and nil n values.

5.3 Parameter Reliability, Residuals and Model Validation

The pairwise coefficients in the upper-right triangle of the correlation matrix are $\{1, -0.497, -0.177; 1, 0.665; 1\}$. The sensitivity coefficient pairing n_2 and m_1 (0.664) indicates partial inter-dependence but only on the second half of the FF depth. Further, the maximum intrinsic (0.00615) and maximum parameter-effects (0.01019) curvatures are approximately two orders of magnitude smaller than the 95% confidence region curvature (0.61889). These measures confirm the asymptotic and almost-linear behavior of the estimates computed at the converged sub-parameter values and, thus, their reliability (Karolczak & Mickiewicz, 1995; Seber & Wild, 2003). The residuals (upper-left corner in Figure 3) are non-normally distributed according to the Jarque-Bera, skewness, and kurtosis tests, whereas the W/S, D'Agostino and Anderson-Darling tests yield opposite results (Ghasemi & Zahediasl, 2012). This aspect is addressed in the Discussion.

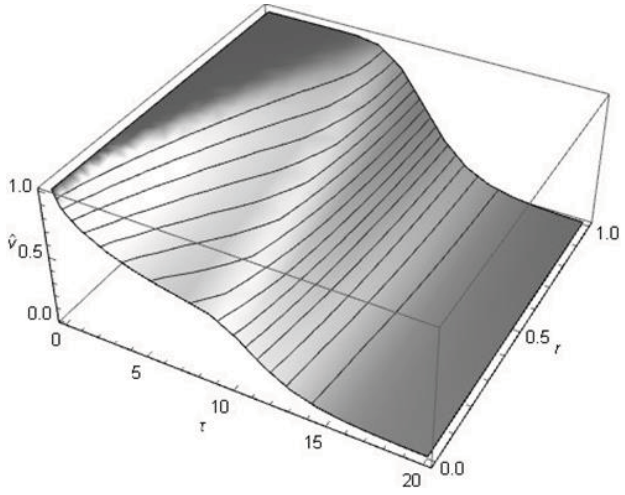


Figure 2. Example of normalized-transient temperature profiles during freezing as predicted by Mathematica's *NDSolve* kernel function, with Bi = 1, ψ = −16, κ = 0.5 and λ = 100

Table 2. Nonlinear regression results estimates and fit measures for *n, m*, and joint sensitivity ($\sigma_{\kappa\lambda}$)

Parameter	Estimate	Standard error	95% Conf. interval		t-value	Bias (%)
$\hat{n}$	3.0826	0.00863	3.0656	3.0995	357.2	-1.08×10^{-3}
$\hat{m}$	0.02992	0.00068	0.0286	0.03124	44.2	7.36×10^{-4}
Constant parameters model						
n_1	2.2264	0.00160	2.2233	2.2296	1381.7	-2.48×10^{-4}
n_2	0.18282	0.00064	0.1816	0.1841	285.4	-8.84×10^{-6}
m_1	0.84361	0.00579	0.8322	0.8549	145.6	1.65×10^{-3}
Adjustable parameters model						
c_1	0.6558	0.00065	0.6543	0.6589	1012.3	-9.82×10^{-8}
c_2	0.0809	0.00049	0.0799	0.0819	163.9	4.31×10^{-8}
Joint sensitivity correlation						

Estimates calculated at a significant probability $p^* < 0.001$

Table 3. Nonlinear regression statistics

Measures	Native model	Full model
RMSE	1.049	0.2233
R_p^2	0.9791	0.9978
AIC	3497.7	612.6†
BIC	3512.9	647.9†
ε (%)	30.16	11.43
ε_m(%)	13.73	6.25
N	1500	150
Parameters	2	6

Statistical measures for native and full models. AIC† and BIC†values calculated using $N = 1\ 500$

Nonlinear regression errors of the ten *K*-fold validations are presented in Table 4. Each validation yielded a set of sub-parameters n_1, n_2, and m_1 calculated with a sample size (*N*) of 1 350 data points. The tabulated errors measures were obtained using these sub-parameters with the ten validation data-splits of the 150-point datasets. Notice the consistency between error measures in this Table (final model) and those in Table 3 (native model).

5.4 Moving Boundary Model Performance

Figure 5 compares FF time profiles predicted by Eq. (20), CNFD model, Eq. (2), Özisik's (1980) analytic solution, and Eq. (1), with the Biot number and sample levels of St and κ as parameters. Though the full model's τ predictions are smaller than Plank's values, notably for low Bi ≤ 0.1, they are consistent with the CNFD model's τ values. Notice that for Bi ≥ 40, the full model's predictions agree with the Özisik solution, which assumes constant thermal properties and a Dirichlet BC.

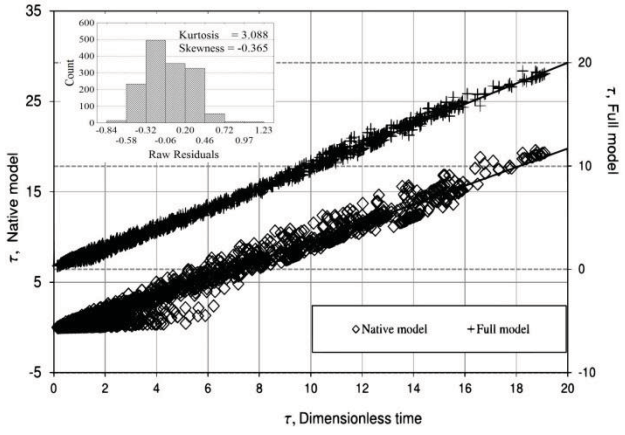


Figure 3. Comparison of native and full model fit responses vs. FF penetration times by the CNFD model (lower plot), and histogram of the raw residuals (upper-left corner plot)

5.5 Thermal Conductivity and Latent Heat Effects

Relative errors in κ ($\delta\kappa/\kappa = \pm 6\%$) generate ($\mp\delta\hat{k}/\hat{k}$) and ($\pm\delta\tau/\tau$) uncertainties. These effects are illustrated as families of s–τ curves in Figure 6 with Bi as parameter. Although $\pm\delta\tau/\tau$ varies along the FF profile, its variability is insignificant ($F = 0.145$, $p* = 0.05$). Figure 7 maps the sensitivity response against ψ and Bi·κ. In the region limited by ($0.06 < \text{Bi}\cdot\kappa < 2$), σ_κ forms a narrow and unexpected pleat or fold (lower-right section of the map). The sensitivity values in this pleat are, nevertheless, positive and of the same magnitude of the stabilized sensitivity values for $\text{Bi}\cdot\kappa > 3$.

Descriptive statistics for σ_κ and σ_λ sensitivities are presented in Table 5. The three main-effects groups detected by Mathematica's GLM analysis in decreasing order of magnitude are:

$$\{\kappa\,(F = 1882.8)\} > \{\text{Bi}\cdot\kappa(F = 326.6), \text{Bi}\cdot\psi\ (F = 238.1), \psi\,(F = 140.8), \psi\cdot\kappa\ (F = 129.8)\} > \{\text{Bi}^2(F = 9.5)\},$$

where all F values are significant at $p* < 0.002$. Factors Bi and λ independently exert no effect on σ_κ.

Uncertainties in λ ($\delta\lambda/\lambda = \pm 3\%$) generate roughly similar errors ($\pm\delta\tau/\tau$) in the FF penetration time shown (Figure 8), where families of s–τ profiles are presented with Bi as parameter but κ and ψ set at fixed levels. The mapped sensitivity (σ_λ) response to λ perturbations forms a concave surface with ψ and Bi in Figure 9. Noteworthy is the σ_λ decline for $0.06 < \text{Bi} < 12$ and subsequent stabilization to levels that depend on ψ. GLM analysis identified the following three main-effects groups on σ_λ, which in decreasing order of magnitude, are:

$$\{\text{Bi}\ (F = 6590)\} > \{\psi\ (F = 2369), \text{Bi}\cdot\psi\ (F = 1784), \text{Bi}^2\ (F = 1356)\} > \{\kappa\ (F = 70.9), \text{Bi}\cdot\kappa\ (F = 36.4)\},$$

where all F values are significant at $p* < 0.001$. Note that λ uncertainties do not affect σ_λ.

A joint sensitivity ($\sigma_{\kappa\lambda}$) range was estimated for test grid T1.3 introducing perturbations centered at κ = 0.5 and λ = 105 for illustration purposes (Figure 10). Note the quick $\sigma_{\kappa\lambda}$ decay with increasing Bi and leveling off to $\sigma_{\kappa\lambda}$ values (almost linearly) depending on ψ. A statistical correlation to predict $\sigma_{\kappa\lambda}$ was developed as:

$$\sigma_{\kappa\lambda} = \frac{c_1(1+\kappa)}{(1+\text{Bi}\cdot\text{St})^{0.5\,c_1}} + c_2\,Ln[1+\text{Bi}], \tag{23}$$

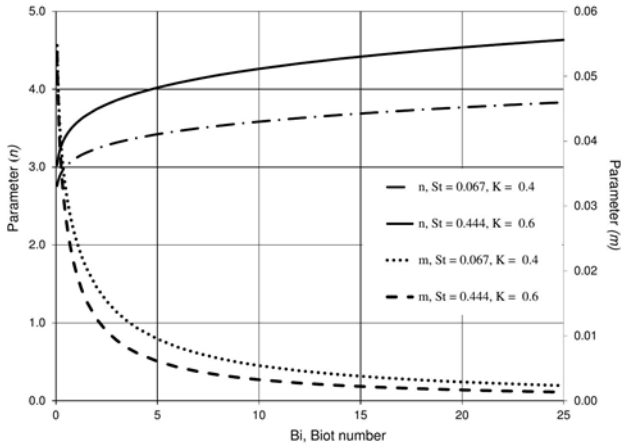


Figure 4. Effect of the Biot number on predicted values of temperature polynomial power (n) and surface temperature parameter (m), with St and κ as parameters

Table 4. *K*-fold validation results including mean (ε), median (ε_m) relative errors, and R_p^2 (N = 150)

K-fold training	Statistical measures			
	R_p^2	RMSE	ε (%)	ε_m (%)
K1	0.9995	0.329	12.71	5.36
K2	0.9962	0.285	11.41	5.53
K3	0.9960	0.282	10.66	4.93
K4	0.9951	0.287	10.02	4.58
K5	0.9962	0.295	12.35	5.89
K6	0.9967	0.286	11.72	5.23
K7	0.9946	0.349	12.97	5.36
K8	0.9963	0.282	10.90	5.44
K9	0.9964	0.268	13.09	5.25
K10	0.9961	0.311	12.52	5.48
Median	0.9959	0.297	11.84	5.42
SD	0.0007	0.025	1.06	0.35
Full model	0.9983	0.297	11.83	5.36

which is valid for $\hat{\varepsilon}_\kappa = \hat{\varepsilon}_\lambda$ and for grid T1.2 conditions. Nonlinear regression estimates and statistics describing this correlation are presented in Table 2. The error measures supporting the fit include $R_p^2 = 0.9988$, RMSE = 0.0248, mean ε = 2.77%, ε_m = 2.35%, maximum intrinsic and parameter-effects curvatures are 0.00011 and 0.00044, respectively, compared to a 95% curvature confidence-region of 0.57704. The pairwise sensitivity coefficient (c_1, c_2) is -0.3585.

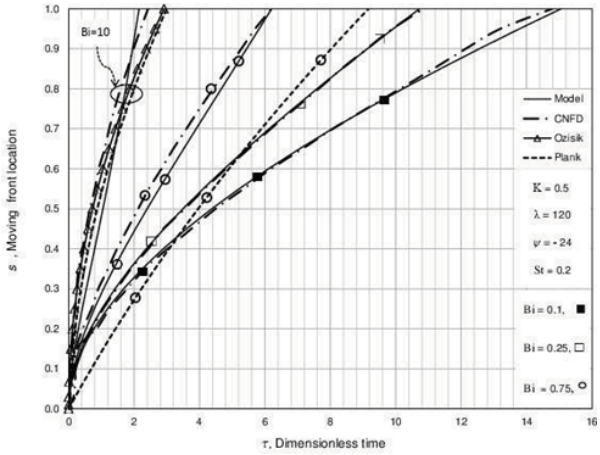


Figure 5. Comparison of freezing front predictions by Eq. (20) with selected published and CNFD freezing front models

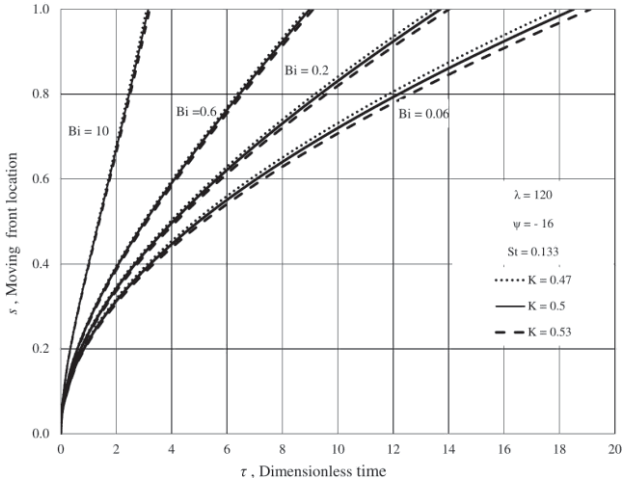


Figure 6. Effect of the conductivity parameter κ (±6%) deviations on the freezing front movement

Table 5. Descriptive statistics of independent and joint sensitivities. MAD is 1.483 × (median of absolute deviations)

	Descriptive statistics				
Sensitivity	Median	MAD	IQR	Minimum	Maximum
σ_K	0.348	0.152	0.220-0.425	0.027	0.950
σ_λ	0.967	0.032	0.877-0.985	0.617	0.991
$\sigma_{K\lambda}$	0.664	0.086	0.611-0.712	0.539	0.804

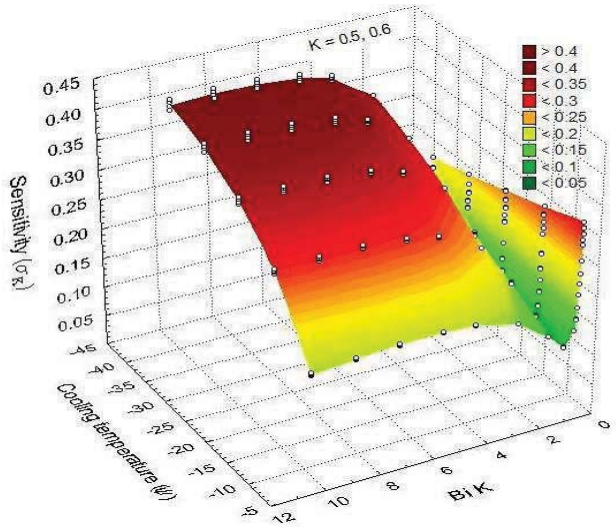


Figure 7. Sensitivity (σ_K) as influenced by Bi κ and ψ, for κ = 0.5 and 0.6. Surface data smoothed by Statistica wafer-fit function

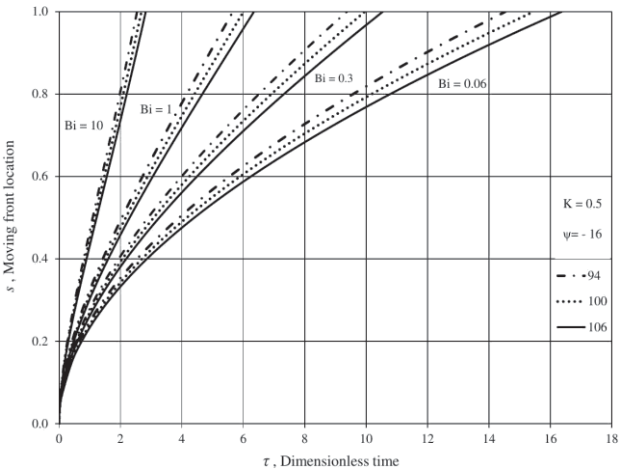


Figure 8. Effect of dimensionless latent heat (λ) (±3%) deviations on the freezing front movement

5. Discussion

Although numerical analysis enables solving food freezing problems with realistic assumptions, it may not be an efficient approach to quantifying the uncertainties introduced by estimated variable thermal properties (Özisik, 1980). We have thus developed an approximate analytical model to map these effects on the FF against relevant input properties, material and cooling conditions. The mentioned effects were calculated as sensitivities driven by independent or joint κ and λ uncertainties (σ_κ, σ_λ, and $\sigma_{\kappa\lambda}$, respectively). Together, these sensitivities vary between 0.1 and 1.

For low to medium freezing rates and as Bi increases from 0.06 to approximately 6, the κ-driven sensitivity range is $0.1 \leq \sigma_\kappa \leq 0.6$. Sensitivities σ_λ and $\sigma_{\kappa\lambda}$, in turn, decrease from approximately 1 to ≤ 0.7 and from roughly 0.8 to ≤ 0.55, respectively. For high freezing rates (Bi > 8), all sensitivities stabilize to levels controlled by ψ for σ_κ and $\sigma_{\kappa\lambda}$, and by ψ·κ for σ_λ. This means that the greater the cooling temperature (ψ), the closer the thermal properties are to their frozen state values and the faster the sensitivities stabilize.

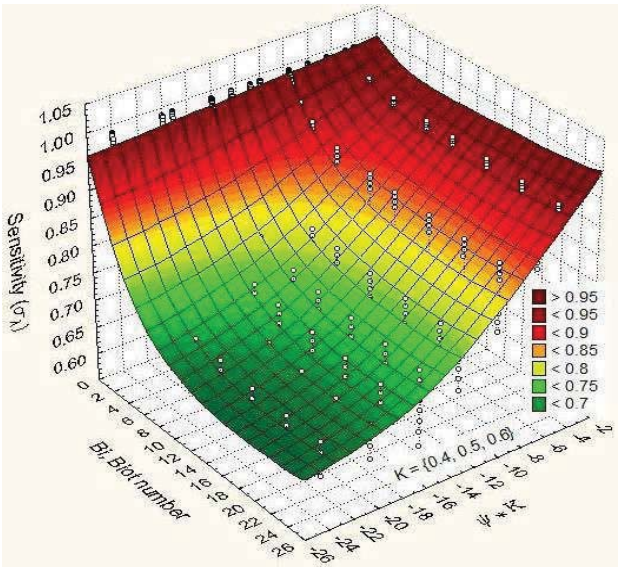


Figure 9. Dimensionless latent-heat driven sensitivity (σ_λ) against Bi and ψ·κ. Surface data smoothed by Statistica distance-weighted least-squares function

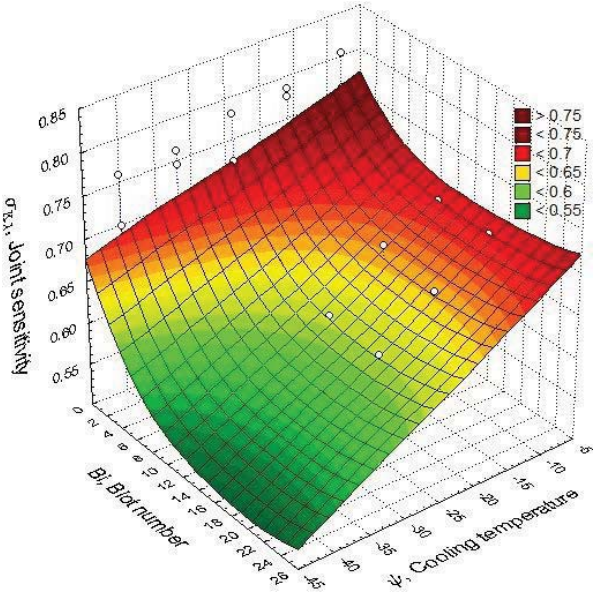


Figure 10. Effect of Bi and ψ on the joint sensitivity ($\sigma_{\kappa\lambda}$). Surface data smoothed by Statistica negative exponential function

Lacking comparable sensitivity maps, we evaluate our σ_κ against Mittal et al.'s (1993) sensitivity values obtained from semi-empirical versions of Plank's Eq. (1). The median σ_κ of 0.348 (IQR = 0.205) is consistent with Mittal et al.'s absolute value range of |-0.69 to -0.06|. Consider also that Plank's theoretical sensitivity relative to k_f is $\overline{\sigma}_{kf} = -\mathrm{Bi}/(2+\mathrm{Bi})$. For high Bi > 8, σ_κ stabilizes to levels depending on ψ·κ (Figure 7), which agrees with the theoretical $\overline{\sigma}_{kf}$ behavior against Bi. There is no contradiction with the negative sign of Mittal et al.'s or Plank's sensitivities because relative deviations in the κ drive changes of opposite sign in the fully frozen thermal

conductivity (k_f). Plank's mean $|\overline{\sigma}_{kf}|$is 0.794 (spanning from 0.029 to 0.926) in the Bi range of 0.06 to 25. Note that $\sigma_\kappa < \overline{\sigma}_{kf}$. This is due to a Plank sensitivity that depends only on Bi, without accounting for either ψ or any buffering effect of the Stefan number.

Our median σ_λ (0.967, IQR = 0.107) is consistent with published values (0.684 to 1.172) (Mittal et al., 1993) and with the theoretical λ-driven Plank sensitivity of 1. As the convection factors (Bi and $|\psi|\cdot\kappa$) increase, σ_λ drops at an ever-decreasing rate but stabilizes for Bi > 8 at levels that depend on $\psi\cdot\kappa$. Therefore, the σ_λ map reveals that the closer is the thermal layer to the fully frozen state or total heat removal, the smaller is the λ uncertainty effect on the FF. Otherwise stated, the slower the convective freezing rate, the closer the FF is to mirror the λ uncertainty.

The joint κ and λ median sensitivity ($\sigma_{\kappa\lambda}$) falls between the independent σ_κ and $\sigma_\lambda$values. This result is consistent with the law of error propagation (Tellingheusen, 2001). Notice the dominating λ-perturbation influence on the $\sigma_{\kappa\lambda}$ response (Figures 9 and 10). Although the $\sigma_{\kappa\lambda}$ sensitivity correlation was developed for equal relative errors in κ and λ, it yields a unique insight into the combined variable thermal property influence on the FF nevertheless. The odd σ_κ pleat at the low end of ψ and $\mathrm{Bi}\cdot\kappa$ ranges (i.e. mild cooling rate) stems from the balancing of the two rational terms containing the FF location (s) in Eq. (20). Together they generate a slight inflection point in the first half of the FF. This subtle slope change becomes amplified at the end of the s–τ profile (s = 1), where σ_κ is calculated. Beyond this inflection point, the s–τ curvature remains monotonic.

Several HBI analyses of simplified Stefan problems have either assumed or estimated the temperature polynomial power (n) from known analytical solutions (Goodman, 1958; Özisik, 1980; Mitchell & Myers, 2012). For a realistic problem formulation, neither case applies. However, it is more coherent with the phase change physics to extract n and m information from the FF data via nonlinear regression. This unique approach enabled us to estimate constant $\hat{n}$ and $\hat{m}$ but, more importantly, of n and m correlations with the input data (Bi, ψ, λ, and κ). The lack of comprehensive, structured, and reliable experimental FF data justified generating these by a numerical solution of the energy equation.

Whereas our constant $\hat{n}$ estimate is 3.08, the adjustable n correlation predicted values ranging from approximately 2.8 to 5.3 depending on Bi, St, and ψ levels. In comparison, Mitchell and Myers' (2012) HBI analysis yields $\hat{n} \approx 3.6$ assuming constant properties. As Bi increases above 8, the shift of n towards a quintic power is consistent with Schwartzberg's (1977) freezing-simulation temperature profiles. The higher the n value above 2, the more pronounced is the temperature profile convexity with a plateaued top. A drawback of the temperature polynomial assumption is that, for Bi values below 0.1, the temperature profile cannot plateau unless n is less than 1, which violates the energy balance at the interface. Last, if n is defined as time dependent (Mitchell & Myers, 2012), the solution would most likely require numerical methods, preventing thus an analytical expression for the FF.

The parameter m was introduced into Eq. (13) to modulate the convective Bi effect. An m parameter magnitude of $\mathcal{O}\,[St = 10^{-1}]$ was expected because, in phase change analysis, St reduces the Bi convective effect. Indeed, for most foodstuffs, St values range between 0.068 and 0.444 (Schwartzberg, 1977). To develop Eq. (22), we conjectured that m would correlate with Bi, St, and κ. First, a power term of Bi could enhance the first-order rational Bi expression in Eq. (13). Second, Eq. (13) misses the buffering effect St has on Bi, and thus the incorporation of the Stefan number. Third, for Bi > 0.1 the thermal conductivity (via κ) should influence the slab temperature profile.

All nonlinear regression statistics in Tables 2 to 4 show significant reductions in parameter, curvature, and regression error measures. The (small magnitude of the intrinsic and parameter-effects) curvature measures relative to the 95% curvature confidence-region confirm the asymptotic and almost linear behavior of the sub-parameters at their converged values. Parameter reliability is thus corroborated (Karolczak & Mickiewicz, 1995; Seber & Wild, 2003).

Whereas the full model is in close agreement with the numerical simulation FF penetration time values because of the fit, it is also consistent with Özisik's (1980) analytic solution for Bi values above 8. Plank's predictions are consistently higher than our predictions because they are based on the complete heat release at the initial freezing point. The robust statistical measures supporting the improved fit quality include the minimized AIC, BIC, RMSE, mean and median relative errors (ε and ε_m), and R_p^2 (Table 3). The median relative regression error (ε_m) was reduced by half to 5.36%. These error measures and graphical assessment of the fit response vs. FF data together endorse the final model accuracy.

Though the normality tests give mixed results, the distribution graph of residuals is not radically non-normal. The large sample size of the FF data supports the reliability of the estimates under the central limit theorem

(Beck & Arnold, 1977; Ghasemi & Zahediasl, 2012). Our FF model accuracy is based on conservative error measures (Tables 3 and 4) that are averages of ten *K*-fold data splits and not of the whole dataset (Jung & Hu, 2015).

Together, the robustness of our FF model and sensitivity analysis are supported by (a) the physical significance of property parameters (κ and λ), (b) final model accuracy, (c) consistent predictions with published average freezing-time sensitivities, and (d) coherent patterns for high Biot numbers and cooling temperature. Our sensitivity maps may be applied to the cooling and subcooling freezing process stages. Particular attention must be given to error estimation for low to medium freezing rate processes (Bi < 8), because of the rapid sensitivity change with the Biot number and cooling temperature (ψ or ψ·κ).

Future studies could invoke alternative convective-surface temperature models (relaxing the PSS assumption) that account for temperature-dependent thermal properties. These models should lead to consistently monotonic s–τ profiles without anomalies. Time dependence of the temperature polynomial power (n) could be pursued, but it may lead to an irreducible energy PDE. This investigation could also be extended to other regular geometries and account for a temperature-dependent density.

7. Conclusions

Our approximate model enabled us to quantify and map systematically the effects of temperature-dependent thermal properties on the FF with acceptable accuracy. We show that the Biot number, cooling temperature, and thermal conductivity markedly and nonlinearly control these FF effects. While the thermal conductivity effect exhibits the greatest variability, the dimensionless latent heat effect is greater and essentially generates a direct and proportional perturbation on the FF. Nonlinear methods and prior information can thus be effective in constructing reliable mathematical models for phase change food processes together with its parameterization via nonlinear regression.

List of Symbols

AIC	Akaike information criterion
Bi	Biot number, h L/k_f
BIC	Bayesian information criterion
c_p	volumetric heat capacity below T_i , dimensional
$\widehat{c_p}$	dimensionless effective and volumetric heat capacity below T_i , (c_p/c_{pf})
h	surface heat transfer coefficient, dimensional
ΔH	latent heat of moist food material, at the initial freezing point, adjusted by bound water content, dimensional
k	thermal conductivity below T_i , dimensional
$\hat{k}$	dimensionless thermal conductivity, (k/k_f)
N	sample size
L	slab half thickness, dimensional
R^2	coefficient of determination
R_p^2	coefficient of determination adjusted for number of parameters
RMSE	root means square error
St	Stefan number, $-\psi/(c_p(T_i - T_a))$
t	dimensional time
T	dimensional food slab temperature
u	Kirchhoff-transformed temperature, dimensionless
v	re-scaled dimensionless temperature, $(T - T_a)/(T_o - T_i)$
X	dimensional freezing front or moving boundary location
x	dimensional spatial coordinate

Greek symbols

α	dimensional thermal diffusivity below T_i
ε	normalized regression error, $\lvert 1 - \tau_{model} / \tau_{CNFD} \rvert$
θ	dimensionless temperature, $(T - T_i)/(T_o - T_i)$
κ	dimensionless thermal conductivity parameter, $(1 - k_u/k_f)$
λ	dimensionless latent heat, $\Delta H/(c_{pf}(T_o - T_i))$
σ_κ	sensitivity due to κ perturbations, $(\delta\tau/\tau)$ $(\kappa/\delta\kappa)$
σ_λ	sensitivity due to λ perturbations, $(\delta\tau/\tau)$ $(\lambda/\delta\lambda)$
τ	dimensionless time, $\alpha_f t/L^2$
ψ	dimensionless cooling medium temperature, $(T_a - T_i)/(T_o - T_i)$

Subscripts

a	external cooling medium
f	fully frozen state
i	initial freezing point
m	median
o	freezing point of pure water
u	unfrozen food state
w	cooled surface

Acknowledgments

VMCh is grateful for the insights regarding nonlinear analysis of Dr. Alexey Brobick, Department of Astrophysics at the University of Lund, Sweden.

Funding: This research received no external funding.

Conflict of interest: The author declares no conflict of interest.

References

Arboretti-Giancristofaro, R., & Salmaso, L. (2003). Model performance analysis and model validation in logistic regression. *Statistica, 2*, 375-396. https://doi.org/10.6092/issn.1973-2201/358

Archontoulis, S., & Miguez, F. (2015). Nonlinear regression models and applications in agricultural research. *Agronomy Journal, 102*(2), 786-796. https://doi.org/10.2134/agronj2012.0506

Baudouy, B. (2002). Integral method for transient HE II heat transfer in a semi-infinite medium. *AIP Conference Proceedings, 613*, 1349-1355. https://doi.org/10.1063/1.1472164

Bazant, M. (2018). Ch. 2: Pseudosteady approximation: moving boundary. In *Analysis of transport phenomena I: Mathematical methods*. Retrieved from https://courses.edx.org/courses/course-v1:MITx+10.50.1x+3T2018/course/

Beck, J., & Arnold, K. (1977). *Parameter estimation in engineering and science*. New York, NY: John Wiley & Sons.

Bellera, C., & Hanley, J. (2007). A method is presented to plan the required sample size when estimating regression-based reference limits. *Journal of Clinical Epidemiology, 60*, 610-615. https://doi.org/10.1016/j.jclinepi.2006.09.004

Carnahan, B., Luther, H., & Wilkes, J. (1969). *Applied numerical methods*. New York, NY: John Wiley & Sons.

Chavarria, V. M. (2019). *Food-slab freezing front data* [Data set]. Harvard Dataverse, V1. https://doi.org/10.7910/DVN/LKTEJQ

Cho, S., & Sunderland, J. (1974). Phase change with temperature-dependent thermal conductivity. *Journal of Heat Transfer - Transactions of the ASME, 96*, 214-217. https://doi.org/10.1115/1.3450167

Cottrell (2006). Temperature measurement. Retrieved from https://web.mst.edu/~cottrell/ME240/Resources/Temperature/Temperature.pdf

Delgado, A., & Sun, D.-W. (2001). Heat and mass transfer models for predicting freezing processes: A review. *Journal of Food Engineering, 47*(3), 157-174. https://doi.org/10.1016/S0260-8774(00)00112-6

Farid, M. (2002). The moving boundary problems from melting and freezing to drying and frying of food. *Chemical Engineering and Processing, 41*, 1-10. https://doi.org/10.1016/S0255-2701(00)00153-7

Fricke, B., & Becker, B. (2001). Evaluation of thermo-physical property models for foods. *Heating, Ventilation and Air Conditioning, 7*(4), 311-330. https://doi.org/10.1080/10789669.2001.10391278

Ghasemi, A., & Zahediasl, S. (2012). Normality tests for statistical analysis: A guide for non-statisticians. *International Journal of Endocrinology and Metabolism, 10*, 486-499. http://dx.doi.org/10.5812/ijem.3505

Goodman, T. (1958). The heat-balance integral method and its application to problems involving a change of phase. *Transactions of the American Society of Mechanical Engineers, 80*, 335-342.

Heldman, D., & Singh, R. (1981). *Food Process Engineering* (2nd ed.) Westport, CT: AVI. https://doi.org/10.1007/978-94-010-9337-8

Hu, H., & Argyropoulos, S. (1996). Mathematical modeling of solidification and melting: A review. *Modeling and Simulation in Material Science and Engineering, 4*, 371-396. https://doi.org/10.1088/0965-0393/4/4/004

Jung, Y., & Hu, J. (2015). A *K*-fold averaging cross-validation procedure. *Journal of Nonparametric Statistics, 27*(2), 167-179. https://doi.org/10.1080/10485252.2015.1010532

Karolczak, M., & Mickiewicz, A. (1995). Why to calculate, when to use, and how to understand curvature measures of nonlinearity. *Current Separations, 14*, 10-16.

Kucera, A. (1985). *Mathematics of moving boundary problems in diffusion* (Doctoral dissertation, University of Wollongong, Australia). Retrieved from http://ro.uow.edu.au/theses/1553

Kumar, A., & Singh, R. (2018). A moving boundary problem with variable specific heat and thermal conductivity. *Journal of King Saud University of Science*, 1-6. http://dx.doi.org/10.1016/j.jksus.2018.05.28

López-Leiva, M., & Hallström, B. (2003). The original Plank equation and its use in the development of food freezing rate predictions. *Journal of Food Engineering, 58*(3), 267-275. https://doi.org/10.1016/S0260-8774(02)00385-0

Lunardini, V. (1988). *Heat conduction with freezing or thawing* (Monograph 88-1). Hanover, NH: U.S. Army Cold Regions Research and Engineering Laboratory.

Mitchell, S., & Myers, T. (2012). Application of heat balance integral methods to one-dimensional phase change problems. *International Journal of Differential Equations.* https://dx.doi.org/10.1155/2012/187902

Mittal, G., Hanenian, R., & Mellikarjunan, P. (1993). Evaluation of freezing time prediction models for meat patties. *Canadian Agricultural Engineering, 35*(1), 75-81.

Özisik, M. (1980). *Heat Conduction*. New York, NY: John Wiley & Sons.

Pham, Q. (1986). Simplified equation for predicting the freezing time of foodstuffs. *International Journal of Food Science & Technology, 21*(2), 209-219. https://doi.org/10.1111/j.1365-2621.1986.tb00442.x

Pham, Q., & Willix, J. (1990). Effect of Biot number and freezing rate on accuracy of some food freezing time prediction methods. *Journal of Food Science, 55*(5), 1429-1434. https://doi.org/10.1111/j.1365-2621.1990.tb03951.x

Rajeev, R. (2014). Homotopy perturbation method for a Stefan problem with variable latent heat. *Thermal Science, 18*(2), 391-398. https://doi.org/10.2298/TSCI110627008R

Ramos, M., Cerrato, Y., & Gutierrez, J. (1994). An exact solution for the finite Stefan problem with temperature-dependent thermal conductivity and specific heat. *International Journal of Refrigeration, 17*(2), 130-134. https://doi.org/10.1016/0140-7007(94)90054-X

Rao, M., Rizvi, S., & Datta, A. (2005). *Engineering properties of foods* (3rd ed.). Boca Ratón, FL: CRC Press.

Schwartzberg, H. (1976). Effective heat capacities for the freezing and thawing of food. *Journal of Food Science, 41*(1), 152-156. https://doi.org/10.1111/j.1365-2621.1976.tb01123.x

Schwartzberg, H. (1977). The prediction of freezing and thawing temperature vs. time through the use of effective heat capacity equations. *Proceedings of the Joint Meeting of Commissions C1 and C2. International Institute of Refrigeration*, 311-317.

Schwartzberg, H., Singh, R., & Sarkar, A. (2007). Freezing and thawing - Computation methods and thermal properties correlation. In S. Yanniotis & B. Sunden (Eds.), *Heat transfer in Food Processing.* https://doi.org/10.2495/978-1-85312-932-2/03

Seber G., & Wild, C. (2003). *Nonlinear Regression*. Hoboken, NJ: John Wiley & Sons.

Singh, J., Gupta, K., & Ral, K. (2011). Variational iteration method to solve moving boundary problem with temperature dependent physical properties. *Thermal Science, 15*(2), 229-239. https://doi.org/10.2298/TSCI100226024S

Solomon, A., Wilson, D., & Alexiades, V. (1981). *The quasi-stationary approximation for the Stefan problem with convective boundary condition* (Technical Report ORNL/CSD-84). Oak Ridge, TN: Oak Ridge National Laboratory. https://doi.org/10.2172/6218971

Tellingheusen, J. (2001). Statistical error propagation. *Journal of Physical Chemistry-US, 105*, 3917-3921. https://doi.org/10.1021/jp003484u

Voller, V., Swenson, J., & Paola, C. (2004). An analytical solution for a Stefan problem with variable latent heat. *International Journal of Heat and Mass Transfer, 47*(24), 5387-5390. https://doi.org/10.1016/j.ijheatmasstransfer.2004.07.007

Wood, A. (2001). A new look at the heat balance integral method. *Applied Mathematical Modeling, 25*, 815-824. https://doi.org/10.1016/S0307-904X(01)00016-6

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Antimicrobial Effects of Sulfadimethoxine on *Salmonella*, *Escherichia coli* and Aerobic plate count (APC) in Small-Scale Broiler Operations

Wannee Tangkham[1] & Frederick LeMieux[1]

[1]College of Agricultural Sciences, McNeese State University, Lake Charles, LA, United States

Correspondence: Wannee Tangkham, College of Agricultural Sciences, McNeese State University, United States. E-mail: wtangkham@mcneese.edu

Received: September 7, 2019 Accepted: October 4, 2019 Online Published: November 20, 2019

doi:10.5539/jfr.v8n6p147 URL: https://doi.org/10.5539/jfr.v8n6p147

Abstract

Poultry is a source of *Salmonella* and *Escherichia coli.* Antibiotics can be used to reduce the enumeration and prevalence of these bacteria. The objective of this study was to determine the effects of sulfadimethoxine antibiotic on the enumeration and prevalence of *Salmonella*, *Escherichia coli* and aerobic plate count in broilers. Broilers (n = 600) were allotted to two treatments, each with twelve replications. The treatments were control (drinking water without antibiotic) and with antibiotic at 0.05% (wt/vol) sulfadimethoxine. After a six-week period, the enumeration of *Salmonella* in the experimental treatment was detected at 2.55 log CFU/g. This value is not different ($P>0.05$) than that detected in the control at 2.81 log CFU/g. With respect to prevalence, there was a difference ($P<0.05$) between the experimental treatment at 90.0% and the control at 100%. The enumeration of *E. coli* in the experimental treatment was detected at 3.97 log CFU/g. This value is lower ($P<0.05$) than that detected in the control treatment at 4.37 log CFU/g. With respect to prevalence, there was no difference ($P>0.05$) between the experimental treatment at 100% and the control at 100%. The enumeration of aerobic plate count in the antibiotic treatment were detected at 6.62 log CFU/g. This value is lower ($P<0.05$) than that detected in the control at 7.50 log CFU/g. With respect to prevalence, there was no difference ($P>0.05$) between treatments. Our overall findings suggest that the use of the antibiotic sulfadimethoxine can reduce the number of *E. coli*, *Salmonella* and aerobic plate count in the small-scale broiler operations.

Keyword: sulfadimethoxine, *Salmonella*, *E. coli*, aerobic plate count, small-scale

1. Introduction

Poultry is a host for the bacteria *Salmonella* (Lee, Runyon, Herrman, Phillips, & Hsieh, 2015) and *Escherichia coli* (Nolan, 2019) which can be a source of human illness (Haleem, Al-bakri, & Al-Hiyaly, 2013). Increased attention has been given to reducing the level of these bacteria in pre- and post-harvest poultry with the aim to reduce the level and incidence of raw product contamination.

Antimicrobial therapy is an important tool in reducing microorganisms in poultry production (Aarestrup, 2015) and enhances growth productivity (Page & Gautier, 2012). However, the use of antibiotics in animal production may produce resistant bacteria which could limit the effectiveness of antibiotics in humans (Simonsen et al., 1998; Klare et al., 1999; Van, London, Driessen, & Stobberingh, 2001). Salmonellosis is one of the most common foodborne diseases caused by *Salmonella* in poultry and can transmitted to humans (Authority, 2016; Antunes, Mourão, Campos, & Peixe, 2016). Colibacillosis is an infectious disease caused by *E. coli* which may produce morbidity and mortality in poultry (Piercy & West, 1976; DeRosa, Ficken, & Barnes, 1992; Lutful, 2010). Aerobic plate count (APC) is commonly used to assess the microbial load of poultry and the counts can be used to determine the quality, safety and shelf life of poultry products (Haleem et al., 2013; Rouger, Tresse, & Zagorec, 2017). Growth of spoilage bacteria lead to defects in meat products and can be responsible for unwanted taste, color, odor, and texture. An APC count at 7 log CFU/g or greater is used to define food spoilage (Zhang et al., 2012; Höll, Behr, & Vogel, 2016) which is associated with food-borne illness (Rouger et al., 2017). Therefore, there is a need to find an alternative antibiotic for prevention of *Salmonella* and *E. coli* infections and reducing the number of APC in poultry production.

Sulfonamide is commonly used to treat upper respiratory (Delaplane, 1945), coccidial infections caused by

Eimeria tenella and *Eimeria necatrix* (Waletzky & Hughes, 1946; Grumbles & Delaplane, 1948) and promote growth in poultry (Whitehill, Oleson, & Hutchings, 1950; Aarestrup, 2000). The commonly used sulfonamide in poultry production is sulfadimethoxine and therefore is appropriate for in vivo testing (FDA, 2013). Sulfadimethoxine can be used to treat coccodiosis (Orton & Hambly, 1971), fowl cholera, and coryza in poultry (Vree & Hekster, 1987; Wang, MacNeil, & Kay, 2012). In addition, sulfadimethoxine improves weight gain and final body weight (Davami, Peterson, Jones, & Ilardi,1987). Previous studies showed that sulfadimethoxine can reduce the number of *Campylobacter* spp. and *C. jejuni* in growing broilers (Tangkham, Janes, & LeMieux, 2016a) and turkeys (Alexandra, 2009). Therefore, most previous studies have concentrated on the transmission routes from commercial flock farm to carcasses after slaughter and retail products with limited information on the effects of production practices within small-scale poultry operations. The purpose of this study is to use antimicrobial therapy techniques to control bacterial contamination in poultry. Specifically, this study examines the effects of sulfadimethoxine antibiotic on the enumeration of *Salmonella*, *E. coli* and aerobic plate count in growing broilers.

2. Method

2.1 Broiler Production

The research experiment was approved by the McNeese State University Institutional Animal Care and Use Committee prior to data collection. Broilers were obtained from the McNeese State University Research Farm in Lake Charles, Louisiana. Birds (n = 600 Ross x Ross) were allotted to one of two treatments: 1) control (drinking water without antibiotic) and 2) drinking water + 0.05% (wt/vol) sulfadimethoxine (Durvet Inc., Blue Springs, Missouri). Drinking water was refreshed every day in both treatment groups. Feces was collected to determine *Salmonella*, *Escherichia coli* and aerobic plate counts from January 2014 to May 2014. Birds were housed in a controlled environment and maintained in Petersime®Battery Cages (32°C) with raised wire flooring (Petersime Incubator Co., Gettysburg, OH). Each cage was divided into 12 pens of equal size of 74.7 cm × 99.1 cm × 24.13 cm (Tangkham et al., 2016a; Tangkham, Janes, LeMieux, 2016b). Each pen housed twenty-five birds. Individual water and feed troughs were provided for each pen and supplied ad libitum. Birds were provided a commercial 18% protein chick grower crumbles with no antibiotics. The housing system was emptied of birds, feed, and litter and cleaned with hot water wash and disinfected. Animal care givers monitored feed and water and removed litter trays daily. Normal pest and rodent control were maintained throughout the experiment. The temperature and % RH during time period was 32°C and 58%, respectively.

2.2 Bacterial Isolation and Identification

The microorganisms were determined following the standards of the Association of Official Analytical Chemists (AOAC, 2000). Each week, fecal samples via swabbing were randomly collected from individual broilers (n = 600). To determine the enumeration (log CFU/g) and prevalence (%) of *E. coli* and *Salmonella*, samples were plated on brilliant green agar. For aerobic plate count, samples were plated on nutrient agar.

Samples were plated on 3M™ Petrifilm to determine the enumeration (log CFU/g) of *E. coli* and APC. *Salmonella* was isolated with brilliant green agar. Plates were incubated in a horizontal position, clear side up in stacks of no more than 20 plates at 37°C for 24-48 h. Results were obtained by selecting a countable plate (30-300 colonies) and the colonies were counted and reported as CFU/g.

2.3 Statistical Analysis

Statistical analysis was performed using SAS windows (SAS, 2003). The Proc GLM procedures were used to evaluate the significance differences of the obtained data. The PDIFF option of LSMEANS was employed to determine significance ($P<0.05$) among treatments. All data are presented as means with standard deviation (SD) and a significance level of was used for statistical analysis of means from treatments.

3. Results and Discussion

3.1 Enumeration of Salmonella

Feces was collected and plated to determine the enumeration of *Salmonella*. The enumeration of *Salmonella* ranged from 0-4.25 log CFU/g. *Salmonella* increased from week 1 through week 3 in both the control and antibiotic treatments (Figure 1). Specifically, the counts of *Salmonella* in the control treatment increased from an initial value of 1.22 log CFU/g in week one to a maximum value of 4.25 log CFU/g in week three (Figure 1). In the antibiotic treatment, the initial value was not detected in week one but increased to 4.02 log CFU/g in week three (Figure 1). Our study supported previous studies that poultry is a source of *Salmonella*, which leads to contamination of diverse foodstuffs (Barrow, Jones, Smith, & Wigley, 2012; Mazengia et al., 2014; Crump, Sjolund, Gordon, & Parry, 2015; Cosby et al., 2015).

For the overall experiment, there was no difference (P>0.05) in the enumeration of *Salmonella* in the antibiotic treatment and the control treatment in weeks 1 through 6. However, the counts of *Salmonella* in the antibiotic treatment of 2.55 log CFU/g was lower than in the control treatment at 2.81 log CFU/g. Similar, to previous studies (Seiffert, Hilty, Perreten, & Endimiani, 2013; Mazengia et al., 2014; Aarestrup, 2015) which indicated that the use of antibiotics had significantly lower rates of recovery of Salmonella. These results suggest that the antibiotic sulfadimethoxine, as applied in this study reduces the enumeration of *Salmonella* in small-scale poultry farming.

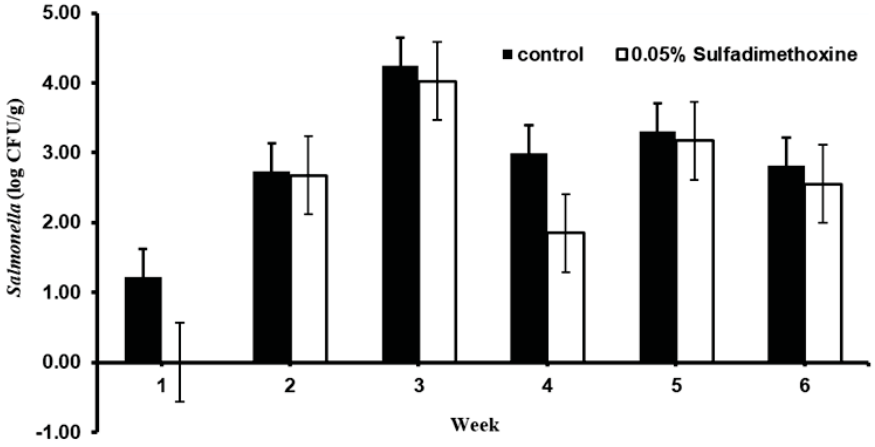


Figure 1. *Salmonella* counts in live broilers from the control and antibiotic treatments from weeks 1 through 6
Data are means from two replications. SEM=3.590

3.2 Enumeration of E. coli

The number of *E. coli* ranged from 3.67-4.55 log CFU/g. There was a small decrease in the enumeration of *E. coli* in the control treatment in weeks 1 through 6 from 4.39 log CFU/g to 4.37 log CFU/g. At week 6, the counts of *E. coli* decreased in the antibiotic treatment from 4.35 log CFU/g to 3.97 log CFU/g (Figure 2). These results were similar to the previous study (Tessi, Salsi, Caffer, & Moguilevsky, 1997) which found that the use of sulfamethoxazole-trimethoprim as an antibiotic can inhibit the growth of *E. coli*. Other studies by Huff, Huff, Rath, Balog, & Donoghue (2002) used a bacteriophage aerosol spray as an antibiotic to reduce the mortality rate of *E. coli* and their results showed that there was a significant decrease in mortality when the birds were challenged with *E. coli* immediately after bacteriophage administration. Other researchers (Al-Ghamdi, El-Morsy, Al-Mustafa, Al-Ramadhan, & Hanif, 1999) also found that ampicillin and tetracycline can be used to inhibit the growth of *E. coli* from chickens. For the overall experiment, the enumeration of *E. coli* in the antibiotic treatment was lower (P<0.05) 3.97 log CFU/g than the control treatment at 4.37 log CFU/g. These results suggest that the antibiotic sulfadimethoxine, as applied in this study reduces the enumeration of *E. coli* in small-scale poultry farming.

3.3 Enumeration of Aerobic Plate Count

After a six-week period, the enumeration of aerobic plate count ranged 6.46-7.93 log CFU/g. These data were related to the previous study by Haleem et al. (2013) who tested the counts of microflora at 6.55 log CFU/g in poultry meat. Our study showed that the counts of APC steadily increased from week 1 through week 6 in the control treatment from an initial value of 6.72 log CFU/g to a maximum value of 7.5 log CFU/g (Figure 3). This may be due to the elevated initial viable count of APC (Haleem et al., 2013) and microbial spoilage occurs because of the growth and metabolic activities of spoiling bacteria (Zhang, et al., 2012; Höll et al., 2016; Rouger et al., 2017). For the overall experiment, the enumeration of aerobic plate count in the antibiotic treatment was significantly lower (6.62 log CFU/g) than in the control treatment (7.50 log CFU/g). Therefore, our results found that the antibiotic sulfadimethoxine, as applied in this study reduces the enumeration of aerobic plate count in small-scale poultry farming.

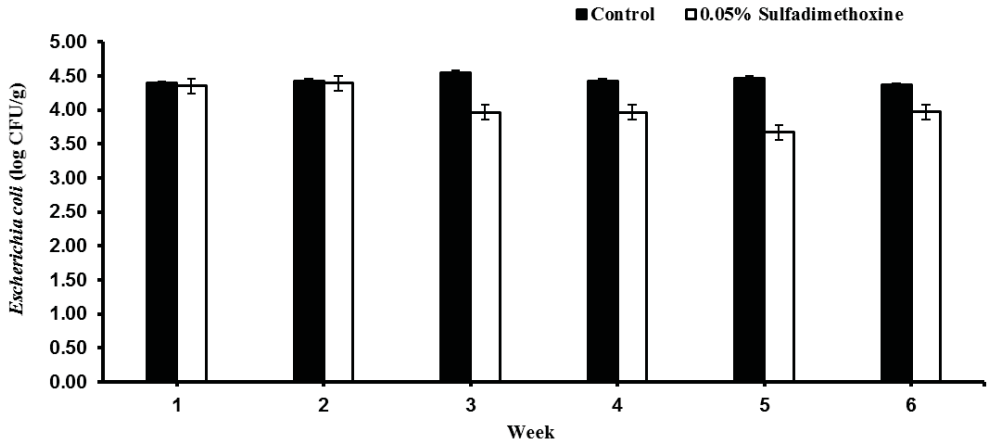


Figure 2. *E. coli* counts in live broilers from the control and antibiotic treatments from weeks 1 through 6
Data are means from two replications. SEM=3.410

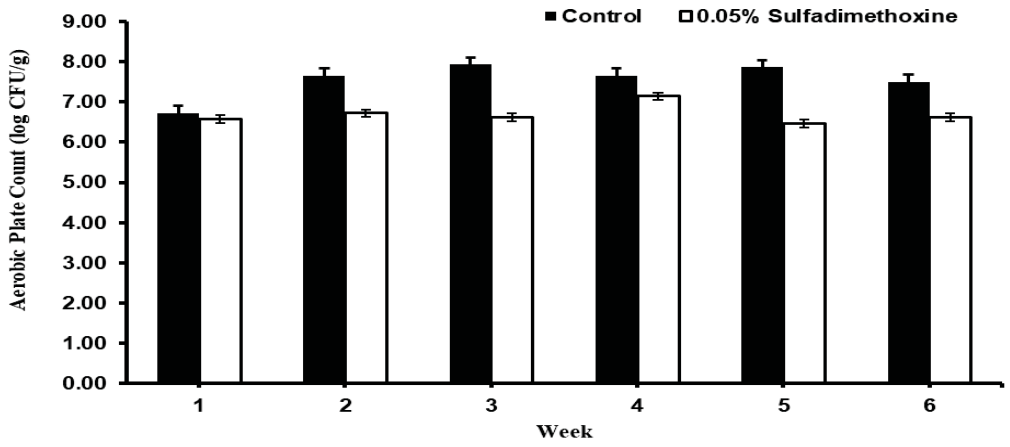


Figure 3. APC counts in live broilers from the control and antibiotic treatments from weeks 1 through 6
Data are means from two replications. SEM= 6.820

3.4 Prevalence of Salmonella, E. coli and Aerobic Plate Count

The prevalence of *Salmonella, E. coli* and aerobic plate counts were randomly tested from individual broilers (n = 300) in both treatments. At week 1, the prevalence of aerobic plate count was detected from both treatments at 100%. For *Salmonella* and *E. coli* were found at 96.7% in the control treatment (Table 1). In week 2, the prevalence of *Salmonella, E. coli* and aerobic plate count were detected 100% in both control and experimental treatments. In week 3, the prevalence of *Salmonella, E. coli* and aerobic plate count were declined in the control and the antibiotic treatment ($P<0.05$) (Table 1). Specifically, they were lower ($P<0.05$) in the antibiotic treatment at 83.3%, 76.7%, and 93.3% of *Salmonella*, *E. coli* and aerobic plate count, respectively. These finding showed that the antibiotic sulfadimethoxine can reduce the prevalence of these microorganisms in broilers especially in week 3 (Table 1). Overall, for the six-week period of testing, the prevalence of *Salmonella* in the antibiotic treatment was lower ($P<0.05$) than in the control treatment (Table 1). Similar, to the studies of Hanson, Kaneene, Paduangtod, Hirokawa, & Zeno (2002) who concluded that tetracycline, nalidixic acid, florfenicol, ampicillin, and ceftiofur were able to decrease the prevalence of *Salmonella*. No difference ($P>0.05$) was found in both treatments on the prevalence of *E. coli* and aerobic plate count.

Table 1. The prevalence of *Salmonella, Escherichia coli* and aerobic plate count in live broilers (n = 600) from the control and antibiotic treatments from weeks 1 through 6

	Control			0.05% sulfadimethoxine		
Week	No. (%) *Salmonella*	No. (%) *E.coli*	No. (%) APC	No. (%) *Salmonella*	No. (%) *E. coli*	No. (%) APC
1	96.7	96.7	100	100	100	100
2	100	100	100	100	100	100
3	86.7	80.0	100	83.3	76.7	93.3
4	96.7	96.7	100	93.3	93.3	100
5	100	100	100	96.7	100	100
6	100	100	100	90.0	100	100

4. Conclusions

This study revealed that poultry is a source of *Salmonella* and *E. coli* in small-scale poultry farming. This may contribute to cross-contamination of meat carcasses after slaughter and retail products. Therefore, the use of sulfadimethoxine as an antibiotic can reduce the enumeration of *Salmonella, Escherichia coli* and aerobic plate count in small-scale broiler operations.

Acknowledgements

This work was partly funded by the McNeese State University Juliet Hardtner Women in Science and Technology Endowed Professorship. We thank Dr. Ray Neyland and Mrs. Jan Prudhomme who provided assistance for this study.

References

Aarestrup, F. M. (2000). Occurrence, selection and spread of resistance to antimicrobial agents used for growth promotion for food animals in Denmark. *APMIS101*, 1-48. https://doi.org/10.1111/j.1600-0463.2000.tb05380.x

Aarestrup, F. M. (2015). The livestock reservoir for antimicrobial resistance: a personal view on changing patterns of risks, effects of interventions and the way forward. *Philosophical Transactions of the Royal Society B: Biological, 370*, 1-13. https://doi.org/10.1098/rstb.2014.0085

Alexandra, J. S. (2009). *Campylobacter* colonization of the turkey intestine in the context of microbial community development. *Applied and Environmental Microbiology, 75*(11), 3564-3571. https://doi.org/10.1098/rstb.2014.0085

Al-Ghamdi, M. S., El-Morsy, F., Al-Mustafa, Z. H., Al-Ramadhan, M., & Hanifm, M. (1999). Antibiotic resistance of *Escherichia coli* isolated from poultry workers, patients and chicken in the eastern province of Saudi Arabia. *Tropical Medicine & International Health, 4*, 278-283. https://doi.org/10.1046/j.1365-3156.1999.00392.x

Antunes, P., Mourão, J., Campos, J., & Peixe, L. (2016). Salmonellosis: the role of poultry meat. *Clinical Microbiology and Infection, 22*, 110-121. https://doi.org/10.1016/j.cmi.2015.12.004

Association of official analytical chemists (AOAC). (2000). Official Methods of Analysis. 17th ed. Gaithersburg, MD, USA.

Authority, E. F. S. (2016). The European union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *European Food Safety Authority Journal, 14*, 4634. https://doi.org/10.2903/j.efsa.2016.4634

Barrow, P. A., Jones, M. A., Smith, A. L., & Wigley, P. (2012). The long view: *Salmonella*- the last forty years. *Avian Pathology, 41*, 413-20. https://doi.org/10.1080/03079457.2012.718071

content in poultry meat in local Iraqi markets. *Journal of Microbiology Research, 3*(6), 205-207. https://doi.org/10.5923/j.microbiology.20130306.02

Cosby, D. E., Cox, N. A, Harrison, M. A., Wilson, J. L., Buhr, R. J., & Fedorka-Cray, P. J. (2015). *Salmonella* and antimicrobial resistance in broilers: a review. *Journal of Applied Poultry Research, 24*, 408-26. https://doi.org/10.3382/japr/pfv038

Crump, J. A., Sjolund, K. M., Gordon, M. A., & Parry, C. M. (2015). Epidemiology, clinical presentation,

laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. *Clinical Microbiology Reviews, 28*, 901-37. https://doi.org/10.1128/CMR.00002-15

Davami, A., Peterson, R. A., Jones, W. T., & Ilardi, R. L. (1987). Compatibility of sulfadimethoxine and ormetoprim with lasalocid and monensin on performance of male broiler chickens. *Poultry Science, 66*(2), 373-375. https://doi.org/10.3382/ps.0660373

Delaplane, J. P. (1945). Sulfaquinoxaline in preventing upper respiratory infections of chickens inoculated with infective field material containing Pasteurella avicida. *American Journal of Veterinary Research, 6*, 207-208.

DeRosa, M., Ficken, M. D., & Barnes, H. J. (1992). Acute airsacculitis in untreated and cyclophosphamide-pretreated broiler chickens inoculated with *Escherichia coli* or *Escherichia coli* cell free culture filtrate. *Veterinary Pathology, 29*, 68-78. https://doi.org/10.1177/030098589202900109

Food and Drug Administration (FDA). (2013). NADA 200-031 Sulfadimethoxine antibacterial soluble powder.

Grumbles, L. C., & Delaplane, J. P. (1948). Relative activity of sulfamethazine and sulfaquinoxaline against coccidia of chickens under field conditions. *Poultry Science, 27*, 411-419. https://doi.org/10.3382/ps.0270411

Haleem, A. M., Al-Bakri, S. A., & Al-Hiyaly, S. A. K. (2013). Determination of microbial

Hanson, R., Kaneene, J. B., Padungtod, P., Hirokawa, K., & Zeno, C. (2002). Prevalence of *Salmonella* and *E. coli*, and their resistance to antimicrobial agents, in farming communities in northern Thailand, *Southeast Asian Journal of Tropical Medicine, 33*(Suppl. 3), 120-126.

Höll, L., Behr, J., & Vogel, R. F. (2016). Identification and growth dynamics of meat spoilage microorganisms in modified atmosphere packaged poultry meat by MALDI-TOF MS. *Food Microbioloy, 60*, 84-91. https://doi.org/10.1016/j.fm.2016.07.003

Huff, W. E., Huff, G. R., Rath, N. C., Balog, J. M., & Donoghue, A. M. (2002). Prevention of *Escherichia coli* infection in broiler chickens with a bacteriophage aerosol spray. *Poultry Science, 81*, 1486-1491. https://doi.org/10.1093/ps/81.10.1486

Klare, I., Badstübner, D., Konstabel, C., Böhme, G., Claus, H., & Witte, W. (1999). Decreased incidence of vanA-type vancomycin-resistant enterocci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. *Microbial Drug Resistance, 5*, 45-52. https://doi.org/10.1089/mdr.1999.5.45

Lee, M. K., Runyon, M., Herrman, J. T., Phillips, R., & Hsieh, J. (2015). Review of *Salmonella* detection and identification methods: Aspects of rapid emergency response and food safety. *Food Control, 47*, 264-76. https://doi.org/10.1016/j.foodcont.2014.07.011

Lutful, K. S. M. (2010). Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *International journal of environmental research and public health, 7*, 89-114. https://doi.org/10.3390/ijerph7010089

Mazengia, E., Samadpour, M., Hill, H. W., Greeson, K., Tenney, K., Liao, G., … Meschke, J. S. (2014). Prevalence, concentrations, and antibiotic sensitivities of *Salmonella* serovars in poultry from retail establishments in Seattle, Washington. *Journal of Food Protection, 77*, 885-93. https://doi.org/10.4315/0362-028X.JFP-13-394

Nolan, L. K. (2019). Overview of Colibacillosis in poultry. University of Georgia. Retrieved from https://www.merckvetmanual.com/poultry/colibacillosis/overview-of-colibacillosis-in-poultry

Orton, C. T., & Hambly, L. R. (1971). Efficacy studies on potentiated sulfadimethoxine as a chicken coccidiostat. *Poultry Science, 50*(5), 1341-1346. https://doi.org/10.3382/ps.0501341

Page, S. W., & Gautier, P. (2012). Use of antimicrobial agents in livestock. *Revue Scientifique et Technique, 31*, 145-88. https://doi.org/10.20506/rst.31.1.2106

Piercy, D. W. T., & West, B. (1976). Experimental *Escherichia coli* infections in broiler chickens: Course of the disease induced by inoculation via the air sac route. *Journal of Comparative Pathology, 86*, 203-210. https://doi.org/10.1016/0021-9975(76)90044-x

Rouger, A., Tresse, O. I. D., & Zagorec, M. (2017). Review bacterial contaminants of poultry meat: sources, species, and dynamics. *Microorganisms, 5*, 50. https://doi.org/10.3390/microorganisms5030050

Seiffert, S. N., Hilty, M., Perreten, V., & Endimiani, A. (2013). Extended-spectrum cephalosporin-resistant gram-negative organisms in livestock: an emerging problem for human health?. *Drug Resistance Updates, 16*, 22-45.

Simonsen, G. S., Haaheim, H., Dahl, K. H., Kruse, H., Løvseth, A., Olsvik, Ø., & Sundsfjord, A. (1998). Transmission of vanAType vancomycin-resistant enterococci and vanA resistance elements between chicken and humans at avoparcin-exposed farms. *Microbial Drug Resistance, 4*, 313-318. https://doi.org/10.1089/mdr.1998.4.313

Statistical Analysis Software (SAS). (2003). SAS User's Guide Version 9.1.3. Cary, NC.: SAS Institute Inc.

Tangkham, W., Janes, M., & LeMieux, F. (2016a). The effects of sulfadimethoxine administered to control *Campylobacter jejuni* in small-scale broiler operations. *Poultry, Fisheries and Wildlife Sciences, 4*, 166. https://doi.org/10.4172/2375-446X.1000166

Tangkham, W., Janes, M., & LeMieux, F. (2016b). Prevalence and distribution of *Campylobacter jejuni* in small-scale indoor and outdoor poultry production. *Journal of Food Protection, 79*(1), 75-81. https://doi.org/10.4315/0362-028X.JFP-15-331

Tessi, M. A., Salsi, M. S., Caffer, M. I., & Moguilevsky, M. A. (1997). Drug resistance of Enterobacteriaceae isolated from chicken carcasses. *Journal of Food Protection, 60*, 1001-1005. https://doi.org/10.4315/0362-028X-60.8.1001

Van Den Bogaard, A. E., London, N., Driessen, C., & Stobberingh, E. E. (2001). Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers, and poultry slaughterers. *The Journal of Antimicrobial Chemotherapy, 47*, 763-771. https://doi.org/10.1093/jac/47.6.763

Vree, T. B., & Hekster, Y. A. (1987). Clinical pharmacokinetics of sulfonamides and their metabolites. *H. Schonfeld, ed. 37*, 1-9. https://doi.org/10.1159/isbn.978-3-318-03212-3

Waletzky, E., & Hughes, C. O. (1946). The relative activity of sulfonamides and other compounds in avian coccidiosis. *American Journal of Veterinary Research, 7*, 365-373.

Wang, J., MacNeil, D. J., & Kay, F. J. (2012). *Chemical analysis of antibiotic residues in food* (1stedn). John Wiley and Sons Press, 242. https://doi.org/10.1002/9781118067208

Whitehill, A. R., Oleson, J. J., & Hutchings, B. L. (1950). Stimulatory effect of aureomycin on the growth of chicks. *Proceedings of the Society for Experimental Biology and Medicine, 74*, 11-13. https://doi.org/10.3181/00379727-74-17793

Zhang, Q. Q., Han, Y. Q., Cao, J. X., Xu, X. L., Zhou, G. H., & Zhang, W. Y. (2012). The spoilage of air-packaged broiler meat during storage at normal and fluctuating storage temperatures. *Poultry Science, 91*, 208-214. https://doi.org/10.3382/ps.2011-01519

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Adult Level of Physical Activity in Urban (Abobo, Cocody) and Rural (Yocoboué) Area in Côte d'Ivoire

Nina Laurette Ahouéfa[1], Audrey Herbert Yepié[1], Jean Jacques Diagou[1] & Louise Anin-Atchibri[1]

[1]Laboratory of Nutrition and Food Safety, UFR of Sciences and Food Technologies, Nangui Abrogoua University, Abidjan, Côte d'Ivoire

Correspondence: Ahouéfa Nina Laurette, Laboratory of Nutrition and Food Safety, UFR of Sciences and Food Technologies, Nangui Abrogoua University, Abidjan, 02 BP 801 Abidjan 02, Côte d'Ivoire. Tel: 225-0978-8910. E-mail: laurette.nina2017@gmail.com

Received: August 13, 2019 Accepted: October 29, 2019 Online Published: November 20, 2019

doi:10.5539/jfr.v8n6p154 URL: https://doi.org/10.5539/jfr.v8n6p154

Abstract

The physical activity (PA) plays an important role in the fight against the chronic noncommunicable diseases.The objective of this study was to assess the level of physical activity in an adult population in rural and urban area in Côte d'Ivoire. To achieve this goal, a group of 1046 participants was submitted to the Global Physical Activity Questionnaire (GPAQ). The data analysis was done according to the GPAQ guidelines. The prevalence of the recommended level of the physical activity during the work, the leisure and the displacement was calculated.This assessment has showed that the intense Physical Activity (PA) decreased with age, from 34% (19-29 years) to 14% (50-60 years).Then the results have showed that the workers had the highest intensity Physical Activity Level (NAP) at 70%, versus 13% for the office workers.Moreover, more the Level of Study (LS) was higher, more the physical activity practiced decreased, whatever the area of living. Finally, it has been shown that men were more active than women. Also, the level of physical activity was higher in rural than in urban areas, and the limited physical activity level was present in the adult population. This work has shown that the level of physical activity and the contexts of practice vary according to the socio-demographic characteristics. At that fact, targeted interventions to promote the physical activity are necessary.

Keywords: assessment, physical activity, Global Questionnaire on Physical Activity (GPAQ), rural, urban, adult, Côte d'Ivoire

1. Introduction

The inactivity is the fourth leading cause of death in the world and is now presented as a true pandemic (Kohl et al., 2012). In fact, the change in lifestyle, the sedentarization of professional activities, the motorization of the means of transport and the automation of housework, have been accompanied by a decrease in the energy expenditure of the population (HCSP, 2000; INSERM, 2008). However, the practice of a regular and adapted physical activity, as well as the limitation of the sedentary activities, constitute with the adoption of a balanced diet, the major factors of acquisition and maintenance of a good state of health. The physical activity is involved, at the same title than the eating habits, in the prevention of a large number of chronic diseases (cardiovascular diseases, diabetes, obesity, cancer, etc.) (INSERM, 2008). Thus, with the 2008 Health Nutrition Barometer, the level of physical activity and that of the sedentarity of the population were assessed using the World Physical Activity Questionnaire (GPAQ) of the World Health Organization (OMS, 2005). It permits to study the contexts of practice (work, leisure, displacement) and the levels of physical activity (high, medium, and limited) during a typical week. In this context, the promotion of the physical activity and the fight against the sedentarity are the major objectives, particularly in the fight against the noncommunicable diseases (OMS, 2005).

Several surveys conducted at the initiative of the health sector have focused on the physical activity and the sedentary practices of the French population (Barnett et al., 2008). However, in the countries of sub-Saharan Africa, a few data on the level of physical activity of the populations are available (Guthold et al., 2008). In Côte d'Ivoire, some studies have estimated the prevalence of the physical activity at 66.7% using the IPAQ method, which is recognized as overestimating the data (Guthold et al., 2008). Also the study conducted by Pepe (2017) has only highlighted the importance of the physical activity as a means of fighting against the noncommunicable diseases. Thereby, the objective of this study was to assess the level of physical activity according to the

socio-demographic characteristics of a rural (Yocoboué) and urban (Abobo and Cocody) adult population in Côte d'Ivoire.

2. Material and Methods

2.1 Study Area

The study was conducted in Côte d'Ivoire, in two (2) communes of Abidjan (Abobo and Cocody), and in a village in the south-east of Côte d'Ivoire (Yocoboué).

2.2 Type of Study, Target Population and Sampling

A descriptive and analytical cross-sectional study was conducted from August 22 to November 19, 2018, and has involved adults. The study population was composed of one thousand one hundred and forty-six (1146) apparently healthy subjects (without declared diseases), aged from 18 to 60 years, including 688 women and 458 men, living in Abidjan (Abobo and Cocody) and in a south-east village of Côte d'Ivoire (Yocoboué).

The subjects were selected according to the simple random method (Giezendanner, 2012) in 6 areas of each urban (Abobo and Cocody) and rural (Yocoboué) region. We used the formula of Giezendanner (2012) for calculating the sample size. The pregnant and lactating women were excluded from the study. The study participants must have resided in the areas of selection for at least 6 months. They must have been previously informed of the purpose of the study and have given their consent of participation.

2.3 Technics and Collection Tools

The data collection technics used were the questionnaire survey for adults. The questionnaire inspired by the WHO STEPS instrument (WHO, 2005) collected information on the physical activity practice and the socio-demographic characteristics such as age, gender, work status,place of residence and the level of education.

2.4 Measuring the Level of Physical Activity

To assess the level of physical activity (PAN) in the study population, the Global Physical Activity Questionnaire (GPAQ) was used. The GPAQ questionnaire, developed by the WHO, contains 16 questions and collects information on the practice of physical activity during activities performed at work, at leisure or a displacement. The activities at work include paid and unpaid work and the housework. The leisure activities include sport and those related to displacement of at least 10 minutes on foot, by bike or in wheel skates. Each participant was asked to describe their weekly physical activity during his professional activities, displacements and leisure. The GPAQ analysis guide provided by WHO was used to analyze the data (Maceraet Pratt, 2000).

2.5 Statistical Analysis

The data analysis was performed using the IBM SPSS statistical analysis software version 20 (SPSS for windows, SPSS Inc., Chicago, IL). The results were presented under the form of percentage. The comparisons of proportions were made by the Pearson chi-square test at 0.05 significance level

3. Results

3.1 Socio-demographic Characteristics of the Study Population

The socio-demographic characteristics of the study population are presented in Table 1. The average age of adults was 38.72 ± 18.5 years, ranging from 18 to 60 years. The modal class is between 30 and 39 years old (49%).Of the 1146 participants, 688 (60.03%) were female, 768 (67%) come from urban areas of Abidjan (Abobo and Cocody) and 33% from rural areas (Yocoboué).

67.36% were educated and the most representative socio professional category of this study was the liberal activity with 47.73% of the participants. The category of civil servants was presented in two groups, namely clerical agents and workers. Workers were present only in urban areas (Abobo 8% and Cocody 5%).

Table 1. Socio-demographic characteristics of the study population

	Rural area		Urban area			
Total (n= 1146)	**Yocoboué (n=378)**		**Abobo (n=384)**		**Cocody (n=384)**	
	n	**%**	**n**	**%**	**n**	**%**
Sex						
Men	151		163		144	
Women	227		221		240	
Total	378		384		384	
Averageage	39,76±17,9		37,77±18,69		38,63±18,92	
Age range (year)						
▪ 18-29	137	36,3	146	38	116	30,2
▪ 30-39	188	49,7	188	49	185	48,2
▪ 40-49	30	8	40	10,4	51	13,3
▪ 50-60	23	6	10	2,6	32	8,3
Education level						
▪ Uneducated	208	55	106	27,6	60	15,6
▪ Educated	170	45	278	72,4	324	84,4
▪ Primairy	106	28	95	24,7	50	13
▪ Secondairy	60	16	102	26,5	146	38
▪ Superior	4	1	81	21,1	128	33,3
Professional status						
▪ Unemployed	38	10	62	16	29	7,0
▪ Housewives	54	14	46	12	8	2,0
▪ Oficials	71	19	84	22	189	49,0
▪ Office Agent	71	19	54	14	171	45,0
▪ Workers	-	-	30	8	18	5,0
▪ Liberal activity	215	57	192	50	140	36,0

3.2 Contexts of Practice according to the Physical Activity Level

The distribution of the level of the physical activity according to the context of practice (high, moderate, limited) is shown in Figure 1. It shows that among the populations with a high level of physical activity, the work accounts for the most part of the total physical activity, with respectively 64.8% in Yocoboué against and 68.2% in Abobo and 24.7% in Cocody. While the displacement-related physical activity is observed in moderate-level in Yocoboué populations at 48.2%, in high-level in populations of Abobo at 48.2% and in limited-level in populations of Cocody at 40.4%.

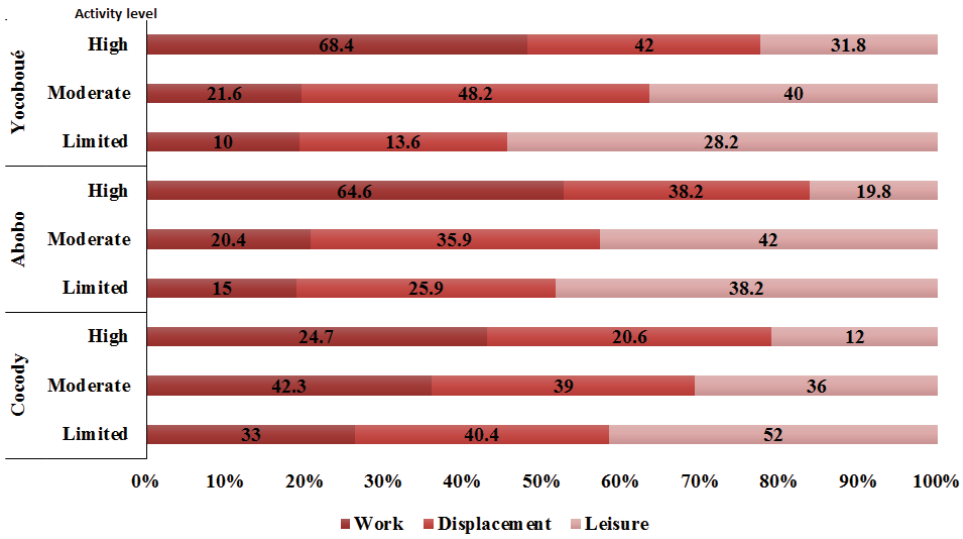


Figure 1. Contexts of practice according to the physical activity level

3.3 Level of "High" Physical Activity according to the Age

The age distribution of the study population according to the physical activity level is shown in figure 2. According to the categorization of the physical activity defined by the WHO from the GPAQ questionnaire, the level of intense physical activity decreased with age. Respectively 22.0%, 18.0%, 10.8% and 3.9% for the age groups [18-29years], [30-39 years], [40-49 years] and [50-60 years] among the inhabitants of Cocody. Then 35.0%, 20.0%, 19.5% and 8.0% for the age groups [18-29 years], [30-39 years], [40-49 years] and [50-60 years] among the inhabitants of Abobo. Finally, 45.2%, 40.6%, 27.2%, and 21.9% for the age groups [18-29 years], [30-39 years], [40-49years] and [50-60 years] for the populations of Yocoboué.

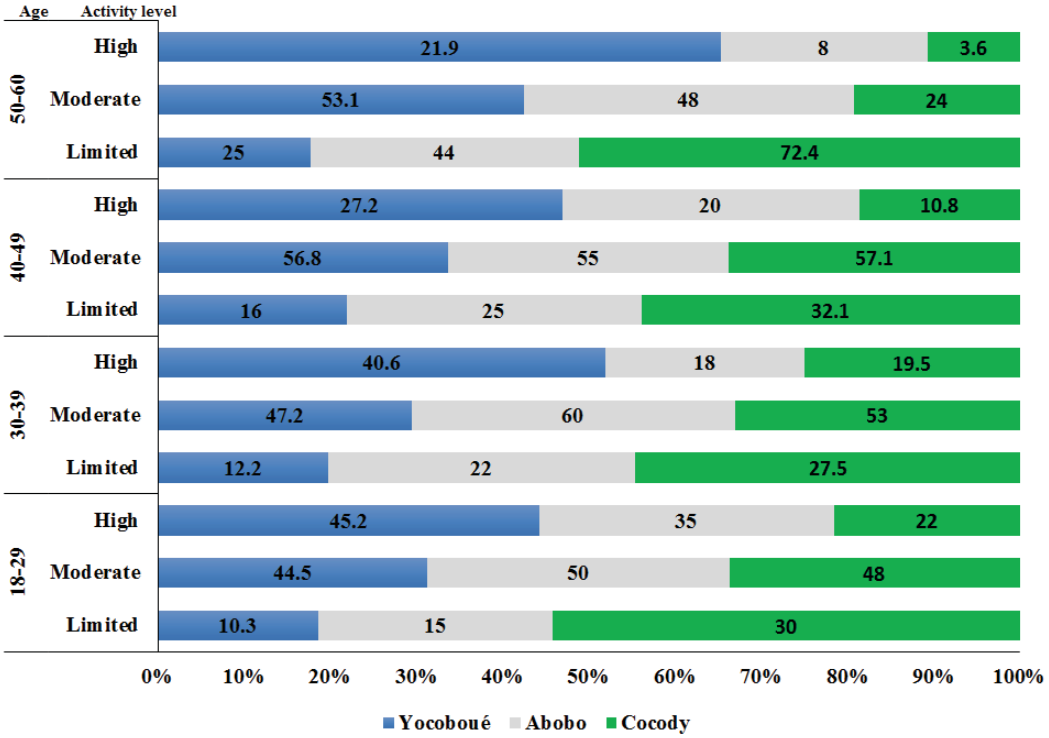


Figure 2. Age distribution of the study population according to the physical activity level

3.4 Physical Activity Level According the Socio-professional Category

The figure 3 shows the physical activity level (PAL) of the study participants according to their socio-professional category. From all the socio-professional categories of the entire sample, the workers were those who had the most intense PAL (70.8%), followed by the liberal activities leaders (42.7%), the housewives (36%), the unemployed (28%) and finally the office workers (13%). The intense physical activity level (PAL) was higher among people practicing a liberal activity in Yocoboué (rural area), with 75% of prevalence, than in urban areas (Abobo 32.6% and Cocody 7.1%). The housewives of Yocoboué (rural area) had an intense Physical Activity more important (59, 7%) than those of urban area of Abobo (13%) and Cocody (8,5%). For the office agents, we noted that those of Cocody (79.4%) and Abobo (29%) had a limited physical activity level (PAL) more important than those of Yocoboué (18.29%). The physical activity of the unemployed is mostly moderate in Yocoboué (48%) and Abobo (37.5%) and rather limited (60%) in Cocody

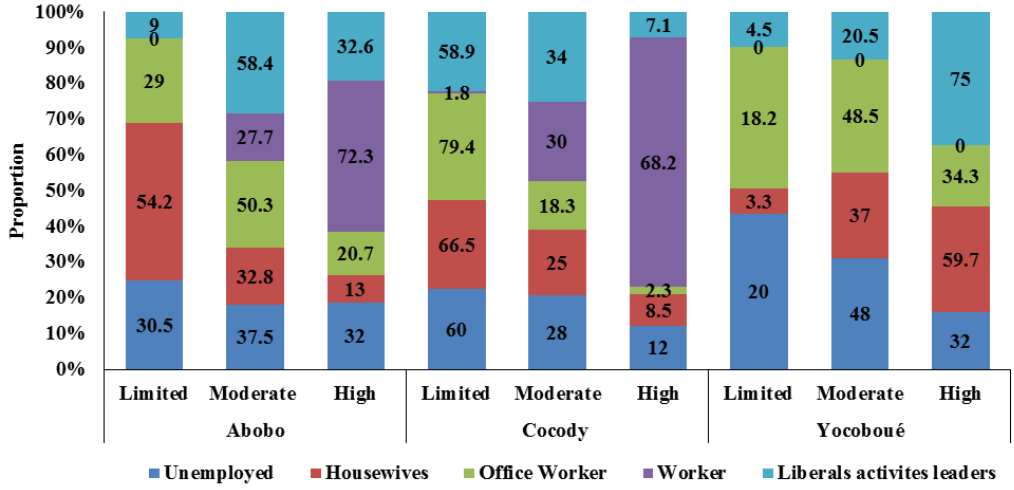


Figure 3. Distribution of the physical activity level (PAL) according to the socio-professional

3.5 Level of "High" Physical Activity according to the Sex

The level of physical activity according to the sex is shown in Figure 4. The intensive Physical activity in men is more important than in women, from rural areas to urban areas. However, it should be noted that the men of Yocoboué (rural) are more active than those of the urban areas of Abobo (50.6%) and Cocody (10.6%). The urban women are less active than those of the rural areas, and the men of the urban areas are much more physically active.

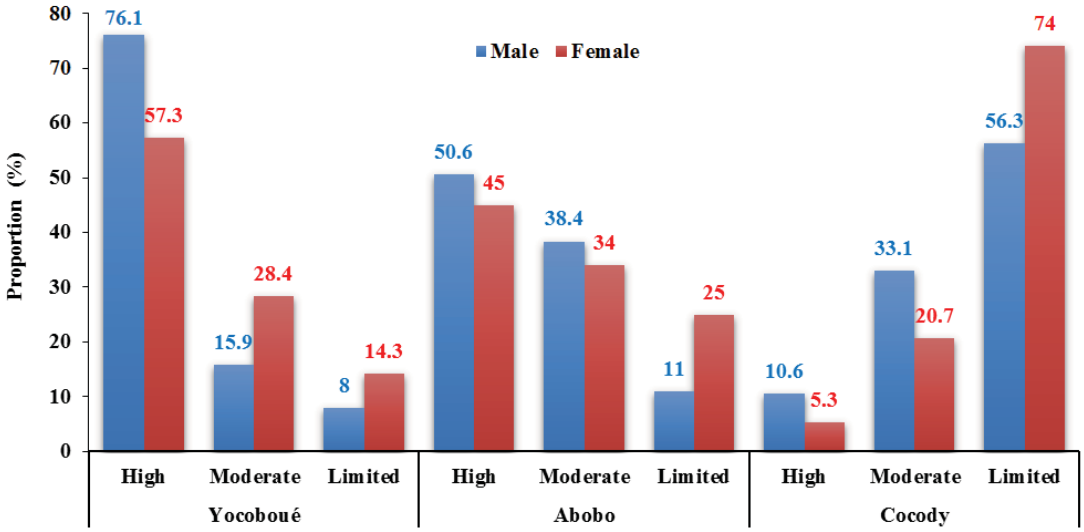


Figure 4. Distribution of the physical activity level (PAL) according to the sex

3.6 Physical Activity according to Study Level

The Figure 5 shows the distribution of the physical activity among the study participants according to the study level. The physical activity of the participants with no education was more important in allthe areas of residence, but was much more pronounced in rural areas (Yocoboué:84.2%) than in urban areas (Abobo: 78% and Cocody: 76%). It can be noted that for all areas of residence, the practice of intense physical activity at work is inversely related to the degree of education and is also more frequent when the person belongs to the category of the workers than the employees.

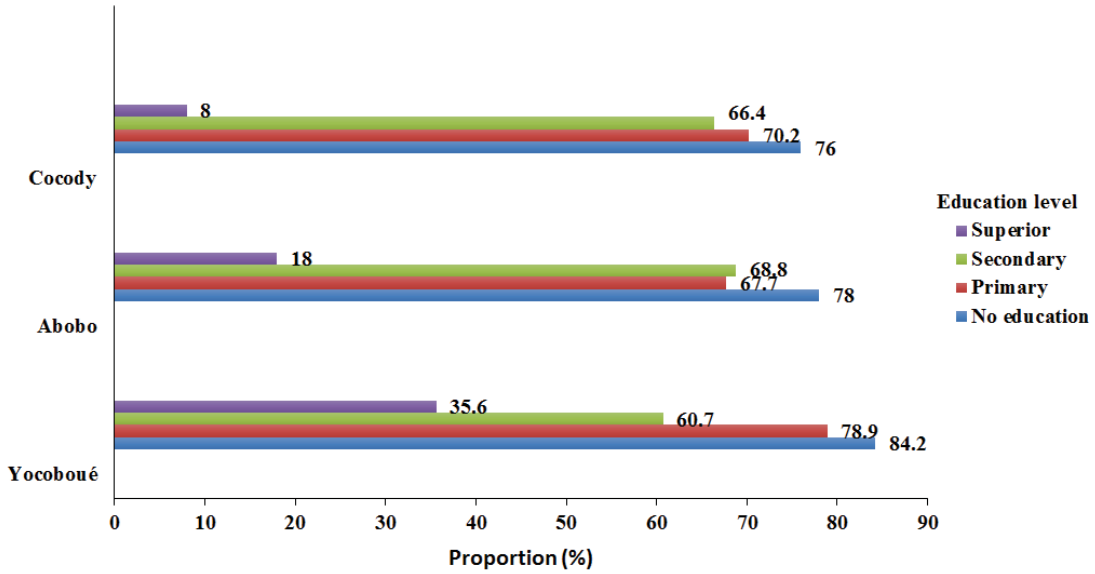


Figure 5. Distribution of the physical activity level according to the study level

4. Discussion

4.1 Practice Contexts according to the Physical Activity Level

The determination of the physical activity level of the population is a concern in many countries (Maceraet Pratt, 2000) because of the importance of the physical activity in the fight against the chronic diseases, especially in their prevention but also in their management (INSERM, 2008). The Measure of the physical activity level according to the contexts provides a better understanding of the overall physical activity (Berrigan et al., 2006) and (Jacobi et al., 2009).

The consideration of the physical activity in it globality give a better understanding of the fact that the physical activity is practiced in different contexts and can produce effects that are themselves different.

The decrease of the physical activity at work and the displacements from the rural areas to the urban areas may be a reflection of the urbanization with the increase in the mechanization of transport and the sedentarization of the workstations, which is progressively installed (Stamatakis, 2007). This study is consistent with that of Stamatakis (2007) who has showed that the data collected between 1991 and 2004 in England showed a downward trend in physical activity at work.

4.2 Physical Activity Level according the Age

The study has also showed that the intense physical activity decreased with age for the entire population. The decrease in intense physical activity with age can be explained by a biological or environmental mechanism. The biological hypothesis is further reinforced by the discovery of dopamine that acts on specific areas of the brain. This hormone is related to the motivation for the locomotion (Sallis and Owen, 1999; U.S., 1996). According to these same authors, other non-biological factors such as environmental (living environment), psychological, social (marital status) and physical (health) variables are also related to the physical activity (Sallis and Owen, 1999; US, 1996). The study corroborates those of the District Control and Prevention Center of Colombia (2004) and of Caspersen et al. (2000) on the decline of age-related physical activity. However, a study conducted on a population of Nigerian civil servants revealed no significant difference between 20 and 64 years of age (Forrest et al., 2001).

4.3 Physical Activity Level according the Socio-professional Category

The study has also showed that workers were the most active of all the socio-professional categories; after them we had persons with liberal activities and those with weak activity as office workers. This could be explained by the fact that the workers make enormous efforts in their work. Office workers have activities that require less effort (Oyeyeni et al., 2016). In developed countries people's hobbies tend to include sports and games, however in Côte d'Ivoire as in Nigeria, hobbies tend to be passive with small physical activity (PA) (Oyeyeni et al., 2016).

4.4 Physical Activity Level according to the Study Level

The results have showed that the level of education influenced the physical activity level (NAP). This could be

explained by the fact that low-educated people move regularly or are likely to move by walk and / or by bike than those with a high level of education. People with a high level of education may own a transport vehicle that significantly reduces their level of physical activity (Jurakic et al., 2009).

This study is consistent with those in Mexico, which have showed a higher percentage of adults working in agriculture and fishing, and who were in a higher category of physical activity than those engaged in lower-intensity occupational activities, the latter also having a low or a moderate physical activity level (Salmon et al., 2000).

4.5 Physical Activity Level according the Sex

The results have showed that the intense physical activity is more marked in men than in women. This is due to the fact that women generally are engaged in physical activities that range from mild to moderate activity; however, men are engaged in moderate to intense activities (Assahet et al., 2015). Also the African culture have the particularity of making women likely to be employed in work such as: organize the house, do housework, which makes them work less. And generally in this culture women have hobbies that tend to keep them at home, while men tend to indulge in leisure activities that require a physical effort (Walter and Randt, 2011). Our study is in agreement with that of Kiruanda et al. (2016) conducted among peri-urban and rural populations in eastern Uganda where the prevalence of the sedentary in women was higher than that of men. On the other hand, the studies carried out by Oyeyemi et al. (2013) in Nigeria and Kanyoni and philipps (2009) in Rwanda found that women were more active than men.

The urban participants were found to be less active than those in rural areas. This could be due to the fact that the rural communities with their socio-economic status, practiced intense physical activity, such as the agriculture, the main source of income, and a type of active transportation. The urban communities can exercise less intense professional activities or not, or adopt a sedentary lifestyle, due to the fact of their less physically active professional activities and of their motorized mode of transportation (Assah et al., 2015).

Most countries in sub-Saharan Africa, especially those with rapid evolution, are in full phase of demographic and epidemiological development. These development processes have resulted in changes in the social capital of societies, work patterns, and lifestyles that have reduced the physical activity levels (Zewnicki et al., 2003).

5. Conclusion

The evaluation of the physical activity level has showed that the physical activity is practiced in different contexts in urban, peri-urban and rural areas. Also, the rural population (Yocoboué) was more active than that of the peri-urban area (Abobo) with moderate physical activity, and that of the urban area (Cocody) with limited physical activity. The low level of physical activity was noticed in the female population. Concerning the socio-occupational category, the office workers had a low physical activity level. For the level of education, the high-educated population had a low level of physical activity. These data suggest that urban residents, women, office workers, and those with low levels of education are targets for the interventions and the physical activity promotion.

References

Assah, F., Mbanya, J. C., Ekelund, U., Wareham, N., & Brage, S. (2015). Patterns and correlates of objectively measured free-living physical activity in adults in rural and urban Cameroon. *J Epidemiol Community Health, 69*(7), 700-707. https://doi.org/10.1136/jech-2014-205154

Barnett, T. A., Gauvin, L., Craig, C. L., & Katzmarzyk, P. T. (2008). Distinct trajectories of leisure time physical activity and predictors of trajectory class membership: a 22 year cohort study. *Interational Journal of Behavioral Nutrition and Physical Activity, 5*, 57. https://doi.org/10.1186/1479-5868-5-57

Berrigan, D., Troiano, R. P., McNeel, T., Disogra, C., & Ballard, B. R. (2006). Active transportation increases adherence to activity recommendations. *American Journal of Preventive Medicine, 31*(3), 210-216. https://doi.org/10.1016/j.amepre.2006.04.007

Caspersen, C. J., Powell, K. E., & Christenson, G. M. (1985). Physical activity, execice, and physical fitness: definition and distinctions for health-related research. *Public health reports, 100*(2), 126-131.

Forrest, K., Bunker, C., Kriska, A., Ukoli, F., Huston, S., & Markovic, N. (2001) Physical activity and cardiovascular risk factors in a developing population. *Medicine and Science in Sports and Exercise, 33*, 1598-604. https://doi.org/10.1097/00005768-200109000-00025

Giezendanner, D. (2012). Taille d'un échantillon aléatoire et Marge d'erreur. En ligne: Retrieved from http://icietla-ge.ch/voir/IMG/pdf/7-taille-d_un-echantillon-aleatoire-et-marge-d_erreur-cms-spip.pdf

Guthold, R., Ono, T., Strong K. L., Chatterji, S., & Morabia, A. (2008). Worldwide variability in physical inactivity a 51-country survey. *Am J Prev Med, 34*(6), 486-94. https://doi.org/10.1016/j.amepre.2008.02.013

Haut Comité de la Santé Publique (HCSP). Pour une politique nutritionnelle de santé publique en France. Enjeux et propositions. Rennes: Edition ENSP; 2000.

Institut national de la santé et de la recherche médicale (INSERM). Activité physique: contextes et effets sur la santé. Expertise collective. Paris: INSERM, coll.2008.

Jacobi, D., Charles, M. A., & Tafflet, M. (2009). Relationships of self reported physical activity domains with accelerometry recordings in French adults. *European Journal of Epidemiology, 24*(4), 171-179. https://doi.org/0.4000/cybergeo.26711

Kanyoni, M., & Phillips, J. S. (2009). Factors associated with physical activity levels among older adults in selected institutions in Rwanda. *J Commun H Sci., 4*(1), 8-14.

Kirunda, B. E., Wamani, H., Fadnes L. T., Broeck J. V. D., & Thorkild, T. (2016). Objectively Assessed Physical Activity and Associated Factors Among Adults in Peri-Urban and Rural Eastern Uganda: A Population-based Study. *Journal of Physical Activity and Health, 13*, 1243-1254. https://doi.org/10.1123/jpah.2016-0025

Kohl, Hr., Craig, C., & Lambert E. (2012). The pandemic of physical inactivity: global action for public health. *Lancet, 380*(9838), 294-305. https://doi.org/10.1016/S0140 6736(12)608988

Macera, C. A., & Pratt, M. (2000). Public health surveillance of physical activity. *Research Quarterly for Exercise and Sport, 71*, 97-103. https://doi.org/10.1080/02701367.2000.11082792

Organisation Mondiale de la Santé (OMS) (2005). Questionnaire mondial sur la pratique d'activités physiques (GPAQ). Guide pour l'analyse. Retrieved from http://www.who.int/chp/steps/GPAQ_Analysis_Guide_FR.pdf

Oyeyemi, A. L., Oyeyeni, A. Y., Adegoke, B. O., Oyetoke, F. O., Aliyu, H. N., & Aliyu, S. U. (2011). Cross cultural adaptation of the international physical activity Questionnaire: Reliability and validity of the Hausa version in Nigeria. *BMC MedicalResearchMethodology, 11*(1). https://doi.org/10.1186/1471-2288-11-156

Pepe, H. D. (2017). Les activités physiques comme moyen de lutte contre les maladies non transmissibles en Côte d'Ivoire, mémoire, Master Projet et développement des territoires, Septembre 2017.

Sallis, J. F., & Owen, N. (2002). Physical Activity and Behavioral Medicine. *Thousand Oaks, CA: Sage*, 110-133.

Salmon, J., Owen, N., Bauman, A., Schmitz M, K., Booth, M. (2000). Leisure-time, occupational, and household physical activity among professional, skilled, and less-skilled workers and homemakers. *Prev Med., 30*(3), 191-9.https://doi.org/10.1006/pmed.1999.0619

Stamatakis, E., Hamer, M., & Lawlor, D. A. (1995). Physical activity, mortality, and cardiovascular disease: is domestic physical activity beneficial? The Scottish Health Survey 1995, 1998, and 2003.young women. *Am J PrevMed, 25*, 140-143. https://doi.org/10.1093/aje/kwp042

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES (1996). *Physical Activity and Health: A Report of the Surgeon General*. Atlanta, GA: Centers for Disease Control, pp. 215-217, 234-236.

Walter, C. M., & Randt, R. D. U. (2011). Socio-cultural barriers to physical activity among black isixhosa speaking professional women in the Nelson Mandela metropolitan municipality. *S Afr J Res Sport PhysEduc Recreation, 33*(2), 143-5. https://doi.org/10.4314/sajrs.v33i2.69698

WHO (2005). Chronic diseases and Health promotion. STEPwise approch to surveilance young women. *Am J Prev Med, 25*, 140-143.

Zewnicki, R., Vanden Auweele, Y., & De Bourdeaudhuij, I. (2003). Addressing over reporting on the International Physical Activity Questionnaire (IPAQ) telephone survey with a population sample. *Public Health Nutr, 6*(3), 299-305. https://doi.org/10.1079/PHN2002427

Journal of Food Research; Vol. 8, No. 6; 2019
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Reviewer Acknowledgements

Journal of Food Research wishes to acknowledge the following individuals for their assistance with peer review of manuscripts for this issue. Their help and contributions in maintaining the quality of the journal are greatly appreciated.

Journal of Food Research is recruiting reviewers for the journal. If you are interested in becoming a reviewer, we welcome you to join us. Please find the application form and details at http://www.ccsenet.org/journal/index.php/jfr/editor/recruitment and e-mail the completed application form to jfr@ccsenet.org.

Reviewers for Volume 8, Number 6

Antonello Santini, University of Napoli "Federico II", Italy

Bojana Filipcev, University of Novi Sad, Serbia

Cheryl Rosita Rock, California State University, United States

Corina-aurelia Zugravu, University of Medicine and Pharmacy Carol Davila, Romania

Diego A. Moreno-Fernández, CEBAS-CSIC, Spain

Emma Chiavaro, University of Parma, Italy

Jose Maria Zubeldia, Gestión Sanitaria de Canarias – Gobierno de Canarias, Spain

Juliano De Dea Lindner, Federal University of Santa Catarina (UFSC), Brazil

Winny Routray, McGill University, Canada

ACADEMIC AND OPEN ACCESS

Canadian Center of Science and Education

CCSENET.ORG

➢ CALL FOR MANUSCRIPTS

Journal of Food Research (JFR) is a peer-reviewed, open access journal, published by Canadian Center of Science and Education. It publishes original research and applied articles in all areas of food studies. Authors are encouraged to submit complete unpublished and original works, which are not under review in any other journals. We are seeking submissions for forthcoming issues. All manuscripts should be written in English. Manuscripts from 3000–8000 words in length are preferred. All manuscripts should be prepared in MS-Word format, and submitted online, or sent to: jfr@ccsenet.org

Paper Selection and Publishing Process

a) Submission acknowledgement. If you submit manuscript online, you will receive a submission acknowledgement letter sent by the online system automatically. For email submission, the editor or editorial assistant sends an e-mail of confirmation to the submission's author within one to three working days. If you fail to receive this confirmation, please check your bulk email box or contact the editorial assistant.

b) Basic review. The editor or editorial assistant determines whether the manuscript fits the journal's focus and scope. And then check the similarity rate (CrossCheck, powered by iThenticate). Any manuscripts out of the journal's scope or containing plagiarism, including self-plagiarism are rejected.

c) Peer Review. We use a double-blind system for peer review; both reviewers' and authors' identities remain anonymous. The submitted manuscript will be reviewed by at least two experts: one editorial staff member as well as one to three external reviewers. The review process may take two to four weeks.

d) Make the decision. The decision to accept or reject an article is based on the suggestions of reviewers. If differences of opinion occur between reviewers, the editor-in-chief will weigh all comments and arrive at a balanced decision based on all comments, or a second round of peer review may be initiated.

e) Notification of the result of review. The result of review will be sent to the corresponding author and forwarded to other authors and reviewers.

f) Pay the publication fee. If the submission is accepted, the authors revise paper and pay the publication fee.

g) E-journal is available. E-journal in PDF is available on the journal's webpage, free of charge for download. If you need the printed journals by post, please order at: http://www.ccsenet.org/journal/index.php/jfr/store/hardCopies

h) Publication notice. The authors and readers will be notified and invited to visit our website for the newly published articles.

More Information

E-mail: jfr@ccsenet.org

Website: http://jfr.ccsenet.org

Paper Submission Guide: http://jfr-author.ccsenet.org

Recruitment for Reviewers: http://www.ccsenet.org/journal/index.php/jfr/editor/recruitment

➢ JOURNAL STORE

To order back issues, please contact the editorial assistant and ask about the availability of journals. You may pay by credit card, PayPal, and bank transfer. If you have any questions regarding payment, please do not hesitate to contact the editorial assistant.

Price: $40.00 USD/copy Shipping fee: $20.00 USD/copy

ABOUT CCSE

The Canadian Center of Science and Education (CCSE) is a private for-profit organization delivering support and services to educators and researchers in Canada and around the world.

The Canadian Center of Science and Education was established in 2006. In partnership with research institutions, community organizations, enterprises, and foundations, CCSE provides a variety of programs to support and promote education and research development, including educational programs for students, financial support for researchers, international education projects, and scientific publications.

CCSE publishes scholarly journals in a wide range of academic fields, including the social sciences, the humanities, the natural sciences, the biological and medical sciences, education, economics, and management. These journals deliver original, peer-reviewed research from international scholars to a worldwide audience. All our journals are available in electronic form in conjunction with their print editions. All journals are available for free download online.

Mission

To work for future generations

Values

Scientific integrity and excellence

Respect and equity in the workplace

CONTACT US

9140 Leslie St. Suite 110
Beaver Creek, Ontario, L4B 0A9
Canada
Tel: 1-416-642-2606
Fax: 1-416-642-2608
E-mail: info@ccsenet.org
Website: www.ccsenet.org

The journal is peer-reviewed
The journal is open-access to the full text
The journal is included in:

AGRICOLA	Global Health	SHERPA/RoMEO
AGRIS	Google Scholar	Standard Periodical Directory
BASE	InfoBase Index	Ulrich's
CAB Abstracts	JournalFinder	WorldCat
Chemical Abstracts Service	JournalTOCs	
Electronic Journals Library	PKP Open Archives Harvester	

Journal of Food Research

Bimonthly

Publisher Canadian Center of Science and Education
Address 9140 Leslie St. Suite 110, Beaver Creek, Ontario, L4B 0A9, Canada
Telephone 1-416-642-2606
Fax 1-416-642-2608
E-mail jfr@ccsenet.org
Website http://jfr.ccsenet.org

ISSN 1927-0887