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Changes in Quality Attributes Related to Browning during Storage of Litchi Juice Fermented by *Lactobacillus*

Xingxing Yuan[1, 2], Yuanshan Yu[1], Yujuan Xu[1], Gengsheng Xiao[1] & Jijun Wu[1]

[1]Sericultural & Agri-Food Research Institute, Guangdong Academy of Agricultural Sciences/Key Laboratory of Functional Foods, Ministry of Agriculture/Guangdong Key Laboratory of Agricultural Products Processing, Guangzhou, 510610 China

[2]College of Food Science and Engineering, Jiang Xi Agricultural University, Nanchang, 330045 China

Correspondence: Yuanshan Yu, Sericultural & Agri-Food Research Institute, Guangdong Academy of Agricultural Sciences/Key Laboratory of Functional Foods, Ministry of Agriculture/Guangdong Key Laboratory of Agricultural Products Processing, Guangzhou, 510610 China. Tel: 86-159-7559-6649. E-mail: yuyuanshan2016@qq.com

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Abstract

Litchi juice fermented by *Lactobacillus casei* was heated (95°C, 1 min) and stored in a dark place at 25°C. Changes in quality attributes (color, 5-hydroxymethylfurfural (5-HMF) phenolic compounds, antioxidant capacity, sugars, free amino acids, and others) related to browning in fermented litchi juice were investigated during the six months of storage. Noticeable visual changes due to browning were observed during storage of fermented litchi juice, especially in the upper part of the juice bottle, and the value of color difference ($\triangle E$) increased to 7.12±0.04 after six months of storage. The 5-HMF content increased with the increase in storage time, which rose from 0 to 2.31±0.16 mg/L after six months of storage. Five soluble phenolic compounds (rutin, narcissoside, quercetin, kaempferol-rutinose-rhamnoside, and isorhamnetin-rutinose-rhamnoside) were identified in fermented litchi juice, none of which showed a significant decrease (P>0.05), whereas a tendency for total phenolic content to decrease was observed during storage of fermented litchi juice. Adding 0.3 g/L of sodium sulfite can inhibit the browning reaction in fermented litchi juice and decrease the formation of 5-HMF as well as the loss of total phenolics.

Keywords: fermented litchi juice, pasteurization, browning, 5-HMF, phenolic compounds

Practical Applications

Non-enzymatic browning is a major factor for quality deterioration during storage of litchi juice. Changes in quality attributes (Color, 5-HMF, phenolic compounds, antioxidant capacity, sugar, free amino acids and so on) related to browning during storage of fermented litchi juice was investigated during 6 months of storage. It was found that adding sodium sulfite can inhibit the browning of fermented litchi juice and the formation of 5-HMF and reduce the loss of total phenolics. Results could provide some data to develop a science-based anti-browning agent for litchi juice.

1. Introduction

Litchi (*Litchi chinensis*Sonn.) is cultivated in sub-tropic or tropicregions, in particular south China, Thailand and India (Zhang et al., 2016, Chaikham et al., 2017). With its bright red pericarp, translucent white flesh, exotic flavor, and particular nutritional qualities, litchi has become one of the world's most popular fruits (Holcroft et al., 1996). Apart from being consumed fresh, litchi fruit is also processed into juice, canned litchi, and is dried. Litchi juice typically contains sugar, widely appreciated flavor,minerals, vitamins, and various phenolics, and whichcan compete in the juice market; recently litchi juice, fermented by *Lactobacillus*, which increases its health benefits with probiotics has emerged on the market (Ibrahim & Mohamed, 2015). However, non-enzymatic browning is the major cause of quality deterioration during storage of probiotic litchi juice.

It was reported that non-enzymatic browning reactions was brought aboutby maillard-associated reactions, ascorbic acid degradation, and acid-catalyzed sugar degradation. And carbonyl compounds, which is intermediates of ascorbic acid and sugar degradation, can polymerize or react with amino acids and participate in

maillard-associated reactions to form brown-colored compounds (Buedo, Elustondo, & Urbicain, 2000, Quayson & Ayernor, 2007, Damasceno et al., 2008). Color changes, loss of reducing sugar and ascorbic acid, and formation of 5-hydroxymethylfurfural (5-HMF) could be observed, which will affectthe quality of fruit juices and reduced purchase desire of consumers (Ibarz, Pagán, & Garza, 1999). These undesirable and complex reactions produce a wide variety of end-products, such asfurans, pyrroles, ketones, and other compounds, and can cause off-flavors and bad color (Fustier et al., 2011).

At present, ultrafiltration, concentration, and adding a protective agent have usually been used to avoid non-enzymatic browning, but since the technologies of ultrafiltration and concentration are costly and consume a lot of energy, it is important to seek suitable protective agent for different juice systems (Borneman, Kmen, & Nijhuis, 2001; Hernández et al., 2009; Fustier et al., 2011; Wu, Hu, & Zheng, 2014). Browning during storage of juice beverages has already been extensively reported, but there is still a limited understanding to this problem, especially in litchi juice (Ibrahim & Mohamed, 2015). In this study, changes in quality attributes (color, 5-HMF, phenolic compounds, antioxidant capacity, sugar, free amino acids, and others) related to browning during storage of fermented litchi juice by *Lactobacillus* were investigated, which could provide some data that could help to develop an anti-browning agent for litchi juice beverage.

2. Materials and Methods

2.1 Preparation of Starter Culture

Lactobacillus casei(GIM1. 204) preserved in our laboratorywas activated (30 °Cfor 12h) in MRS broth (HaiboBioTechnology Co. Ltd., Qingdao, China),andthen the cell pellet was used to inoculate litchi juice.

2.2 Preparation Offermented Litchi Juice

Litchi juice (cv. Huaizhi) was presented by Guangdong BosunHealth FoodCo. Ltd., Guangzhou, China). After the litchi juicewas incubated at 30 °C for 18 h with an initial 5.0 Log CFU/mL of *Lactobacillus casei*, the 2 g/L of xanthan gum (Shunqi Biotechnology Co. Ltd., China) was also added to the litchi juice for sensory characteristics. Thexanthan gum was dispersed using stirrer and homogenizer, thesamples werepasteurized (105 °C, 30s) in a Laboratory UHT Sterilization Device (Shanghai Pilotech Equipment Co. Ltd., China), and then filled into lipped glass bottle.

2.3 Storage and Sampling

The fermented litchi juice in the glass bottle was stored in a dark place at 25 °C for 6 months and removed at three months interval for further analysis.

2.4 Color Assessment

The juice color was measured in the reflectance mode for 3 times at 25°C (UltraScan VIS, HunterLab, Reston, America). The L^*, a^*, and b^* value was measured and the total color difference (ΔE) was calculated by Equation 1.

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

where ΔE is the total color difference between a sample and control (0 d storage), L^*, a^*, and b^* are respectivelythe lightness, redness, and yellowness of a sample, and L_0^*, a_0^*, and b_0^* are respectively the lightness, redness, and yellowness of control (samples for 0 d storage).

2.5 Determination of Ph, Total Soluble Solids (TSS), and Titratable Acidity

The pH of juice samples was measured using a pH meter (Metrohm744, Herisau, Netherland) at 25 ±1 °C. A digital refractometer (Model RP-101, Atago, Co., Ltd., Tokyo, Japan) was used to measure the TSS. The automatic titrimeter (Metrohm Co. Ltd.) was used to analyze the titratable acidity, and the results were expressed as g citric acid equivalents per liter.

2.6 Determination of Ascorbic Acid and 5-HMF

The ascorbic acid was measured according to the methods of Yu et al. (2014) using the HPLC system (Shimadzu Co., Japan).

The 5-HMF was analyzed by HPLC method. The juice sample was mixed with methanol at the ratio of 1:1 (v/v), then centrifuged for 5 min to collect supernatant with 10000×*g* at 4°C. The supernatant filtered using 0.22μm of nitrocellulose membrane (Beijing Bomex Co., Beijing, China) was then used for further HPLC analysis. The 5-HMF was separated using Agilent ZORBAX SB- Aq (4.6 * 250 mm) column at 30 °Cusing 50% (v/v)

acetonitrile aqueous solution as the elution at a flow rate 1.0 mol/L and quantified using external standards with a UV-VIS detector at 280 nm.

2.7 Determination of Sugar, Total Polyphenols, and Antioxidant Capacity

Sugars (fructose, glucose, and sucrose) were analyzed using HPLC according to the methods of Yu et al. (2015). Total polyphenols were determined using the Folin-Ciocalteu method (Yu et al., 2014; Aydin et al. 2017). The antioxidant capacity of juice sample was determined by oxygen radical absorbance capacity (ORAC). The ORAC assay refer to the methods of Ou et al. (2001) and Yu et al. (2014), and the result was expressed as mM Trolox equivalent (TE)/L.

2.8 HPLC Analysis of Phenolic Compounds

The juice sample was mixed with absolute ethanol using a ratio of 1:2 (v/v) and sonicated with a 200 W ultrasound power at 40KHz for 20 min at room temperature. Subsequently, the mixture was centrifuged at 10,000 rpm for 10 minto collect the supernatant, and then the supernatant passed through a 0.22μm nitrocellulose membrane (Beijing Bomex Co., Beijing, China) were used for further HPLC analysis.An Agilent 1200 RRLC system coupled with Agilent6530 TOF-MS was used. Sample was separated in an Agilent Poroshell 120 EC-C18 column (3.0 × 50 mm, 2.7 μm) using the mobile phase consisted of (A) 0.4% (v/v) acetic acid and (B) according the methods of Yang et al. (2017). The identification of phenolic compounds was determined by using authentic standards and by comparing itsfragmentation pattern of deprotonated and product ions, while quantification was performed by external calibration with standards of phenolic compounds.

2.9 Determination of Free Amino Acids

The free amino acids compositions of litchi juice samples were measured according to the methods of Yu et al. (2015) using amino acids analyzer (Hitachi Ltd., Japan).

2.10 Statistical Analysis

Duncan's multiple range tests were used to determine statistically significant differences of variables at 95% confidence. One-way analysis of variance was accomplished with the software SPSS Statistics 19.0 (IBM Co., USA).

3. Results and Discussion

3.1 Changes in TSS, pH, Titratable Acidity, Ascorbic Acid, Andsugar

The TSS, pH, and titratable acidity of fermented litchi juice were 15.82±0.16°Brix, 4.53±0.14, and 2.00±0.06 g of citricacid per 1L, respectively (Table 1). During storage of 6 months, no significant changes (P>0.05) for the TSS, pH，titratable acidity of fermented litchi juice was observed, which may related to the inactivation of indigenous microorganism during thermal pasteurization of fermentation litchi juice. The glucose and fructose were the dominant sugars in fermentation litchi juice, reaching 71.32±1.04, and 66.23±1.12, respectively (Table 1). No sucrose and ascorbic acid was detected in fermentation litchi juice. It had been reported that sucrose could be hydrolyzed into fructose and glucose during fermentation of *L. casei* (Zheng et al., 2014). Reports had shown that 230 mg/Lof ascorbic acid was detected in the fresh litchi juice (Zheng et al., 2014), while ascorbic acid was not detected in the pasteurized fermentation litchi juice, which could be attributed to aerobical and anaerobical degradation of ascorbic acid during litchi juice processing and storage (Kennedy et al., 1992; Kabasakaliset al., 2000).

Table 1. Changes in total soluble solids (TSS, °Brix), pH, titratable acidity (TA, g/L), sugar (g/L), and 5-HMF (mg/L) in fermented litchi juice during storage

Storage times	0 day	3 months	6 months
TSS	15.82±0.16[a]	16.06±0.12[a]	15.93±0.18[a]
pH	4.53±0.14[a]	4.36±0.13[a]	4.30±0.11[a]
Titratable acidity	2.00±0.06[a]	2.02±0.02[a]	2.02±0.01[a]
fructose	66.23±1.12[c]	76.35±1.53[a]	70.45±1.34[b]
glucose	71.32±1.04 [c]	79.66±1.28[a]	74.74±1.33 [b]
5-HMF	N. D. [a]	0.95±0.07[b]	2.31±0.16[a]

N.D., Contents below the detection limit. The detection limit of 5-HMF was 0.02 mg/L.

[a,b,c] Different letters represent a significant difference within the same row (P <0.05).

3.2 Changes in Color and 5-HMF

During storage of fermented litchi juice, a noticeable visual browning was observed, especially in the upper part of the juice bottle (Figure 1), which may be attributed to more oxygen in the top of bottle (Molnar-Perl & Friedman, 1988; Buedo, Elustondo, & Urbicain, 2000). Table 2 presented the changes in color parameters of fermented litchi juice during storage. No significant change (P>0.05) for the fermented litchi juice was observed in L^* value after 6 months of storage (Table 2), while the $a*$, $b*$, and ΔE value for the fermented litchi juice showed a tendency to increase and the ΔE value reached 5.11 and 7.12 after 3 and 6 months, respectively (Table 2). It can be a noticeable visual difference as the ΔE value was more than 3.0 (Cao et al., 2012).

Table 2. Change in color for fermented litchi juice during storage

Time (months)	$L*$	$a*$	$b*$	ΔE
0	48.71±1.32 [a]	-1.72±0.32[c]	2.05±0.01[c]	——
3	47.68±1.13[a]	-0.96±0.30[b]	6.99±0.08[b]	5.11±0.11
6	46.31±1.25 [a]	-0.59±0.22[a]	8.64±0.11[a]	7.12±0.04

[a,b,c] Different letters represented a significant difference within the same column (p<0.05)

Non-enzymatic browning could be a major factor of quality deterioration for the fermented litchi juice showing a noticeable visual browning. It was reported that Maillard reactions are involved in the formation of brown pigments, and 5-HMF is an intermediate product of the maillard reaction (Sapers, 1993; Capuano & Fogliano, 2011; Lee et al., 2014). Accordingly, the formation of 5-HMF during storage of fermented litchi juice was observed in the study, and the content of 5-HMF increased with the increase of storage times, which reached 2.31 mg/L after 6 months storage (Table 1).

3.3 Changes in Total Polyphenols and Antioxidant Capacity

Table 3 presented the changes in total phenolic content, and antioxidant capacity (ORAC value) of fermented litchi juice during storage. The total phenolics content showed a reduction tendency during storage, and which decreased 22% after 6 months storage. It was reported that total phenolics loss of pasteurized juice during storage was mainly due to the phenolics oxidation degradation and polymerization (between phenolics or phenolics and proteins) (Cao et al., 2011).

No significant decrease (P >0.05) in the ORAC value of fermented litchi juice was observed after 6 months storage, even though the ORAC value in fermented litchi juice showed a 9% reduction after 6 months storage (Table 3). The major compounds with the oxygen radical absorbance capacity in litchi juice was phenolics, and some studies showedthattotal phenolic contentand the antioxidant capacity were correlated (Mccue & Shetty, 2005; Klopotek, Otto & Bohm, 2005; Perez-Gregorio et al, 2011). In this study, the data trends for total phenolic content and antioxidant capacity (ORAC value) during fermented litchi juice storage not showed positively associated (Table 3), which may be due that the products of phenolics oxidation degradation and polymerization also have some antioxidant capacity. Studies showed that antioxidant capacity of phenolics depend on their chemical structure and can be affected by the group attached to a basic aglycon (Jakobek et al., 2009; Perez-Gregorio et al, 2011). Therefore, this could be the subject of further research, because a new antioxidant compound, which exhibits super high antioxidant capacity, much stronger than the capacity of existing antioxidants, may be formed during storage of fermented litchi juice.

Table 3. Changes in total phenolics (mg/L), and antioxidant capacity (ORAC value, mM TE/L) for fermented litchi juice during storage

Storage times	0 day	3 months	6 months
Total phenolics	229.78±4.13[a]	194.39±2.69[b]	179.00±2.49[c]
Antioxidant capacity	15.26±0.31[a]	15.21±0.19[a]	13.82±0.29[b]

[a,b,c] Different letters represented a significant difference within the same row (p<0.05)

3.4 Change in Soluble Phenolic Compounds

There were five soluble phenolic compounds identified in fermented litchi juice by the standards and published data (Zhang et al. 2016), including Rutin, Narcissoside, Quercetin, Kaempferol-rutinose-rhamnoside, and Isorhamnetin-rutinose-rhamnoside. Phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds. Phenolic acids, flavonoids and tannins are regarded as the main phenolic compounds in fruits (Rodríguez et al., 2009;

Aydin & Mammadov, 2017). Most naturally occurring phenolic compounds are present as conjugates with mono- and polysaccharides, linked to one or more of the phenolic groups, and may also occur as functional derivatives such as esters and methyl esters (Bonoli et al., 2004; Rodríguez et al., 2009). The five soluble phenolic compounds identified in fermented litchi juice is flavonoids, soluble phenolic acids and tannins was not observed and identified. All content of five phenolic compounds identified in fermented litchi juicedid not show significant decrease (Table 4) (P>0.05), whereas total phenolicscontent showed a decrease tendency during storage (Table 3), indicating oxidation degradation or polymerization of some insoluble-bound phenolic compounds could be the main factor that caused the decrease in total phenolics.

Table 4. Change in phenolic compounds for fermented litchi juice during storage

Time (months)	Rutin (mg/L)	Narcissoside (mg/L)	Quercetin (mg/L)	Kaempferol- rutinose - rhamnoside (mg/L)	Isorhamnetin - rutinose-rhamnoside(mg/L)
0	10.52±0.28[a]	4.74±0.7[a]	115.31±4.54[a]	16.08±1.64[a]	3.96±0.48[a]
3	10.84±0.54[a]	4.84±0.64[a]	112.7±3.52[a]	14.58±2.01[a]	3.93±0.33[a]
6	9.85±0.48[a]	4.34±0.47[a]	112.6±3.28[a]	14.75±0.33[a]	3.95±0.51[a]

[a,b,c] Different letters represented a significant difference within the same column (p<0.05)

3.5 Changes in Free Amino Acids (AA)

There are 22 kinds of free amino acids that were detected. Most of free AA had no significant changes after 6 months of storage (P>0.05), just 8 kinds of free amino acids (Ser, Glu, Ala,Val, γ-ABA, Orn, 1Mehis, α-AAA) showed a tendency of decrease during storage (Table 5). Silvan et al. reported that some free amino acids was the one of reactants participated in maillard reaction, the results indicated that the loss of some free amino acids may be related to the maillard reaction and the browning of fermentation litchi juices during storage (Azandouz & Puigserver, 1999; Jiang et al., 2017).

Table 5. Changes in free amino acids (mg/L) fermented litchi juice during storage

Time (months)	0	3	6
Asp	137.32±1.82[a]	129.42±2.06[a]	133.48±1.91[a]
Thr	17.09±1.22[a]	15.91±1.43[a]	16.34±0.86[a]
Ser	76.94±2.33[a]	73.11±3.96[a]	73.95±2.93[a]
Glu	62.73±1.33[a]	57.34±0.96[b]	58.22±1.4[b]
Gly	16.84±1.02[a]	16.71±1.48[a]	16.49±1.32[a]
Ala	559.29±13.9[a]	547.57±12.6[a]	545.25±19.5[a]
Val	67.68±3.56[a]	63.05±3.27[a]	64.10±3.24[a]
Met	18.56±2.25[a]	17.65±2.77[a]	17.27±2.38[a]
Cysthi	12.94±1.04[a]	11.97±1.69[a]	11.98±1.42[a]
Ile	15.48±2.39[a]	14.01±2.46[a]	14.48±2.58[a]
Leu	5.78±1.21[a]	6.13±1.18[a]	6.29±1.32[a]
Tyr	23.61±2.76[a]	23.32±2.81[a]	24.47±2.54[a]
Phe	12.92±1.53[a]	11.97±1.48[a]	12.25±1.29[a]
β-Ala	8.53±0.48[a]	7.59±0.43[b]	6.91±0.31[c]
γ-ABA	831.35±12.08[a]	809.32±11.64[b]	797.95±9.69[c]
EOHNH2	15.75±0.47[a]	15.98±0.52[a]	14.88±0.59[a]
Orn	68.26±0.94[a]	61.46±0.86[b]	63.71±0.72[b]
Lys	24.33±1.43[a]	21.72±1.21[a]	23.17±0.99[a]
1Mehis	30.38±1.22[a]	24.06±0.98[b]	21.10±1.07[c]
Pro	24.06±1.01[a]	23.42±0.98[a]	22.68±1.21[a]
α-ABA	13.35±1.06[a]	13.89±0.79[a]	13.25±1.03[a]
α-AAA	16.32±0.71[a]	4.69±0.73[b]	N. D[.c]

[a,b,c] Different letters represented a significant difference within the same row (p<0.05)

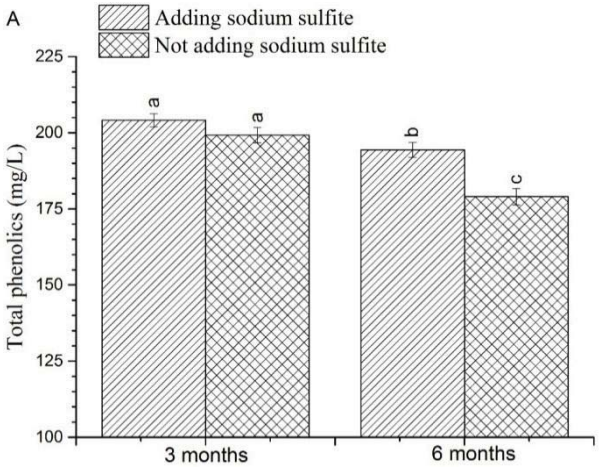
[N.D.], Contents below the detection limit. The detection limit of α-AAA was 0.05 mg/L.

3.6 Effect of Adding Sodium Sulfite on Quality Attributes Related to Browning during Storage

Sulfite is widely used to prevent browning reactions of fruit juices (Zhou, Zhang & Xin, 2004; Wu, 2014). In this

study, thequality attributes related to browning was also analyzed for the fermented litchi juice added with 0.3 g/L of sodium sulfite. Compared with fermented litchi juice not added with sodium sulfite, the ΔE value showed a slower increase and less than 3.0 (Figure 2A), and noticeable visual browning was not observed in the fermented litchi juice added with 0.3 g/L of sodium sulfite during storage of 6 months (Figure 1). Moreover, the increase of 5-HMF and loss of total phenolics was less in the fermented litchi juice added with 0.3 g/L of sodium sulfite as compared with fermented litchi juice not added with sodium sulfite during storage of 6 months (Figure 2B and 2C). No significant different (P>0.05) was observed in TSS, pH，titratable acidity, sugar, soluble phenolic compounds, and free amino acids after 3 or 6 months of storage (data not presented).

Figure 1. Change in color for fermented litchi juice adding without (A) or with (B) 0.3 g/L of sodium sulfite during storage



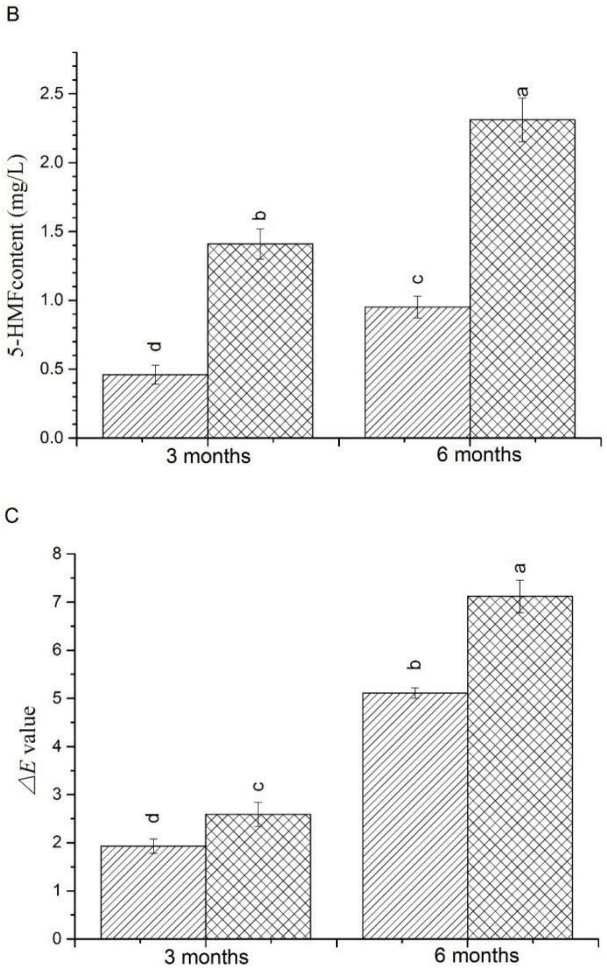


Figure 2. Change in total phenolics (A), 5-HMF (B)and Δ*E*value (C) for fermented litchi juice adding without or with 0.3 g/L of sodium sulfite during storage.

4. Conclusion

Pasteurized (95 °C, 1 min) fermented litchi juice with *Lactobacillus casei* showed a noticeable visual browning during storage at 25 °C, especially in the upper part of the juice bottle. The non-enzymatic browning is the major quality deterioration during storage of fermented litchi juice, which are related to ascorbic acid (AA) degradation, Maillard-associated reactions, intermediates of AA and sugar degradation, and so on. It was found that sodium sulfite can decrease the formation of 5-HMF and the loss of total phenolics, and inhibited browning reaction of fermented litchi juice. Analysis of quality attributes related to browning during storage showed sodium sulfite or other similar anti-browning agent should be using to inhibit the formation of 5-HMF and the loss of total phenolics, and decrease the browning of fermented litchi juice.

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Author Contributions

Yuanshan Yu, Xinxin Yuan and Yujuan Xu designed the experiments, analyzed the data and reviewed the manuscript; Gengsheng Xiao and Jijun Wu collected the data and wrote the manuscript. All of the authors completed and authorized the definitive manuscript.

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Physico-chemical Characteristics of Selected Jackfruit (*Artocarpus Heterophyllus Lam*) Varieties

J. Balamaze[1], J. H. Muyonga[1] & Y. B. Byaruhanga[1]

[1]Department of Food Technology and Nutrition, School of Food Technology, Nutrition and Bio Engineering, Makerere University, P. O. Box 7062 Kampala, Uganda

Correspondence: Yusuf Byaruhanga, Department of Food Technology and Nutrition, School of Food Technology, Nutrition and Bio Engineering, Makerere University, P. O. Box 7062 Kampala, Uganda. Tel: 256-772-445-113. E-mail: ybbyaru@gmail.com / ybbyaruhanga@caes.mak.ac.ug

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Abstract

This study was aimed at establishing the variation in physico-chemical properties between and within different jackfruit varieties.

Mature jackfruits from trees known to produce orange, yellow and white flakes were harvested and ripened at 28^{o}C and 90% relative humidity. The weight, length and circumference of ripe fruits were measured. The fruits were cut into three equal sections: top, middle and bottom. Each section was separated into flakes, rind, abortive perigones, consolidated perigones, seeds and core. The percent weight of different fruit parts, and flake physico-chemical characteristics were determined.

The average weight, length, circumference and maturation period for jackfruit varieties studied were 12 kg, 47 cm, 76 cm and 104 days, respectively. The mean proportions of flakes, rind, abortive perigones, consolidated perigones, seeds and core were 24.7, 20.0, 14.5 14.3, 13.1 and 11.3%, respectively. Ascorbic acid (AA), carotenoids, total soluble solids (TSS), TSS: acid ratio, color and texture varied significantly among jackfruit sections. Color did not vary among sections of yellow and white flaked varieties. Flake texture (hardness) was 12.1, 10.5 and 6.6 N/mm for the white, yellow and orange flaked varieties, respectively. Flake textural resilience was in the order: white > yellow > orange flakes. Principle component analysis showed white flakes correlated positively with flake resilience, hardness and color lightness. Orange flakes were associated with high carotenoids and TSS content. Yellow flakes exhibited intermediate properties between those of white and orange flakes. Jackfruit flake color and section can be used as flake quality predictor.

Keywords: jackfruit, *Artocarpus heterophyllus lam*, physico-chemical, texture, fruit characteristics

1. Introduction

Jackfruit (*Artocarpus heterophyllus lam*) is the largest tree born fruit, constituting edible flakes and an inedible portion both of which can be processed into value added products. The flakes can be processed into products like juice, jam, dried chips, fruit leathers and wines, while the inedible portion could be utilized as a source of pectin. Pectin is important ingredient in the food and pharmaceutical industries where it can be used as a gelling agent, stabilizer, thickener or emulsifier (Beguma, Azizb, Uddinb, & Yusof, 2014).

The propagation of jackfruit is mainly through seed planting. Besides, jackfruit undergoes extensive cross pollination (Mitra & Mani, 2000), which leads to a lot of variation in fruit characteristics. Such variation may affect the quality of products processed from fruits from different jackfruit varieties. According to Balamaze, Muyonga & Byaruhanga (2019), there are mainly three jackfruit varieties based on flake color in Uganda. Mostly, jackfruit flakes are mainly consumed fresh with the inedible fruit portions wasted or fed to animals. Farmers with jackfruit trees reported losses of up to 20% during peak harvest season (Balamaze et al., 2019). It is therefore, necessary that alternative approaches for utilization of jackfruit be evaluated to ensure full realization of the economic potential of the different jackfruit varieties. This requires a comprehensive understanding of the physico-chemical properties of the different jackfruit varieties.

Several studies on characterization of jackfruit have been undertaken; Jagaseesh, Reddy, Basavaraj, Swamy, & Laxminarayan (2010) categorized jackfruits based on fruit size, taste, odour of flakes, shape and density of

spines on the rind. Singh & Srivastava (2000) identified jackfruit types based on fruit bearing habits, yield and period the fruit takes to mature. Mitra & Mani (2000) reported jackfruit types with total soluble solutes (TSS) greater than 25° brix to be more suitable for preparing desserts. Mannan, Gazi & Mia (2006) studied variation in physical properties of jackfruits during off season periods and significant variations were found among the germplasms in relation to fruit characteristics. Reddy, Patil, Kumar, & Govindaraju (2004) reported that among the qualitative characters studied, TSS was more variable in jackfruit clones. Ullah & Haque, (2008) reported wide variation in the period a fruit takes to mature ranging between 82 and 160 days. Rosnah, Chia, Chin, Noraziah, & Osman, (2009) reported the changes in the chemical composition as well as variation in pH, TSS and Titratable acidity (TA) of the jackfruit cultivar J33 during ripening and storage. Sakimin, Patre, Jurami, Alami & Aslan (2017) observed a significant increase in firmness for flakes treated with ascorbic acid under cold storage.

In spite of several studies previously conducted on jackfruit, there is a dearth of information on the relationship between jackfruit flake color and their physico-chemical characteristics. A detailed understanding of jackfruit characteristics based on flake color would be an important guide for identifying jackfruit varieties for particular industrial process applications. This study therefore, aimed at establishing whole jackfruit dimensions, fruit part proportions and physico-chemical properties of flakes from different jackfruit varieties.

2. Material and Methods

2.1 Experimental Design

Three jackfruit trees from each variety known to produce yellow, orange and white flakes were selected, marked and secured in the same locality of Malangala sub-county in Mityana District, Uganda. On each of the identified jackfruit trees, female fruit sets were randomly selected and identified according to Ullah and Haque (2008). The fruit sets were coded to identify the fruit by: fruit set date, flake color and number. The code was sealed in an impervious transparent plastic material to prevent moisture destroying the label. For each jackfruit variety studied, a total of 20 coded fruits were selected and divided into 2 sets of 10 fruits each; the first set was for studying the physico-chemical characteristics of the fruit and the second was for determination of maturation period of the different jackfruit varieties. The jackfruit varieties and sections were the independent variables while the jackfruit parts and flake physico-chemical properties were the dependent variables. The experiment and analyses was done in triplicates.

2.2 Sampling

The first set of fruits for determining the maturation period were left on the tree and monitored until they ripened. The number of days, from fruit set, a particular fruit took to mature and ripen were recorded. A ripe fruit was identified by a deep hollow or empty vessel sound produced when tapped by hand.

The second set was for determining fruit part proportions and flakes' physico chemical characteristics. Jackfruits for this purpose were harvested mature green; fruits were considered mature when the last leaf on the fruit stalk turned yellow, rind spine flattened and when the fruit skin color became pale (APAARI, 2012). Three mature fruits from each of the selected trees were harvested avoiding them to hit the ground. The fruits were transported on the same day to the laboratory. The fruits were kept covered in wooden boxes for 2 days at 28^{o}C and 90% RH. This was to allow uniform ripening of the fruits before analysis.

2.3 Whole Fruit Dimension and Section Proportion Determination

Ripe fruits were washed using tap water. The adhering water after washing was flushed off using a jet of compressed air. The fruits were weighed using a weighing scale (Adam equipment CBK32, Johannesburg, South Africa) and the weight (kilograms) was recorded. The fruit length and cross sectional circumference (centimeters) measured from top to bottom and at the middle point of the fruits, respectively were determined using a measuring tape. The fruits were transversely sectioned using a stainless steel knife into three equal sections labeled as top, middle and bottom (Ong, et al., 2006). The distal section of fruit that is attached to the plant was considered as the top section while the proximal section was considered as the bottom. Each section was coded, weighed and then divided into four equal quarters to facilitate separation into parts including: core/rachis, seeds, flakes/bulbs without seeds, abortive perigones/rags, rind and consolidated perigones. The parts were separately weighed and the proportion of each part for a jackfruit section was calculated and expressed as percentage of the total weight of the section.

2.4 Determination of Physical Properties of Jackfruit Flakes

Two flake samples of about 150 g each were separately picked from each section of the three different jackfruit varieties. The two samples were respectively used for determining physical and chemical properties, of the flakes following the procedures described in the following sections.

2.4.1Thickness, Length and Breadth of Flakes

Flake thickness was determined using a micrometer screw gauge (N8/32, New Delhi, India) and the values were recorded in centimeters. The length and breadth of the flakes were taken using Vanier calipers (KBD Tolls Jiangsu China) and values were recorded in centimeters.

2.4.2 Flake Color Measurement

The color intensity parameters L*, a* and b* for the flake were determined using a Lovibond Tintometer L322/92E, Salisbury, England). The lightness value (L*) represents the black-white colors, a* represents the green-red colors and b* represents the blue-yellow colors.

2.4.3 Textural Properties (Hardness and Resilience) of Flakes

A uniform flake sample of 1 cm^2 was used to prepare samples for texture analysis. Textural properties were measured using a texture analyzer (model TA.XT- Plus Stable Micro System, Ltd Godalming, Surrey, UK) with a 2 kg load cell fitted with a light knife blade (A/LKB) probe. The probe was operated in compression mode with a trigger force of 0.049 N, pre-test speed 1mm/sec, test speed 2 mm/sec, a return speed of 10 mm/sec and penetration distance of 0.29 cm. Hardness was considered to be the initial force (N) needed for the TA probe to cut through the flake skin and was expressed in Newtons (N) represented by the peak value on the graph. Resilience was considered to be the total force needed for the TA probe to cut through a penetration depth of 0.29 cm into the flake. Resilience was expressed as the area under the curve.

2.5 Determination of Chemical Properties of Jackfruit Flakes

2.5.1 Titratable Acidity and pH

Titratable acidity was determined according to the method described by Rangana (1995) with modifications. A 50 g portion of jackfruit flakes was homogenized using Robot Blender (H-Biaugeaud R23 Beauduex France). A 10 g sample of the homogenized flakes was mixed with 25 mL of distilled water and titrated against 0.1 N NaOH using phenolphthalein indicator. The end point was a faint pink color and the results were expressed as percentage citric acid equivalent. The pH of the homogenized flakes was measured directly using a pH meter (Model HI 221, HANNA Instruments Ltd, Bedford, UK).

2.5.2 Total Soluble Solids (TSS) and TSS: Acid Ratio

Total soluble solids was determined using a hand held refractometer (Abbe 315RS, Royal Tunbridge Wells, UK). TSS: Acid ratio was calculated by dividing the TSS value by the titratable acid value.

2.5.3 Vitamin C

Vitamin C content was determined according to AOAC (1995) with modifications. One hundred grams of flakes were homogenized using a blender and 100 mL of 4% Trichloroacetic acid were added. The mixture was filtered and 5 mL of filtrate were made-up to 10 mL with 4% Trichloroacetic acid solution. This solution was titrated against 0.01% 2, 6-dichloro-phenol-indophenol solution which was freshly standardized using 0.05% ascorbic acid solution. The end point was marked by a transient pink color which persisted for 15 s. All chemicals and reagents were obtained from Sigma (Sigma-Aldrich, New Jersey, USA).

2.5.4 Total Carotenoids

Total carotenoids content was determined according to Pinheiro-santana, Stringheta, Branda, Paez, & Queiroz (1998) with modifications. Five grams of flakes were ground, in a clean mortar, to a fine pulp and 20 ml of 80% acetone were added. The extract was centrifuged at 5,000 × g for 5 min and the supernatant was transferred to a 100 ml volumetric flask. The residue was ground with additional 20 ml of 80% acetone, centrifuged at 5,000 × g for 5 min and the supernatant was transferred to the same flask. This process was repeated until the residue was colorless. The pestle and mortar were washed thoroughly with 80% acetone and washings were collected in the same flask and filtered through glass wool lined by a layer of sodium sulphate crystals to remove traces of water. The final volume was made-up to 100 ml with 80% acetone and the absorbance was read at 450 nm for the sample and blank (80% acetone). The carotenoids were determined and expressed in mg per 100 g of pulp on fresh sample.

2.5.5 Dry Matter

Dry matter content was determined according to Robert & Bradley (2010) with modification. Accordingly, 2-6 g of sample was weighed and dried overnight at 80^{o}C in an air forced oven (Ov123 Gallakamp, England). The dry matter content was calculated by expressing the dried sample weight as a percentage of the wet sample weight using the equation below.

$$Dry\ matter = \frac{Total\ Dry\ weight\ of\ the\ sample}{Total\ fresh\ weight\ of\ the\ sample} x\ 100$$

2.6 Data Analysis

Statistical analyses were performed using SAS University edition software local host 10080. Means were compared by Analysis of Variance (ANOVA) and separated by Tukey Post hoc test (with $p = 0.05$). In order to visualize and analyze the overall variability present in the data, the same data used for mean separation were subjected to multivariate principal component analysis (PCA). The PCA was performed taking into consideration 14 main parameters i.e. dry matter, titratable acidity, ascorbic acid, total soluble solids, carotenoids, TSS: TA ratio, flake width, length, thickness, hardness, resilience, L*, b* and a*. The general variability in jackfruit flakes characteristics was visualized on a 2D-PCA. New orthogonal coordinates explaining the biggest variability in the same data were selected. Additionally, the loadings, which are distances from the center to a variable location showed the distribution of different variables in the unit plot of the two PCs whereas, the Eigen value scores for each observation (section) showed the location of different jackfruit sections in relation to variables.

3. Results

3.1 Dimensional and Maturation Characteristics of the Whole Jackfruits

Significant differences were observed in the weight length and circumference of the different jackfruit varieties. In terms of weight and circumference, yellow flaked jackfruits were significantly heavier and larger in circumference ($p<0.05$) than the white and orange flaked fruits. The white flaked jackfruits took a significantly longer period to mature than either the yellow or orange flaked jackfruits (Table 1). The yellow flaked fruits were longer than the white and orange. Generally, the orange flaked jackfruit variety was relatively smaller in all aspects and took relatively a shorter period of time to mature compared to the white and the orange flaked varieties.

Table 1. Mean dimensions and maturation time of three jackfruit varieties

Variety/Flake color	Mass (Kg)	Length(cm)	Circumference (cm)	Maturation period (days)
Yellow	17.0^{a*}±5.6†	51.9^{a}±3.6	81.9^{a}±3.3	105.0^{b}±2.0
White	11.0^{b}±2.0	46.1^{b}±2.8	77.3^{c}±1.6	113.0^{a}±1.7
Orange	8.7^{b}±1.5	43.3^{b}±2.5	68.5^{b}±1.6	95.0^{c}±2.0
Overall average	12.0±4.0	47.0±4.5	76.0±7.0	104.0±5.0

*Mean values with the same superscripts in the same column are not significantly different

† ±values are standard deviation of the mean

3.2 Seeds, Core, Perigone, Flake and Rind Proportions of Different Jackfruit Sections

There were significant differences in seeds, core, abortive perigones and flakes proportions among different jackfruit varieties and fruit sections. The middle section of the orange flaked jackfruit variety had more seeds than the bottom and the top. For the white flaked jackfruits, the bottom section had significantly lower seeds than the middle and top sections of the same fruit. Variation in the core proportion based on fruit sections was also observed. The middle section of all jackfruits varieties studied had relatively more core proportion than the bottom and the top sections (Table 2).

The abortive perigones were higher in middle section of yellow flaked fruits while the lowest proportion was observed in top and bottom sections of white and orange flaked fruits, respectively. Flakes were highest in the middle section of orange flaked jackfruits, however, the distribution of flakes among sections did not exhibit a particular trend. The rind and consolidated perigones did not vary significantly among fruit sections (Table 2). The jackfruit type affected some fruit proportions. The mean flake proportion was higher in orange flaked variety (29.6 %) compared to white and yellow which had 23.1% and 21.4 %, respectively. The seeds were generally higher in orange flaked jackfruit while the white flaked jackfruit exhibited the least seed proportion. The proportion of the core was higher in white flaked variety whereas, the abortive perigones were higher in the yellow flaked variety. However, the rind proportion did not vary significantly among fruit varieties.

Table 2. Percent proportion of fruit parts from different fruit of three jackfruit varieties

Variety/Flake color	Section	Seed	Rind	Consolidated perigones	Core	Abortive perigones	Flakes
Yellow	Top	$11.5^{b*}\pm0.3^{\dagger}$	$19.3^{a}\pm0.1$	$15.8^{a}\pm2.1$	$8.5^{g}\pm1.0$	$16.0^{c}\pm0.4$	$19.3^{c}\pm1.2$
	Middle	$13.3^{b}\pm0.2$	$16.6^{a}\pm0.3$	$14.4^{a}\pm2.5$	$13.5^{b}\pm1.9$	$17.8^{a}\pm2.0$	$23.5^{b}\pm7.3$
	Bottom	$12.2^{b}\pm0.6$	$22.8^{a}\pm1.7$	$15.5^{a}\pm1.1$	$11.9^{d}\pm2.6$	$15.5^{c}\pm1.6$	$21.5^{c}\pm1.9$
White	Top	$11.3^{b}\pm0.7$	$20.7^{a}\pm2.4$	$15.0^{a}\pm0.5$	$8.9^{f}\pm0.2$	$12.9^{d}\pm0.7$	$26.6^{b}\pm1.0$
	Middle	$11.2^{b}\pm0.9$	$17.6^{a}\pm0.6$	$13.5^{a}\pm1.0$	$12.7^{b}\pm0.7$	$13.3^{d}\pm0.6$	$25.1^{b}\pm2.2$
	Bottom	$9.1^{c}\pm0.7$	$24.2^{a}\pm2.2$	$17.1^{a}\pm1.1$	$12.0^{c}\pm0.9$	$14.8^{c}\pm0.8$	$17.8^{c}\pm2.2$
Orange	Top	$13.0^{b}\pm0.4$	$18.0^{a}\pm5.1$	$13.0^{a}\pm3.3$	$8.1^{g}\pm1.7$	$14.3^{c}\pm0.5$	$26.7^{b}\pm3.3$
	Middle	$17.3^{a}\pm0.4$	$21.3^{a}\pm6.3$	$15.7^{a}\pm3.9$	$15.0^{a}\pm4.0$	$16.7^{b}\pm0.6$	$39.5^{a}\pm5.2$
	Bottom	$9.1^{c}\pm2.0$	$20.6^{a}\pm6.3$	$14.2^{a}\pm5.0$	$11.0^{e}\pm1.4$	$12.7^{d}\pm0.9$	$22.7^{b}\pm8.7$

*Mean values with the same superscripts in the same column are not significantly different

† ±values are standard deviation of the mean.

Flakes from the white flaked jackfruits were smaller in size than those from the orange or yellow flaked jackfruits. With respect to fruit sections, flakes from the middle section of the yellow flaked jackfruit were larger in size compared to flakes from other sections. There were minor differences in lightness among flakes from different sections, this was exhibited in flakes from the bottom section of white flaked fruits and orange flakes from the different sections. Flake redness on the other hand was highest and significantly varied only in orange flaked jackfruits sections. The degree of yellowness was lower in flakes from the bottom of white flaked jackfruits and higher in top and middle sections of yellow flaked jackfruits. The degree of yellowness did not differ significantly among jackfruit sections of the same fruit (Table 3).

Table 3. Physical properties of flakes from different sections of three jackfruit varieties

Variety/Flake color	Section	Length (cm)	Breadth (cm)	Thickness (cm)	Color		
					L*	a*	b*
	Top	$5.8^{a*}\pm0.6^{\dagger}$	$3.3^{c}\pm0.2$	$0.4^{ab}\pm0.1$	$88.7^{abc}\pm6.7$	$-17.9^{a}\pm4.1$	$92.9^{b}\pm1.0$
Yellow	Middle	$6.0^{a}\pm1.2$	$3.7^{a}\pm0.1$	$0.3^{ab}\pm0.1$	$92.5^{abc}\pm5.1$	$-18.5^{a}\pm1.9$	$92.6^{b}\pm1.1$
	Bottom	$5.4^{a}\pm0.6$	$3.2^{c}\pm0.2$	$0.3^{ab}\pm0.1$	$93.0^{abc}\pm3.3$	$-19.8^{a}\pm2.8$	$91.0^{ab}\pm6.9$
	Top	$5.5^{a}\pm0.9$	$3.0^{b}\pm0.2$	$0.4^{ab}\pm0.1$	$93.1^{abc}\pm8.1$	$-23.6^{ab}\pm1.1$	$86.8^{ab}\pm3.7$
White	Middle	$5.1^{a}\pm0.7$	$3.0^{b}\pm0.2$	$0.5^{ab}\pm0.1$	$95.1^{bc}\pm1.1$	$-23.8^{ab}\pm2.7$	$86.6^{ab}\pm3.9$
	bottom	$5.2^{a}\pm0.6$	$3.1^{b}\pm0.2$	$0.4^{ab}\pm0.1$	$96.0^{c}\pm1.1$	$-24.9^{ab}\pm2.5$	$84.5^{a}\pm7.1$
	Top	$5.3^{a}\pm1.1$	$3.2^{c}\pm0.2$	$0.4^{ab}\pm0.1$	$85.0^{a}\pm8.3$	$-6.3^{c}\pm6.5$	$90.5^{ab}\pm2.8$
Orange	Middle	$5.5^{a}\pm1.1$	$3.4^{a}\pm0.4$	$0.4^{ab}\pm0.1$	$86.9^{b}\pm5.3$	$-7.8^{d}\pm8.9$	$90.3^{ab}\pm4.2$
	Bottom	$5.5^{a}\pm0.8$	$3.5^{a}\pm0.4$	$0.4^{ab}\pm0.1$	$87.4^{ab}\pm5.0$	$-9.4^{cd}\pm8.8$	$87.0^{ab}\pm5.4$

*Mean values with the same superscripts in the same column are not significantly different.

† ±values are standard deviation of the mean.

L*= lightness, a*= redness, b*= yellowness

3.3 Textural Properties of Jackfruit Flakes

Hardness and resilience of the flakes varied significantly among white, yellow and orange flaked jackfruits (Table 4). In general, orange flaked variety was significantly softer with a mean hardness of 6.6 N compared to the yellow and white flaked varieties, which exhibited a mean hardness of 10.5 N and 12.1 N, respectively. Although resilience followed the same trend as flake hardness, it exhibited relatively higher mean force values of 7.7, 11.6 and 13.4 N for orange yellow and white flaked varieties, respectively. Jackfruit sections affected flake hardness and resilience, with flakes from the top sections exhibiting a relatively softer texture than those from the middle and bottom sections of the same fruit variety (Table 4).

Table 4. Textural characteristics of flakes from different sections of three jackfruit types

Variety/Flake color	Section	Hardness (N)	Resilience (N/mm)
Yellow	Top	8.1^{de*} ±0.6†	9.3^{de} ±1.1
	Middle	10.9^{bc} ±1.4	12.0^{bc} ±1.3
	Bottom	12.5^{ab} ±1.0	13.6^{abc} ±1.0
White	Top	9.7^{cd} ±1.4	11.3^{cd} ±1.5
	Middle	12.8^{ab} ±0.9	13.8^{ab} ±1.1
	Bottom	13.9^{a} ±0.8	15.3^{a} ±1.0
Orange	Top	5.5^{f} ±1.0	6.4^{g} ±1.1
	Middle	6.6^{d} ±0.3	7.8^{ef} ±1.3
	Bottom	7.9^{d} ±2.4	9.1^{de} ±2.0

*Mean values with the same superscripts in the same column are not significantly different.

† ±values are standard deviation of the mean.

3.4 Chemical Properties of Jackfruit Flakes

Jackfruit variety affected titratable acidity of flakes. White flaked variety had relatively lower TA than the yellow and orange flaked varieties. Flake pH, on the other hand, remained fairly constant among different jackfruit varieties. Total soluble solutes varied significantly among jackfruit varieties, with orange varieties showing relatively higher total soluble solids values than white flaked variety. With respect to TSS:acid ratio, orange flaked variety exhibited relatively high ratio than yellow and white flaked variety. Regarding ascorbic acid, yellow flaked variety had significantly lower AA than other varieties. Carotenoids were highest in the orange flaked variety. The dry matter content did not vary among flakes from the different jackfruit varieties (Table 5). Titratable acidity varied among different fruit sections. The white flaked variety had significantly lower titratable acidity in the top and middle sections compared to sections of other fruit types. The pH and TSS did not vary significantly among flakes from different fruit sections. TSS:TA ratio decreased from top to bottom sections in all fruit varieties. Ascorbic acid, carotenoids and dry matter content did not vary significantly among fruit sections (Table 5).

Table 5. Chemical composition of flakes from different sections of three jackfruit varieties

Variety/Flake color	Fruit section	Titratable Acid (TA) (g)/100	pH	Total oluble Solids (TSS)	TSS:TA	Ascorbic Acid (mg)/100	Carotenoid (mg)/100	Dry matter Content (g)/100
Yellow	Top	0.38^{a*} ±0.1†	5.1^{ab}±0.5	24.3^{ab}±1.9	63.7^{a} ±3.0	2.1^{bc} ±0.4	0.6^{b} ±0.1	25.7^{a}±5.1
	Middle	0.40^{a} ±0.1	5.0^{ab}±0.6	22.4^{bc}±2.1	56.0^{ab}±3.3	2.0^{bc} ±0.5	0.5^{b} ±0.1	25.5^{a}±2.3
	Bottom	0.44^{a} ±0.1	4.6^{b} ±0.3	20.9^{cd}±2.3	47.5^{c} ±3.9	1.6^{c} ±0.5	0.5^{b} ±0.1	23.9^{a}±7.7
White	Top	0.32^{b} ±0.1	5.2^{ab}±0.3	19.4^{de}±0.8	60.6^{b} ±2.1	3.9^{a} ±1.2	0.4^{b} ±0.1	25.4^{a}±0.3
	Middle	0.36^{b} ±0.1	5.4^{a} ±0.3	18.1^{de}±0.9	50.3^{e} ±2.5	3.6^{ab} ±1.0	0.3^{b} ±0.1	24.7^{a}±2.4
	Bottom	0.39^{a} ±0.2	5.5^{a} ±0.2	17.0^{e}±1.1	43.6^{c} ±1.9	3.2^{ab} ±0.5	0.3^{b} ±0.1	24.2^{a}±2.0
Orange	Top	0.40^{a} ±0.1	4.9^{b} ±0.3	25.8^{a} ±1.0	64.5^{f} ±1.4	4.3^{a} ±1.2	2.4^{a} ±1.1	25.4^{a}±2.8
	Middle	0.42^{a} ±0.1	5.0^{ab}±0.3	25.7^{a} ±1.9	61.2^{b} ±1.5	3.7^{a} ±1.0	2.2^{a} ±1.0	24.5^{a}±3.5
	Bottom	0.43^{a}±0.1	4.8^{b} ±0.3	25.3^{ab}±2.4	58.3^{ab}±2.0	3.2^{ab} ±1.1	1.8^{a} ±1.0	22.3^{a}±5.4

*Mean values with the same superscripts in the same column are not significantly different

† ±values are standard deviation of the mean

TSS:TA Ratio of Total Soluble Solids to Titratable acid

3.5 Relationships between Flake Physico-chemical Properties and Fruit Sections

The relationship was analyzed by subjecting all the flake parameters of different fruit sections of different jackfruit varieties to principle component analysis. Principle component one (PC1) and principle component two (PC2) which explained most of the variability in the flake characteristics (54% and 18%) respectively were selected (Figure 1).

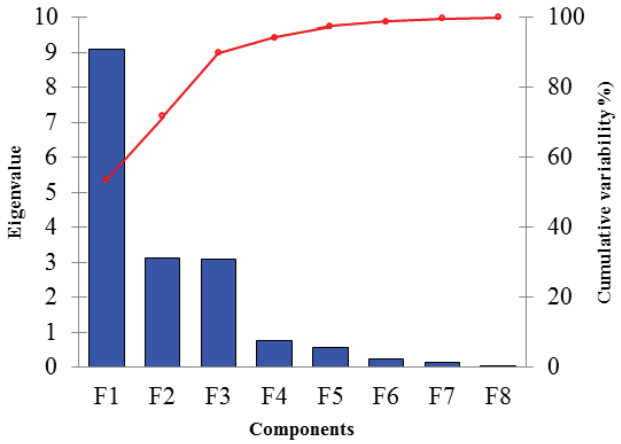

Figure 1. Screen plot of Eigen value versus Principal components

The two principal components provided a general visualization of the interrelationship between the flake physico-chemical properties and sections of different jackfruit PC1 was positively and majorly related to carotenoids, flake redness and TSS and these variables were in proximity to orange jackfruit sections on the bi-plot. Flake textural properties, flake lightness and yellowness occupied a negative location along PC1 and closely associated to white jackfruit sections. On the plot the properties of yellow flaked jackfruit were located between those of white and orange flaked jackfruit varieties (Figure 2). PC1 clearly separated the samples based on their color and textural properties and therefore the 52% variability explained by PC1 was mainly in flake appearance and hardness. PC2 separated samples based on their dimensional characteristics; with flake thickness loaded positively and close to white jackfruit sections while yellow flakes of the yellow flaked variety were broader and elongated. PCA located jackfruit sections into three clusters (Figure 2). The orange sections (Cluster 1) were located on the positive side of PC1 and PC2, the white sections on the negative side of PC1 and positive part of PC2 (Cluster 2) and the yellow sections cluster occupied an intermediate position between white and orange fruit sections (Cluster 3).

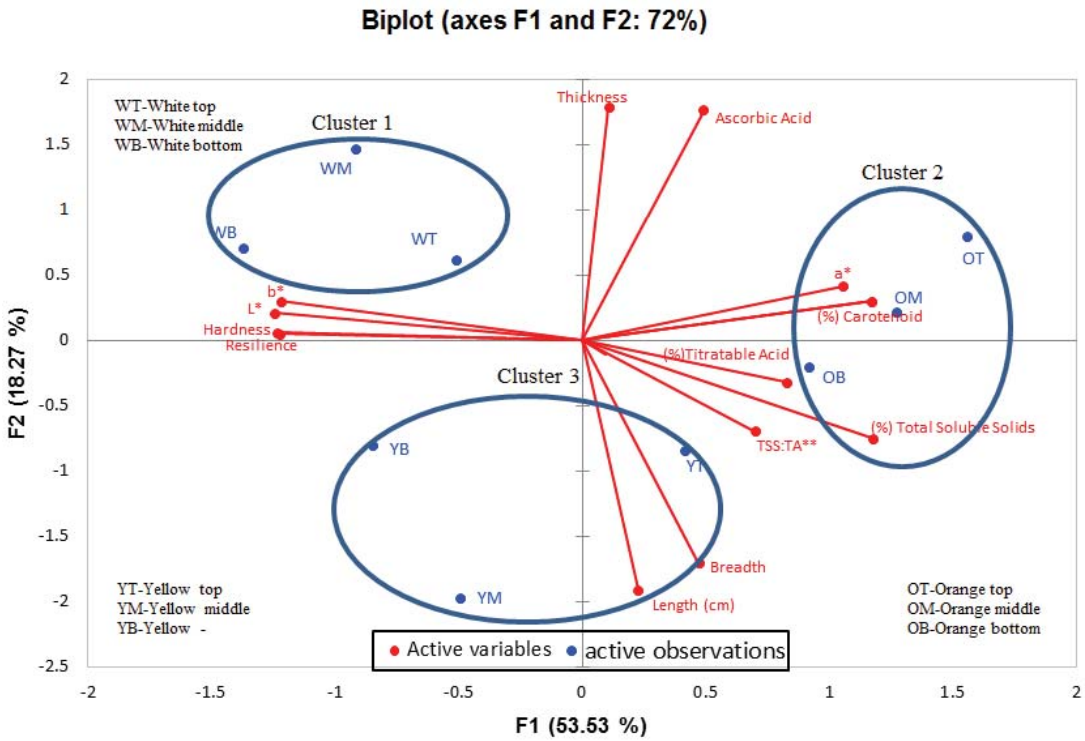

Figure 2. The interrelationship between flake physico-chemical properties and fruit sections

4. Discussion

4.1 Fruit Size, Parts Proportion and Flakes Dimension

The jackfruit circumference of 76 cm observed in this study, was 1.7 times the size of jackfruit circumference reported by Sarker & Zuberi (2011) implying that the jackfruits in the study area were generally large in size than those reported in literature. The yellow flaked jackfruits among the varieties studied had a larger fruit size with an intermediate maturation period compared to the white and orange flaked jackfruits. The difference in fruit size and maturation period observed in this study could possibly be due to genetic variation, growth conditions, weather, soil fertility and tree age. This is consistent with Azad, Jones, & Haq (2007) who emphasized the role of genotypes in jackfruit inter-tree variation.

There was variation in the fruit proportions namely: seeds, core, abortive perigones and flakes among the three jackfruit varieties. It can also be presumed that the yellow fruit type generates more flakes and waste than the white and orange flaked jackfruits due to its larger size. However, the small size orange flaked jackfruits contained more flakes than the relatively larger yellow and white flaked varieties. This discrepancy could be attributed to genetic differences. Jagadeesh et al., (2010) reported variability among jackfruits grown in western Ghat region of India due to genetic variation. Considering flake dimensions, the breadth and thickness varied significantly for the three jackfruit varieties and were consistent with results reported by Jagadeesh et al., (2010). The authors reported 3.32, 5.71 and 0.386 cm for the flake breadth, length and thickness respectively. The variation observed in flake dimensions could be indicative of the greater variation existing among different jackfruit varieties caused probably by differences in genetic makeup.

4.2 Jackfruit Flake Color & Carotenoid Content

The high 'a' value in flakes of the orange flaked jackfruit varieties suggests higher level of carotenoids in orange than yellow or white flakes. This was consistent with the principle component analysis bi-plot which located carotenoids in close proximity with orange flaked sections. The high levels of flake redness for orange flakes could probably be due to the high carotenoids content in orange flaked than in yellow or white flakes. This is in agreement with results reported by Englberger et al. (2006) who reported that darker yellow and orange colors in pandanus fruits are indicative of higher carotenoid levels. Also, high levels of carotenoids in orange flakes could be attributed to accelerated biosynthesis of carotenoids in orange flakes than in other jackfruit types caused by differences in genetic composition. The jackfruit sections did not affect flake color in all jackfruit types. Patil (2003) observed a uniform distribution of carotenoids in flakes from different sections of ripe jackfruits which was consistent with the results from this study.

Minor differences in color lightness, redness and yellowness were observed in white and yellow flaked jackfruit varieties suggesting closeness of the white and yellow flaked jackfruit varieties. Shyamalamma, Chandra, Hegde, & Naryanswamy (2008) reported the existence of genetic diversity in some jackfruit cultivars. The strong colour intensity in the orange flakes suggests greater difference in genetic make up for orange flaked variety as compared to the yellow and white flaked varieties.

4.3 Chemical Composition of Jackfruit Flake

Titratable acidity varied significantly, the low titratable acidity observed in white flaked jackfruit could be attributed to the slow maturation process exhibited by this variety. Maturation leads to ripening and involves utilization of organic acids as sources of carbon skeletons for the synthesis of new compounds during ripening (Batista-Silva et al., 2018). Since white flaked jackfruit takes a relatively longer period to mature, it could imply that the long maturation period caused more organic acid break down in white flaked jackfruit compared to other jackfruit varieties of the same maturity or degree of ripeness. The low acid content observed in this study was consistent with results reported by Gwosami et al. (2011). The authors observed an average TA of 0.65% which was almost twice as much as that exhibited by white flakes. The difference might have been caused by differences in maturation periods.

Total soluble solids content, varied between jackfruit types and was lower in white compared to yellow or orange jackfruit flakes but was in the range of 20-27% reported by Goswami et al., (2011). However, it was slightly lower than 25-41% reported by Reddy et al. (2004) for jackfruit varieties of Indian Kerala region. Accumulation of soluble solids is in fruits postharvest, a result of physiological processes that lead to starch and pectin break-down into smaller soluble molecules (Beckles, 2012). Therefore, the high TSS content observed in orange flakes could be attributed to inherently high starch and pectin content in orange flakes compared to the yellow and white flakes.

The TSS-acid ratio varied with fruit type and section. With respect to fruit sections, the top section flakes in all

jackfruit varieties showed relatively higher TSS- acid ratio than other sections, suggesting that flakes from top sections are tastier and therefore fruit ripening begins from the top section. This was clearly illustrated by PCA bi-plot in which total soluble solids, titratable acidity and TSS- acid ratio, which are the determinant of fruit taste, were located on the positive side of PCA1 together with orange jackfruit sections. This was consistent with Krüger et al. (2012) who reported that fruit flavor is mostly determined by contents of total acids, total soluble solids and their ratio. Mitra and Mani (2000) and Saxena, Bawa, & Raju (2011) stated that jackfruits with TSS greater than 25°brix and 0.3% titratable acidity are considered of high quality for dessert purposes. The results of this study were in agreement with the findings of Ketsa (1988) who reported that small fruits have higher TSS content and therefore high TSS-acid ratio than large fruits. Basing on the TSS:acid ratio, orange flakes could be more suitable for dessert preparations, whereas, the yellow and white flaked jackfruit varieties could be more suited for sweetened or dehydrated products.

The ascorbic acid content, varied significantly ($p<0.05$) between different fruit types, with white and orange flakes exhibiting twice as much ascorbic acid as in yellow flakes. The difference in the vitamin C content observed may be attributed to inherently reduced capacity to metabolize ascorbic acid in the ripe white and orange flaked jackfruit type. Rashim (2003) reported reduction in ascorbic acid content during ripening, which was attributed to degradation of the vitamin during the ripening processes. Therefore, relatively high levels of ascorbic acid in orange and white flaked jackfruit variety may be indicative of reduced capacity of utilizing ascorbic acid as carbon chain source during ripening.

The dry matter content did not vary among the jackfruit types and sections. Dry matter reported in this study was in agreement with values reported by Jagadeesh, et al., (2006) and Ibrahim, Islam, Helali, Alam, & Shafique (2013) but were slightly higher than 17-20% reported by Goswami, Hossain, Kader, & Islam (2011) and Mitra & Mani, (2000) for five jackfruit varieties grown in Bangladesh. Abong et al., (2010) associated high dry matter content of jackfruit flakes with high yields of chips. Therefore, flakes from the three jackfruit varieties could be used to make good quality chips.

4.4 Textural Properties

Flakes from the white flaked variety had lower total soluble solutes and were harder than those from the yellow and orange flaked jackfruit varieties. This was consistent with PCA results which located white flaked section close to flake hardness on one side and orange flakes close to TSS on the other side of the bi-plot. The difference in textural properties among flake types could be attributed to low activities of starch and pectin degrading enzymes in the white flakes compared to yellow and orange flakes. This is in agreement with Huber, Karakut & Jeong (2001) who stated that fruit softening can be attributed to pectin depolymerization and dismembering mediated by pectinase enzymes active in ripe fruit. On the other hand flake resilience and hardness for all the three jackfruit varieties studied increased from top to bottom sections of the fruit, while TSS and TSS: TA increased in the opposite direction (Table 5). The variation in flake hardness among fruit sections could possibly be due to differences in the degree of ripeness of flakes from a particular fruit section. As the fruit ripens there is a general decrease in starch as it is being converted to sugars, this is consistent with an inverse relationship observed between TSS and textural properties of the flakes. Wongmetha, Lih-Shang, & Liang (2015) also reported that as the fruit ripens, starch is degraded to sugars reducing its contribution towards flake hardness. The starch degradation and pectin de-polymerization and dismembering might be taking place at a relatively slower rate in the harder white flaked jackfruits than the yellow or orange flake types. The three jackfruit types therefore, could be suitable for processing different jackfruit based products. The harder textured white flakes could be suitable for processing yoghurt with jackfruit flake pieces. Mesurolle, Saint-Eve, Déléris, & Souchon (2013) highlighted the importance of the harder fruit textural characteristics in processing of yoghurts or toppings with whole fruits or fruit pieces. The softer yellow and orange flakes on the other hand could be suitable for products like juices, fruit leathers and jams or jellies which require prior pulp extraction.

4.5 Relationship between flake characteristics and fruit sections

The bi plot located carotenoids, flake redness and TSS on the positive side of PC1. This signifies that flakes with high carotenoid content are deep red in color and sweeter in taste than those with low carotenoid content. Considering health benefits, orange jackfruit flakes are deep red in color Swami, Thakor, Haldankar & Kalse 2012) and therefore richer in carotenoid content which is an important parameter for selecting varieties with more significant health promoting characteristics. The bi- plot located white sections to the negative side of PC1close to flake textural properties signifying a higher degree of hardness in white flakes than in orange and yellow flakes (Figure 2. This makes white flakes more suitable for use in products such as yogurts, desserts and toppings which require retention of tougher textural characteristics for the ripe fruit (Mesurolle, Saint-Eve,

Déléris, & Souchon, 2013). Yellow jackfruit flakes exhibited characteristics intermediate between the soft orange and hard white flake types. This makes yellow and orange flakes less suitable for minimally processed products because hardiness in fruit texture is one of the prime requirements in minimally processed fruit products (Ohlsson, 1994). In all jackfruit sections studied, flakes extracted from top sections leaned more towards the positive side of PC1 implying that they are tastier, attractive in color, higher in carotenoids and softer in texture. Flakes from bottom section on the other hand, leaned more towards to the negative side of PC1 signifying their being harder in texture and lighter in color. In this regard, flakes from top sections are better suited for products which require pulp extraction whereas those from middle and bottom section are more suitable for minimally processed jackfruit flakes. PCA bi-plot also showed that, sections of the same jackfruit are closely located regardless of variation in the analyzed parameter indicating that jackfruit variety has a greater effect on analyzed parameters than fruit sections.

5. Conclusion

The results suggest a relationship between jackfruit flake colour and some physico-chemical characteristics. Thus, flake color can be used as an indicator for jackfruit physical chemical properties and perhaps a predictor of the jackfruit performance in different processing applications.

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Surveys on the Consumption of Attiéké (Traditional and a Commercial, Garba) in Côte d'Ivoire

Justine Bomo Assanvo[1], Georges N'zi Agbo[1] & 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: 00 225-0778-6755. E-mail: justinebomo2015@gmail.com

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Abstract

Traditional attiéké and commercial attiéké Garba are fermented cassava semolina, steamed and prized by people living in the big cities of Côte d'Ivoire and elsewhere. Attiéké Garba is a derivative of the traditional attiéké resulting from intended manufacturing defects. A consumption survey was therefore conducted in Abidjan (big city) and two departments, Dabou and Jacqueville, major production areas of attiéké and Garba, to assess the importance and determinants of consumption of 4 types of attiéké (Adjoukrou, Ebrié, Alladjan and Garba), consumer preferences and to identify the descriptors of quality that motivate the consumption of these food products. Surveys showed that 99% of respondents consume regularly 1 to 2 times attiéké (traditional or commercial) per day. The consumption of attiéké is related to the organoleptic properties of the different types of attiéké mainly taste (65%), brightness (35%), absence of fibers (33%), color (22%) and odor (21%). The consumption of attiéké Garba is linked to its character of cheap food. Despite the preference of attiéké Adjoukrou (38%) and Ebrié (29%) for their better quality and popularity, the consumption of Garba (10%) is growing. Income, nationality or ethnicity, sex, socio-professional status and educational level have a major influence on the choice of a type of attiéké. The share of consumption of traditional attiéké and commercial attiéké Garba for middle-income consumers (70,000 CFA F <r≤ 200,000 CFA F) and high income (r> 200,000 CFA F) is respectively 23.44% and 7.33% while it is 21.03% and 41.35% respectively for low-income consumers (r <70,000 CFA F).

Keywords: cassava, traditional attiéké, attiéké Garba, consumption, descriptors of quality

1. Introduction

In Côte d'Ivoire, cassava roots are preferentially used in the manufacture of several types of attiéké. According to Assanvo et al. (2017), the consumption of attiéké in Côte d'Ivoire, is estimated today at about 1 300 000 tonnes / year. It continues to grow in view of the growing importance of commercial attiéké Garba in the diet of low-income working populations.

Attiéké is a food produced from a fermented cassava paste, in the form of steamed granules. Originally, attiéké was 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 ethnic groups, Adjoukrou, Ebrié and Alladan remain the largest producers and consumers (Kouadio, Kouakou, Angbo, & Mosso, 1991). Gradually, attiéké has acquired a market value because it constitutes an important source of income for producers. In addition, today this product has spilled over into its home environment and is being consumed all over the country due to its "ready-to-eat" presentation (Assanvo, Agbo, Behi, Coulin, & Farah, 2006). This product is exporting to border countries, some countries in Africa, Europe and the United States through an informal network. On the other hand, other foods with similarity to attiéké appear (Sotomey, Ategbo, Mitchikpe, Gutierrez, & Nago, 2001).

According to Assanvo (2008), attiéké Garba differs from traditional attiéké on several steps of production: reduction of the fermentation time of cassava paste, elimination of drying, granulation and winnowing stages.

These steps ensure the attiéké a better quality. The absence of well-made grains, sensation of grain in mouth and the accentuated color due to the large quantity of oil added during the manufacture of attiéké Garba gave this product the name of speed attiéké. In addition, if traditional attiéké is often eaten as an accompaniment to traditional sauces or the combination of fried or shredded fish and vegetables (onions, tomatoes, chillies) at home or during ceremonies, attiéké Garba is sold for street consumption only, and eaten exclusively with tuna fish. However, despite its poor nutritional and sanitary quality, Garba is being consumed more and more especially in big cities like Abidjan. This phenomenon leads to a substitution of the traditional attiéké by Garba in some cities of Côte d'Ivoire.

Previous studies have focused on production, economic analysis of the traditional cassava processing industry in attiéké in the Abidjan region, conservation methods, biochemical, microbiological and fermentative aspects (N'zore, 1985; Kouadio et al., 1991; Diop, 1992; Aboua, 1998; Fortin, Desmarais, Assovie, & Diallo, 1998; Heuberger, 2005; Coulin, Farah, Assanvo, Spillmann, & Puhan, 2006; Kastner et al., 2006; Kastner et al., 2007; Gnagne, Koffi, Assanvo, & Soro, 2016; Assanvo et al., 2017). Despite the importance of this extensive work on attiéké, few focused on the consumption determinants, reasons and preferences of attiéké and other starchy foods on the Ivorian market. The present study aims at investigating the consumption determinants of traditional attiéké and Garba. The goal is the valorization of the traditional Ivorian attiéké. Specifically, the study compared three traditional attiéké and the Garba through consumer surveys to identify similarities and differences between these four types of attiéké. The appreciation of attiéké consumers was defined by the quality descriptors of the traditional finished product, their importance and their level of consumption and preference for populations compared to other foods marketed in Côte d'Ivoire.

2. Method

2.1 The Different Types of Traditional Attiéke Used for Surveys

Four (4) main types of attiéké systematically corresponding to three traditional attiéké (Ebrié, Adjoukrou and Alladjan) and the commercial attiéké Garba were the subject of a survey on their consumption (table 1). Ebrié, Adjoukrou and Alladjan are ethnic groups of the Southern Côte d'Ivoire.

Table 1. Sample size used for the attiéké consumption survey

City/ Department	Township/ village	Number of neighborhoods investigated	Number of households surveyed	Type of attiéké consumed
Abidjan	Yopougon	7	120	Ebrié, Adjoukrou
	Cocody	8	160	Alladjan, Garba
	Koumassi	11	101	
Dabou	Nouvel Ousrou	3	30	
	Bouboury	3	30	
	Orbaff	3	30	
Jacqueville	Grand-Jack	3	30	
	Ahua	3	30	
	Sassako Begniny	3	30	
Total	9	44	561	

2.2 Study Area

The General Census of Population and Housing (GCPH) of 1998 and 2014 was used as the basis of the survey (Anonymous, 1998, 2014). The RGPH has revealed that the city of Abidjan (economic capital of Côte d'Ivoire) is an area of ethnic diversity. All major cultural groups of Côte d'Ivoire are represented, as well as populations from neighboring countries (Mali, Burkina Faso, Ghana, Nigeria, Niger, Benin, Togo, Guinea, etc ...). This wide representation allowed a large restriction of the consumption survey to the city of Abidjan, particularly the communes of Yopougon, Cocody, Koumassi. These 3 communes comprise 60% of the population of Abidjan according to the GCPH 2014. For completing the data, consumers from 3 villages per department of Dabou Jacqueville were investigated (Figure 1, Table 1). The city of Abidjan (2,877,948 in 1998, 4,395,243 in 2014) and the departments of Dabou (120,304 in 1998, 148,874 in 2014) and Jacqueville (52,871 inhabitants in 1998, 56,308 in 2014), represent large areas of production and regular consumption of traditional attiéké, Abidjan being the area of mass production of attiéké Garba (Figure 1).

2.2 Survey Plan

The survey methods of Dussaix and Grosbras (1996) and Tillé (2001) were adopted for the present study. The

sampling method applied in this consumption survey on attiéké is the three-stage cluster survey, the primary level being the communes or villages surveyed, at the secondary level the neighborhoods and the tertiary level, the households surveyed (Table 1).

In each commune or village, neighborhoods and households were randomly selected. The interlocutors were identified within each household according to their availability to answer the questions. A household has an average of 6 persons. The overall sample size was 561 households interviewed in 44 investigated neighborhoods (Table 1). Surveys were conducted in families using semi-structured interviews. The family represents the sampling unit.

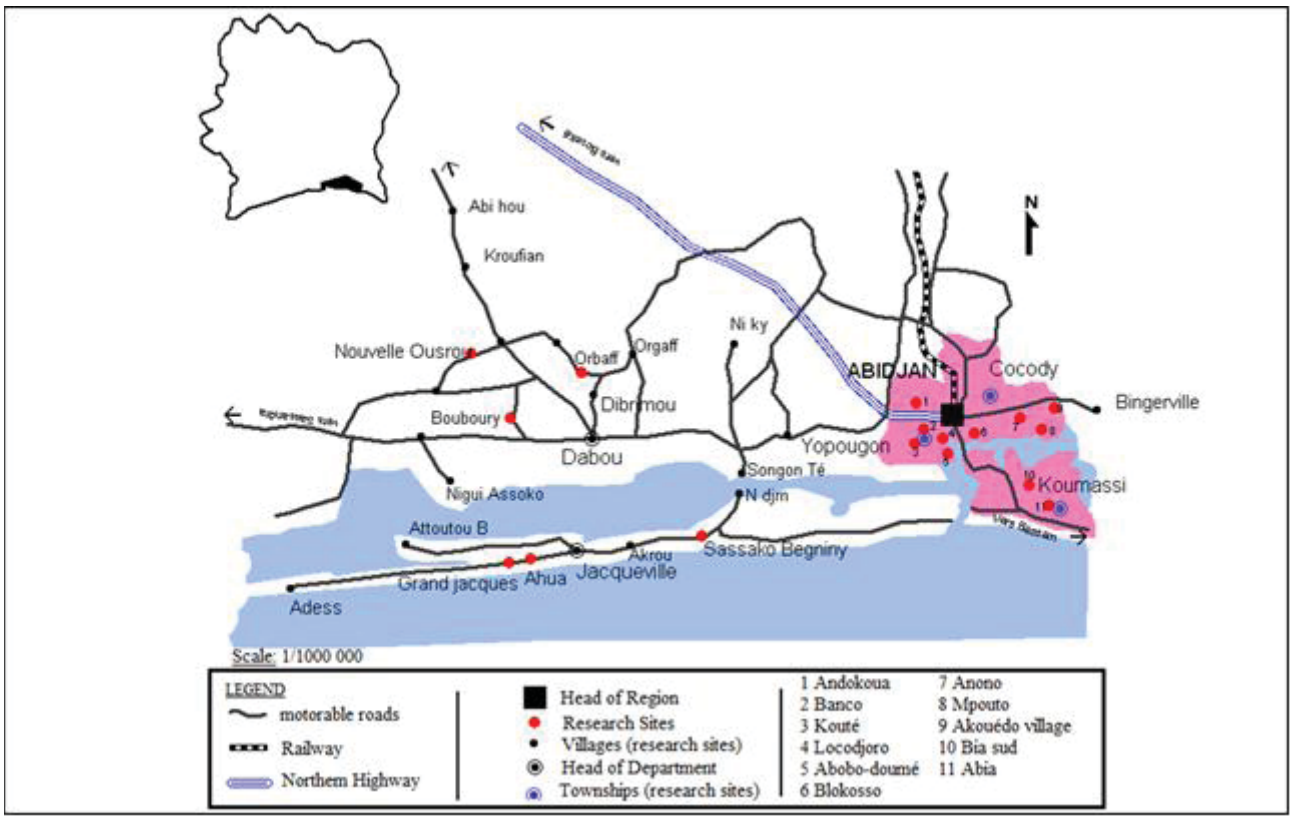

Figure 1. Map of study site presenting the attiéké consumption areas included in the survey

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

2.3 Collection Support (Survey)

A pre-survey was conducted in the village of Adiopodoumé in April 1999 to collect information that was used to prepare the survey questionnaire. This village is located 17 km from Adjamé (Abidjan commune). The survey was repeated in 2006 and then in 2017 for a data update and conducted following production survey (Assanvo, 2008). The choice of the three communes and six villages (primary units) included the density of the populations, the average income level of the resident populations, the importance of the outlets and the geographical position of each municipality. The survey dwelt on the factors of choice of an attiéké type. Thus, socio-demographic and organoleptic characteristics were considered. A standard questionnaire tested beforehand, served as a support for the survey.

The questionnaire consisted of the following three sections:

Section 1: Identification of the respondent. This section collects sociodemographic information on men and women surveyed;

Section 2: Determinants, level, importance, reasons for consumption of types of attiéké and the factors influencing their preference;

Section 3: Importance of descriptors quality according consumers.

2.4 Statistic Analysis

The results were recorded in a database. A frequency calculation was carried out. The preference of one type of attiéké was classified in three reasons according to the definition of consumers. The descriptive statistics of survey data was performed using the software SAS version 8.2.

The level of income (r) expressed in CFA franc was classified into 3 categories: low income (r <70,000 CFA francs), average income (70,000 CFA francs <r <200,000 CFA francs) and high income (r ⩾ 200,000 CFA francs) on the basis of the salaries of civil servants in Cote d'Ivoire.

Differences in consumption between ethnicity or nationality, gender, age, income level, socio-occupational status, level of education, townships or villages and preferences were tested.

3. Results

3.1 Consumption and Preference of Attiéké Types

3.1.1 Attiéké Consumption Level

A total of 561 households were surveyed, of which 62% were women and 38% were men. The age of consumers was 5-80 years and divided into 8 age groups: 5-14 years (10.88%), 15-19 (17.29%), 20-30 (18.18%), 31-40 (17.47%), 41-50 (11.76%), 51-60 (11.23%), 61-70 (8.73%) and 71-80 (4.46%). The surveyed consumer population includes 4 levels of education: Illiterate (13.37%), Primary (40.99%), Secondary (32.27%) and Superior (13.37%).

Apart from other dishes (rice, foutou, placali, etc.), almost all the households surveyed (99%) consumed at least one time attiéké per day. With regard to ethnicity or nationality, Ebrié, Alladjan and Adjoukrou consumed attiéké one (1) to two (2) times per day. The other ethnic groups (Avikam, Aizi, Attié, Abouré, Agni, Baoulé, Senoufo, Abey and other ethnicities or nationalities, Table 2) consumed attiéké on average only once a day or not at all.

For nationalities, 75% of foreign populations living in Côte d'Ivoire (Table 2), consumed Garba more than traditional attiéké. For natives, 40% prefer to eat Garba.

At income level, consumers with incomes 100,000-200,000 CFA francs/month consume attiéké on average only one (1) time per day. Persons with income ≥ 200,000 CFA francs consume once or not at all per day. On the other hand, those with incomes < 70,000 CFA francs, consume an average of two (2) times per day. It appears that the lower the income level, the higher the consumption (Table 3).

The analysis of socio-professional situation of interviewed persons (male or female) revealed the better the socio-professional situation, the lower the consumption of traditional attiéké and attiéké Garba (table 4). Similarly, the higher the level of education, the lower the consumption of attiéké but especially that of Garba highly decreases. In fact, households whose heads of families have a higher level of education consume an average of 1 time day per day or not at all traditional attiéké or Garba. On the other hand, the illiterate households or with primary and secondary education levels consume mostly daily at least 1 time traditional attiéké or commercial attiéké, Garba.

The results of the survey revealed a higher consumption of traditional attiéké for women compared to men (twice a day for women and once a day for men). Consumption of attiéké Garba was higher in men than in women (twice a day for men and once a day for women).

The primary reason for consumption of attiéké was eating habit, staple food or preferred food. The second reason was satiety, good taste and nourishing character. The third reason was the economic nature of attiéké, low cost, availability, well-made appearance, durability and ready-to-eat character of attiéké.

The communes surveyed in Abidjan or the villages in Dabou and Jacqueville were classified by percentage of consumption reasons. Thus in Abidjan, for reason 1, Koumassi (41%) and Yopougon (35%) had the highest percentages. For Cocody, 24% was recorded. For reason 2, Yopougon came first with 39% followed by Koumassi (35%) and Cocody (26%). However, for reason 3, Cocody has the highest percentage of consumption (46%), followed by Koumassi (40%) and Yopougon (14%). In Dabou (65%) and Jacqueville (70%), reason 1 was predominant in all the villages surveyed, followed by reason 2 which represented 25% in Dabou and 20% in Jacqueville.

3.1.2 Preference Level of Attiéké Types

The ranking obtained with the data collected indicates that 38% of the households surveyed prefer to consume attiéké Adjoukrou, 29% attiéké Ebrié, 23% attiéké Alladjan and 10% attiéké Garba (figure 2).

The assessment of the different types of attiéké (Ebrié, Adjoukrou, Alladjan and Garba) by ethnicity or

nationality, excluding the traditional producing ethnic groups (Ebrié, Alladjan and Adjoukrou), showed that the other ethnicities or nationalities prefer consuming the attiéké Adjoukrou (53%) followed by attiéké Ebrié (29%). However, for Attié ethnic group, the preference for the three types of attiéké, Ebrié, Adjoukrou, Alladjan was equal (33.33%). Whether Baoulé have a substantially equal preference for attiéké Ebrié (50%) and attiéké Adjoukrou (40%), the Abouré do not differentiate attiéké Adjoukrou (50%) and attiéké Alladjan (50%). This ethnic group had no preference for attiéké Ebrié and others.

Table 2. Number of times per day of average consumption of studied attiéké (Adjoukrou, Ebrié, Alladjan and Garba) by ethnicity or nationality

Nationality	Ethnic group	Number of households (n=561)	Percentage (%)	Number of times per day average consumption of attiéké (traditional / Garba)
Ivorian	Ebrié	90	16.04	2
	Adjoukrou	50	8.91	2
	Alladjan	50	8.91	2
	Aizi	10	1.78	1
	Avikam	10	1.78	1
	Néo	5	0.89	1
	Abidji	5	0.89	1
	Attié	10	1.78	1
	Abey	10	1.60	1
	Abourey	12	2.14	1
	Appolo or N'zima	15	2.67	1
	Baoulé	36	6.42	1
	Agni	14	2.5	1
	Abron	4	0.71	1
	Dioula	24	4.28	1
	Koyaka	6	1.1	1
	Mahouka	6	1.1	1
	Senoufo	10	1.78	1
	Tangbanan	7	1.25	1
	Yacouba	9	1.60	1
	Gouro	13	2.32	1
	Gagou	5	0.89	1
	Guéré	6	1.1	1
	Wobé	7	1.25	1
	Bété	18	3.21	1
	Dida	10	1.78	1
	Kroumen	8	1.43	1
	Toura	4	0.71	1
	Koulango	6	1.1	1
	Lobi	6	1.1	1
Total Ivorian		466	83.07%	
Burkinabe		10	1.78	1
Malian		9	1.60	1
Guinean		8	1.43	1
Togolese		10	1.78	1
Beninese		9	1.60	1
Nigerian		7	1.25	1
Ghanaian		10	1.78	1
Nigerian		10	1.78	1
Senegalese		9	1.60	1
Cameroonians		8	1.43	1
Congolese		5	0.89	1
Total foreign		95	16.93%	

Table 3. Daily consumption of traditional attiéké and attiéké Garba by income level

Income level of the households surveyed, n=561	Number of households	Percentage (%)	Number of times per day
0-10,000 CFA F (0-17.02 dollar US)	65	11.58	2
10,000-20,000 CFA F (17.02-34.04 dollar US)	86	15.33	2
20,000 -50,000 CFA F (34.04-85.09 dollar US)	137	24.42	2
70,000-100,000 CFA F (119.13-170.19 dollar US)	123	21.95	1
150,000-200,000 CFA F (255.28-340.38 dollar US)-	80	14.25	1
≥ 200,000 CFA F (≥ 340.38 dollar US)	70	12.47	1

Table 4. Daily consumption status of traditional attiéké and commercial attiéké Garba by socio-professional

Socio-professional situation of household managers (male / female)	Number of households	Percentage (%)	Average number of times per day
Frame	69	12.30	0 à 1
Administrative employee	136	24.24	1
Liberal employee	104	18.54	1
Worker	152	27.09	2
Unemployed	100	17.82	2

According to municipalities, the inhabitants of Yopougon consumed attiéké Ebrié (40%) and attiéké Adjoukrou (34%) more than attiéké Alladjan (26%). However, in Cocody, people consumed as much attiéké Adjoukrou (16%) as attiéké Ebrié (15%). The commune of Koumassi had a clear preference for attiéké Adjoukrou (61%) whereas the preference of attiéké Ebrié and Alladjan was 16%. The percentage of preference attributed to attiéké Garba was not very high (7%) but its preference was better perceived than in Yopougon and Cocody. With regard to the villages of Dabou and Jacqueville, almost all the inhabitants preferred consuming attiéké locally produced. Sometimes, the foreign populations of these villages consumed other types of attiéké when they are outside the producing villages.

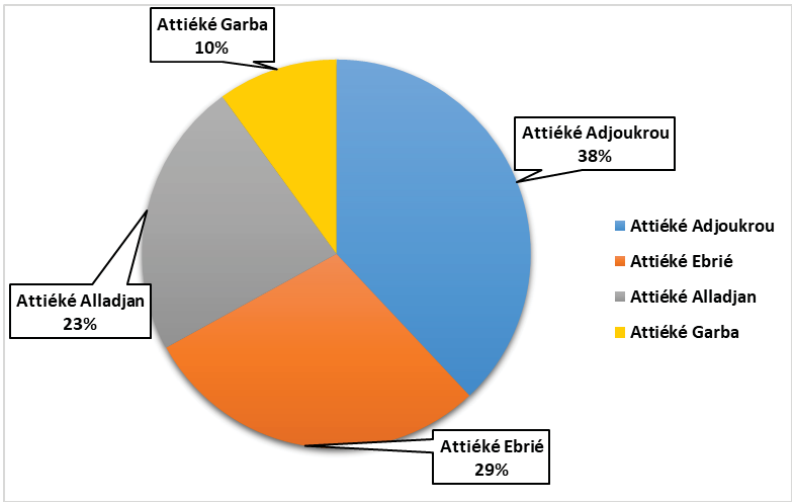

Figure 2. Percentage preference of different types of attiéké (Ebrié, Adjoukrou, Alladjan, and Garba) by the households surveyed

Almost all consumers (99.62%) rejected the acidity of attiéké. The sweetness is the most sought (59.31%), followed by the neutral character (37.26%). The salty character does not matter because salt can be added if needed.

Most households (58%) preferred the cream color of attiéké while 35% preferred the yellow color. Only a few (7%) and especially the Ebrié preferred it off-white. As a general rule, the indigestible character of attiéké is mostly not accepted by usual consumers.

Two important criteria of preference for producers and sellers were the conservation mode and duration of

attiéké. Generally kept in bags or in baskets covered with banana leaves, attiéké can lose its quality if not well cooked and / or preserved.

Attiéké Adjoukrou was more cohesive (sticky) than attiéké Ebrié. By this character (Table 5), it is closer to attiéké Alladjan and is much appreciated by consumers. Its cohesive (sticky) character is appreciated by 70% of consumers. Attiéké Ebrié was less cohesive and it existed in the Ebrié ethnic group two another types called "Agbodjama" (large grains) and "N'tonié" (with small grains) that grains are more stain remover. About 63% of consumers appreciated attiéké Ebrié because of its non-sticky nature. Only 37% of consumers surveyed preferred it cohesive (sticky) texture. Most of Ivorian consumers or other nationalities appreciated the cohesive (sticky) texture of attiéké (traditional and commercial).

Table 5. Preference for cohesive and little cohesive textures according to the types of attiéké

Types of attiéké	Ebrié		Adjoukrou		Alladjan		Garba	
Texture	Cohesion between grains	Little cohesion between grains	Cohesion between grains	Little cohesion between grains	Cohesion between grains	Little cohesion between grains	Cohesion between grains	Little cohesion between grains
Preference (%)	37	63	70	30	55	45	60	40

3.1.3 Share of Traditional Attiéké and Garba in Relation to the Consumption of Food Sold on the Abidjan Market

Low-income households (r <70,000 CFA F) preferred consuming attiéké Garba compared to traditional attiéké, rice, cassava, yam and plantain (Figure 3).

Middle-income consumers (70,000 CFA F <r ⩽ 200,000 CFA F) and high income (r> 200,000 CFA F) preferred eating traditional attiéké and rice (Figure 4) compared to attiéké Garba, cassava in other forms of preparation, yam and plantain (Figure 4).

The share of consumption of traditional attiéké and commercial attiéké Garba for middle-income consumers (70,000 CFA F <r⩽ 200,000 CFA F) and high income (r> 200,000 CFA F) is respectively 23.44% and 7.33% while it is respectively 23.89% and 38.68% for low-income consumers (r <70,000 CFA F).

3.1.4 Classification of Quality Descriptors of Different Types of Attiéké by Households

A list of quality descriptors of attiéké was drawn up among the households surveyed. These descriptors are ranked by importance according to the percentages assigned by the 561 households surveyed. Comparison of percentages showed the importance of taste (65%), bright or translucent appearance (35%), fibers in attiéké (33%), color (22%) and odor (21%) (Figure 5).

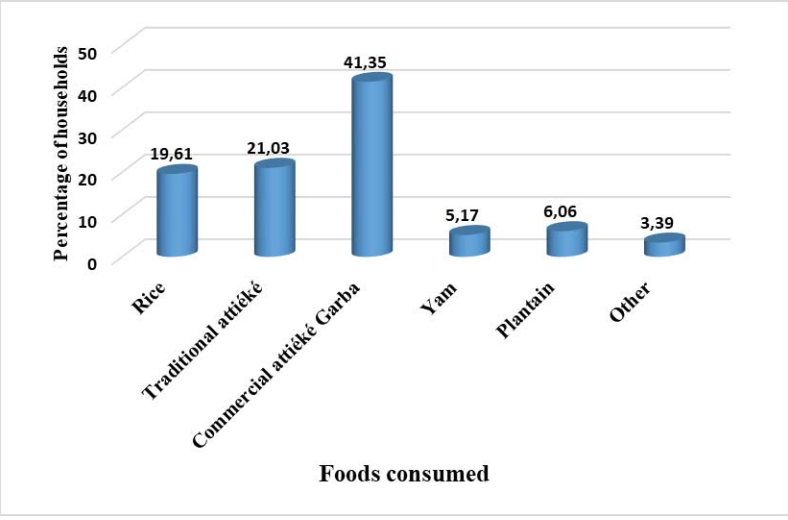

Figure 3. Preference for consumption of traditional attiéké and attiéké garba relative to other foods sold on the market at the level of low-income households

The 561 households attributed to the taste descriptor, a great importance in the choice of good attiéké. On the other hand, for consumers, translucent or bright and fibrous characters were more important than odor. Color, odor, firmness, well-formed grains and rounded grains came in third position. The characters, cohesiveness between grains, good conservation and grain size, appeared as secondary in the perception of the 561 households. Moisture, health, elasticity, well-being and flavor were not important factors for consuming households (Figure 5).

4. Discussion

4.1 Importance and Determinants of Attiéké Consumption

4.1.1 Level and Importance of Attiéké Consumption

The importance and determinants of attiéké consumption were studied in Abidjan, Dabou and Jacqueville. The frequency of regular consumption of attiéké for 99% of households surveyed showed that attiéké was a very important food for consumers. Indeed, the consumption of this food is increasing throughout the country and remains growing (Kouadio et al., 1991). From cultural value, attiéké has gained social value because it is consumed throughout the country due to its ready-to-eat food and energetic value close to rice (Diop, 1992).

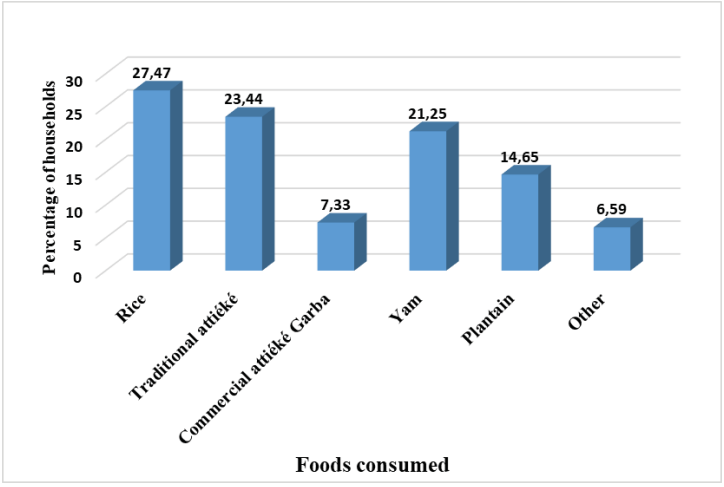

Figure 4. Preference for consumption of traditional attiéké and attiéké Garba compared to other foods sold in the market at the level of high-income and middle-income households

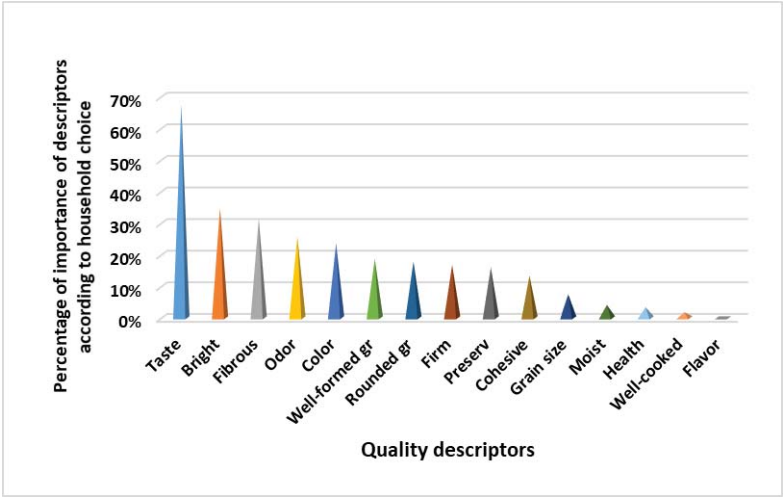

Figure 5. Importance percentage of descriptors of studied attiéké (Adjoukrou, Ebrié, Alladjan and Garba) according to consumers

Well-formed gr: Well-formed Grains, Rounded gr: Rounded grains, Preserv: good food preservation, the bright aspect expresses the translucent character of the attiéké, cohesive: cohesion between grains, health: provides health, Well cooked: well-cooked attiéké

The frequency of consumption in relation to sex showed that women consume attiéké more than men (twice a day). This could be explained by the fact that women are those who do the market and prepare the food. In addition, they often are in charge of the production and sale of attiéké. Only the attiéké Garba is generally sold and consumed by men.

The frequency of consumption in relation to nationality may be justified by the fact that attiéké is not part of the eating habits of these foreign nationalities. These foreign people cannot always make the difference between traditional and commercial products. The main thing for them is the stamina effect (the starch), the energy produced by the semolina and the tuna fish used. On the other hand, Ivorians prefer consuming more attiéké because a large part of the population is producer community. Then, with the better knowledge of the traditional product, they prefer the traditional attiéké to the attiéké Garba.

It was also noted that the higher the socio-occupational situation, the lower the consumption of traditional attiéké and especially attiéké Garba. This statement also includes the level of education. In fact, traditional attiéké or Garba are essentially carbohydrate (Assanvo et al., 2017) and the knowledge of the side effects of the toxic cassava varieties used for manufacturing traditional attiéké or Garba on health dissuades some consumers. Indeed, this food could lead a possible elevation of blood glucose in case of high consumption. This could have a negative impact on the health of diabetics, obese or overweight individuals (Sievenpiper, Chan, Dworatzek, Freeze, & Williams, 2018). The risk of toxicity due to a bad elimination of cyanide in attiéké Garba (Assanvo, 2008) could lead to the appearance of endemic goitre and cretinism (Delange, 1994) or paralysis (Banea, Tylleskar, Tylleskar, Gebre-Medhin, & Rosling, 2000; Banea et al., 2012). The sanitary character of the attiéké Garba can be a source of toxi-infection due to the presence of bacilli (Abe, Assanvo, Sanogo, & Koffi, 2018) or simply a recontamination caused by a bad handling during sales or packaging (Assanvo, 2008)

The frequency of consumption in relation to the ethnic group showed that Adjoukrou, Ebrié and Alladjan ethnic groups consume more attiéké daily than other ethnic groups and nationalities. This result is justified by the fact that this food constitutes the basis of the diet of these coastal populations of Côte d'Ivoire (Kouadio et al., 1991). According to these authors, the Adjoukrou, Alladjan and Ebrié, lagoon peoples, remain the largest producers and consumers of attiéké and constitute the supply base of the wide Abidjan market. According to UNIDO's Interim Report on the Starchy Food Chain (United Nations Industrial Development Organization [UNIDO], 1990), 100 tons of fresh attiéké were produced daily in 1985, for the city of Abidjan alone, by the artisanal sector. Nowadays, because of the growing demand, other ethnic groups or nationalities that are not originally producers, are quite present on the market. These are Attié, Baoulé, Burkinabé and Togolese, among others who specialized in the production and sale of attiéké Garba. The attiéké Garba originally manufactured by Ebrié producers, is commonly referred to as fast attiéké (or attiéké "speed"). However, new producers not belonging to the traditional producing ethnic groups have become specialists. Traditional attiéké and Garba also have economic value. In Côte d'Ivoire, the ever-increasing demand in cities has made it possible to move from family production to market production (Diop, 1992). Now the product occupies an important place in the local economy with its positive spin-offs along the industry. Urban consumption, particularly in Abidjan, is important because attiéké is a ready-to-eat food (Tano & Perrault, 1986; Assanvo, Agbo, Brunnchweiler Beez, Monsan, & Farah, 2018). The popularity of this food has grown in the West African subregion (Burkina Faso, Mali, Ghana, Togo, Benin, Senegal) to the point where it develops in countries like Benin and Ghana, not only networks of production systems but also the appearance of other foods with a similarity to attiéké (Sotomey et al., 2001; Obilie, Tanoh-Debrah, & Amoa-Awua, 2004). In addition, Essia, Kouebou and Djoulde (2003) present attiéké as a semolina highly appreciated by West and Central African populations. For the rest of the world, attiéké has currently crossed the borders of Europe (France, Belgium, Switzerland), America (Canada and USA) and Asia (Japan, China) through an informal network as in the subregion.

4.1.2 The Reasons for the Consumption of Attiéké

The level of income has an impact on the consumption of attiéké. This is because attiéké is cheap and more accessible than other types of foods. The type of attiéké called Garba, being very cheap, is experiencing a marked increase in consumption among people with low incomes, young people and children. However, Tano and Perrault (1986) showed that attiéké will have difficulty replacing imported rice in the dietary habits of mostly urban consumers because of its high cost. According to their study in 1979, attiéké was 62% more expensive than rice and in 1985, 114% more expensive. Today, the cheapest cost of attiéké Garba could lead attiéké to overhang rice, given its ever increasing consumption favored by the growing poverty due to the consequences of the war and the high cost of life that Côte d'Ivoire faced. But for this, the quality of the attiéké Garba should be significantly improved to be consumed by all. Other reasons for consumption are to be considered when referring to the percentages of consumption reasons for attiéké. These are, for example, eating

habits. Cocody, Yopougon and Koumassi communes are major areas of attiéké production and sale. These villages are home to Ebrié villages and some Adjoukrou and Alladjan communities that are set up for on-site production. This justifies the high percentages (35% and 41%) of consumption by eating habit (reason 1) of the communes of Yopougon and Koumassi. This is the case in the villages of Jacqueville (70%) and Dabou (65%). In sum, the high frequency of food consumption is very often linked to eating habits. Massamba & Treche (1995) show that the high frequency of consumption of chikwangue (fermented product of cassava from retting) in the Congo (central plateau), and inhabitants of the Nairi Valley, was due to the fact that the chikwangue is the staple food of these populations.

For reason 2 (Yopougon, 39%, Koumassi, 35% and Cocody, 26%), some consumers prefer traditional attiéké and / or Garba because it satiates more and provides more energy. In these communes, some people consume attiéké for its good taste.

Considering the percentages of reason 3 (Cocody, 46%, Koumassi, 40% and Yopougon, 14%), even though Cocody community contains many high-income people, a segment of the population with a low income lives there too. Also, the presence of "maquis", restaurants, university residences (campus) and schools can justify the high consumption of traditional attiéké and Garba in this area.

4.1.3 Influence of the Preference and Location of a Type of Attiéké on the Consumption

The preference and location of a type of attiéké influence the consumption. Attiéké Adjoukrou appears to be the most appreciated by consumers (38%). The second type of attiéké is the attiéké Ebrié, followed by attiéké Alladjan. This last type of attiéké, in terms of quality, is close to Adjoukrou attiéké but its lesser popularization explains its third rank. Attiéké Garba is less appreciated especially because of its poor quality. It is produced for commercially purpose by new producers who do not always master the production process.

The distribution of consumption of attiéké according to the communes indicated that in Cocody, the consumers surveyed eat both attiéké Ebrié and attiéké Adjoukrou. This can be explained by the presence of Ebrié villages and attiéké Adjoukrou is more sold on the Cocody market for its strong demand in this locality of Abidjan. In Koumassi, attiéké Adjoukrou is also much more appreciated than the Ebrié and Alladjan types. Attiéké Garba seems more popular in Koumassi than in Yopougon and Cocody. A food can be consumed even if not prefer for different reasons (economic, accompaniment, etc). In the villages of Dabou and Jacqueville, the preference is focused on attiéké locally produced. This is due to cultural reason and food habit. The situation is the same in the Ebrié villages

4.2 Importance of Attiéké Quality Descriptors for Consumers' Households

4.2.1 Influence of the Assessment of Attiéké Organoleptic Characteristics of and the Type of Consumers on the preference of attiéké

The preference of a type of attiéké is related to the appreciation of the organoleptic characters. Indeed, according to Lenglet (2006), the three factors of sensory acceptability and consumer palatability are: appearance (shape, color) under vision; the flavor (aroma, flavor) related to odor and taste, and the texture (resistance, consistency) of touch. In the case of attiéké, taste, texture, odor, color, and digestion are particularly important characteristics of consumer choice. Two types of consumers stand out: the first group comprises the most warned consumers who are most often the households belonging to different producing ethnic groups, and especially attiéké sellers. The second group is composed of non-warned consumers, often disregarding attiéké. According to the consumers of group 1, the appearance of grains (shape and texture), odor, taste (acid, neutral, sweet, salty) and color are the main criteria for assessing attiéké. The grains should be round, similar to the shape of the fish eggs. They may be sticky or not sticky linked to the preference of the producing ethnic group. In the case of odor, the statement not pleasant means the product is of poor quality, not fresh or not well cooked. The characters of the taste, very little acid, neutral and slightly sweet, are the most sought. The acidic nature is one of the limiting factors in the choice of attiéké although it is a food resulting from a fermentative process. The salt criterion does not matter because salt can be added when needed. Attiéké Alladjan is known for its salty character, seawater is used during the manufacturing process.

More than half of the consumers surveyed prefer attiéké Adjoukrou because it is more cohesive than attiéké Ebrié. This texture is appreciated by easy hand manipulation. Attiéké Ebrié and its variants "Agbodjama" and "N'tonié", (Assanvo, 2008) were originally reserved for ceremonies. Today, with the demand and the grains cohesive character sought by consumers, a more cohesive attiéké variant than traditional attiéké Ebrié has emerged.

Native consumers of traditional attiéké do not appreciate attiéké Garba. However, this attiéké is more and more

appreciated by a slice of the population living in Côte d'Ivoire. If at the bôginning low or average income justified the consumption of Garba, today this attiéké has entered the diet of the populations (adults, young people, children) living in big cities like Abidjan. It begins to constitute a food habit for these new consumers despite its low quality. At the organoleptic level, attiéké Garba does not meet the standards of traditional producers including the presence of well-made grains, slightly acidic taste, cohesiveness of granules, no wet, elasticity and cream color (Assanvo et al., 2018). The color of Garba is more pronounced because of the large amount of oil added. Hence forth attiéké Garba may be take in account in the food of the populations living in Côte d'Ivoire, even if an improvement of its quality is needed (Assanvo, 2008).

4.2.2 Importance of Quality Descriptors According to Consumers in the Choice of Attiéké during the Purchase

For the purchase of attiéké, the taste (acidulated, slightly sweet and sometimes salty) is over other characters. This character is therefore an important quality descriptor. Appearance (translucent or shiny appearance, well-rounded and well-rounded grain characters, presence of fibers or absence of impurities, color), odor and texture of attiéké, also appear to be of importance for consumers whether the percentages are not as high as in the case of taste.

For attiéké Garba, after two to three days, there appears an unpleasant smell (off flavor) consequence of an alteration of taste. This type of attiéké is designed to be consumed preferably within 24 hours after its preparation.

The importance percentage of the smell is not as high, however this descriptor plays an important role in the choice of the product for purchase. Similarly, it seems easier to understand the importance that the consumer attributes to the appearance of attiéké. The view allows the identification of the product, evaluation of freshness, the quality of texture and color. It is therefore essential in the choice of refusal or acceptance of a food (Barrett, Beaulieu, & Shewfelt, 2010). Thus, the informed Ivorian consumer (producers and sellers) gives great importance to the design of grains in attiéké. For most producers interviewed, the granulation step is very important in the manufacturing process of Ivorian attiéké. The term attiéké is only used when the grains are well formed and rounded. The absence of well-formed grains is reported in the case of "attiéké gari" in Benin (Sotomey et al., 2001) and attiéké in Southwestern Ghana (Obilie et al., 2004). These grains play a role in the firmness (in the mouth) of the product attiéké sought by the consumer.

Regarding the other descriptors (cohesion between grains, grain size, moisture, elasticity, flavor), the lack of knowledge or non-perception of these terms can be at the origin of low percentages of importance during the purchase. These descriptors may not count for the consumer. This hypothesis remains to be verified because according to Monneuse (2003), the organoleptic factors of food are a condition for a food intake. However, the sensory system makes the link between the environment, habits and needs and stimulates food intake according to expressions of preferences or rejections (Eertmans, Baeyens, & Van den Bergh, 2001; Johnson, 2013).

5. Conclusion

At the end of these surveys on consumption, it appears that attiéké is a food consumed by the majority of the surveyed households, all sex and age combined.

The consumption of attiéké Garba is increasing in all social classes. The high frequency of consumption is mainly related to social level and ethnicity. Several reasons gathered in three groups explain the consumption of attiéké: the main reason of consumption is the food habit, staple food or favorite food; the second reason is satiation, good taste and nourishing character. The third reason is the economic nature of attiéké, the low cost, availability, well-made appearance, durability and the "ready to consume" nature of attiéké.

Four major elements explain the reasons for the popularity, the commercial success and the preference of traditional attiéké and attiéké Garba among consumers in Côte d'Ivoire and elsewhere:

- characteristic of food ready to eat;

- fact of being a calorific food, very energetic;

- being an accompaniment that can be associated with different types of dishes (fish, red or white meat, vegetables, sauces);

- organoleptic, hedonic and ideal factors (what we think of the food), intervene directly in the motivation and the act of food consumption.

Among the different types of attiéké, attiéké Adjoukrou is the most appreciated by consumers (38%), followed by attiéké Ebrié. However, the consumption of attiéké Garba (or attiéké "speed") is growing especially for low-income populations.

In general, during the purchase of attiéké, the most important descriptor for consumers is the taste. The appearance, smell and aroma of attiéké also play a determining role in the choice of attiéké to buy.

All these organoleptic characteristics identified after the consumer survey will allow standardization of the traditional manufacturing process of attiéké and give it reliable standards of quality. This will be beneficial for improving the quality of the finished product, health and well-being of consumers.

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Influence of Oryzanols Concentrate on the Oxidation of Methyl Ester Linoleic Acid and Study of Their Oxidation Products

Bruno Irigaray[1], Iván Jachmanián[1] & María A. Grompone[1]

[1]Departamento de Ciencia y Tecnología de los Alimentos, Facultad de Química, UdelaR. Gral Flores 2124, Montevideo, Uruguay

Correspondence: Bruno Irigaray, Departamento de Ciencia y Tecnología de los Alimentos, Facultad de Química, UdelaR. Gral Flores 2124, Montevideo, Uruguay. E-mail: birig@fq.edu.uy

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Abstract

Oryzanols are frequently found in rice bran oil but almost completely removed in the neutralization step when the oil is chemically refined. In this way, oryzanols can be recovered from the soapstocks to generate a concentrate. Thereby, they could be used as antioxidants in lipids for specific purposes. In the present work the antioxidant power of oryzanols concentrate (33% purity) was studied together with pure oryzanols and butylhydroxytoulene (BHT). Methyl esters were prepared from regular sunflower oil without antioxidants to which the antioxidants before mentioned were added in an effective concentration of $3x10^{-3}$ M. The samples were oxidized in a heating block with oxygen flow and the hydroperoxides of linoleic acid methyl ester were analyzed. It was observed that all antioxidants were able to protect the sunflower oil methyl esters from oxidation with respect to methyl esters without antioxidants. Oryzanols presented a notoriously lower protection ability compared to BHT. However, the formation of the linoleic acid methyl ester hydroperoxides and their proportion, for the same oxidation stage, did not show differences between the antioxidants used. Therefore, the oxidation kinetics were similar between the different antioxidants studied.

Keywords: oxidation, methyl esters, linoleic acid, hydroperoxides, oryzanols

1. Introduction

It is interesting to study the evolution of the composition of a system subjected to a process of oxidative rancidity and to compare the variations that occur in the presence of an antioxidant whose antioxidant power is to be analyzed. There are precedents related to the monitoring of the concentration of primary oxidation products (hydroperoxides of linoleic acid) as a function of the oxidation time. Xu & Godber (2001) studied the oxidation of high purity linoleic acid at 37 °C under an oxygen atmosphere in the presence of oryzanols, ferulic acid and α-tocopherol. In similar investigations the formation of hydroperoxides from linoleic acid methyl ester was studied (Chan & Gordon, 1976; Brash, 2000; Mäkinen & Hopia, 2000; Morales, Dobarganes, Marquez Ruiz, & Velasco, 2010).

In the case of linoleic acid, the carbons more likely to generate hydroperoxides are those located in positions 9, 11 and 13 (Figure 1). The study of the products formed from linoleic acid showed that hydroperoxides predominate in positions 9 and 13 while the hydroperoxide in position 11 is not formed in significant amount (Bergström, 1945; Bergström, Blomstrand, & Laurell, 1950). This is due to the fact that the formation of the conjugated double bond gives the free radical greater thermodynamic stability and, therefore, the free radicals available for the subsequent reaction with oxygen and with the free hydrogen radical will be located at carbon 9 and in carbon 13 of the linoleic acid molecule (Bascetta, Gunstone, & Walton, 1983; Brash, 2000).

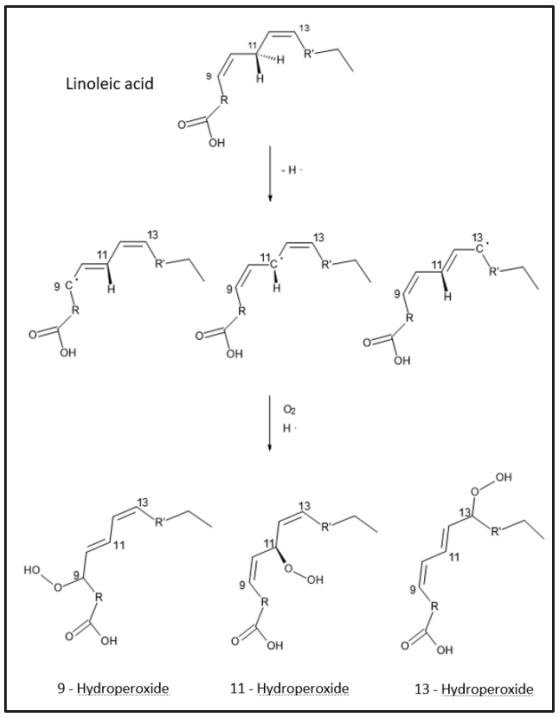

Figure 1. Linoleic acid reaction with molecular oxygen to form 9, 11 and 13-hydroperoxide

The 9 and 13-hydroperoxide of linoleic acid have geometrical isomerism, so that the cis and trans isomers can be formed in each of these positions. This is because when conjugated double bonds are formed, due to their resonance the hydrocarbon chain can be located in a cis or trans position with respect to the other end.

Mäkinen & Hoppia (2000) and Morales et al. (2010) described the formation and identification of the four isomers obtained from methyl ester linoleic acid: methyl 13-hydroperoxide (Z)-9 (E)-11 octadecadienoate, methyl 13-hydroperoxide (E)-9 (E)-11 octadecadienoate, methyl 9 hydroperoxide (Z)-10 (E)-12 octadecadienoate and 9 hydroperoxide (E)-10 (E)-12 octadecadienoate. These compounds were separated by high performance liquid chromatography on a silica column and detected by HPLC analysis with a diode array detector at a wavelength of 234 nm. These authors also identified (at 234 nm) other oxidation products, such as the methyl ester linoleic acid hydroxydienes. When a greater degree of oxidation is reached it is possible to determine, by this technique, some secondary oxidation compounds derived from methyl ester linoleic acid such as ketodienes which are formed from the aforementioned hydroperoxides, which were identified at a wavelength of 268 nm. Therefore, it is possible to study the formation of hydroxyperoxides from linoleic acid (Gardner & Weileder, 1972; Xu & Godber, 2001; Fukushige, Wang, Simpson, Gardner, & Hildebrand, 2005) as well as from linoleic acid methyl ester (Chan & Gordon, 1976, Wang, Ohshima, Ushio, & Koizumi, 1999; Brash, 2000; Mäkinen & Hopia, 2000; Pajunen, Johansson, Hase, & Hopia, 2008, Morales et al., 2010). Approximately 60 % of regular sunflower oil is linoleic acid. This fatty material is low-cost and easily available.

In this work the evolution of hydroperoxide content in a sample of methyl esters obtained by derivatization of commercial regular sunflower oil (SFOME), which was previously purified to eliminate the presence of antioxidants, was studied. These results were compared with those obtained in identical conditions, but with the addition of oryzanols concentrate and other antioxidants. The latter allowed to get more information regarding the antioxidant activity of these compounds by studying the products formed during the oxidation of a lipid sample and the effect of the presence of oryzanols. This is particularly interesting since through this study it was possible to learn how oryzanols protect this type of lipid matrices at temperatures close to room temperature.

2. Materials and Methods

2.1 Preparation of Fatty Acids Methyl Esters

To obtain the methyl esters, regular refined sunflower oil manufactured by the company COUSA (Uruguay) was used. Before doing so, the oil was purified on an alumina column according to the method described by Yoshida

et al. (1992) and Morales et al. (2010).

Once the oil was purified, 2 grams were weighed in a glass vial with a screw cap and 12.5 mL of 2 N KOH solution in methanol was added. The solution was magnetically stirred for 30 minutes at room temperature. Subsequently, 5 mL of petroleum ether was added and the mix stirred for approximately 1 minute. Phase separation was achieved by centrifugation at 3000 rpm. The organic layer was transferred to a second vial flask and washed with 5 mL of distilled water. This step was repeated three times and the sample then dried with anhydrous sodium sulfate. The solvent was evaporated under a stream of nitrogen until constant weight. Sunflower oil methyl esters (SFOME) were stored under nitrogen atmosphere, at freezer temperature (-20 °C) and protected from light.

2.2 Addition of Antioxidants to Fatty Acid Methyl Esters

Sigma-Aldrich quality BHT, an oryzanols standard supplied by Dr. Haiko Hense from the Federal University of Santa Catarina (Brazil) and an oryzanols concentrate obtained in our research facility with 33 % purity were used as antioxidants. The oryzanols concentrate purity was determined by HPLC.

An antioxidant solution in isopropanol or chloroform was prepared and no more than 150 μL added directly on SFOME in order to obtain a final concentration of 3 x 10^{-3} M. After adding the antioxidants, samples were placed in an ultrasound bath for 10 min to ensure complete homogenization.

The SFOME were stored under nitrogen atmosphere, at freezer temperature (-20 ° C) and protected from light until use.

2.3 Oxidation of Fatty Acids Methyl Esters

The oxidation of the SFOME was carried out in a test tube with a screw cap, provided with a capillary tube (0.25 mm internal diameter x 400 cm long) for oxygen supply. A dry block heater for temperature control was used.

The temperature was set at 50 °C and once it was reached, the test tubes containing 1.5 g of SFOME samples were placed. A constant oxygen flow of 6 mL / min was applied so that it bubbled through the samples. At certain time intervals, aliquots of approximately 7 mg were taken. Subsequently, 1 mL of hexane was then added and samples were ready for HPLC analysis.

2.4 Analysis by High Performance Liquid Chromatography

The analysis by high performance liquid chromatography (HPLC) was carried out according to the method reported by Morales et al. (2010). A Shimadzu 20A HPLC, equipped with a Lichrospher-Si column (250 mm x 4.6 mm x 5 μm) and Shimadzu SPD M20A diode array detector was used. Oven temperature was adjusted at 40 °C. An isocratic program was used with hexane:diethylether (82:18, v:v) and the flow was 2 mL/min.

Chromatograms were recorded at a wavelength of 234 nm and 268 nm to determine the SFOME primary and secondary oxidation compounds. Hydroperoxides of methyl ester linoleic acid were identified according Morales et al. (2010).

3. Results and Discussion

3.1 Hydroperoxide Formation Rate

Lipid oxidation process starts with relatively slow kinetics. The advance of the oxidation reaction accelerates the kinetics until at some point the rate of formation of the oxidation compounds strongly increases. As previously mentioned, Mäkinen & Hopia (2000) and Morales et al. (2010) identified by HPLC the four major hydroperoxides of methyl ester linoleic acid (Table 1).

Therefore, the variation of the area corresponding to these peaks as a function of time allowed to compare the kinetics of the oxidation process in different conditions (without the addition of antioxidants or with the presence of antioxidants of different nature).

Table 1. Identification of the four hydroperoxides formed in the oxidation of methyl linoleate

Hydroperoxide	Abbreviation
Methyl 13-hydroperoxy-(Z)-9,(E)-11-octadecadienoate	13H (Z)-9 (E)-11
Methyl 13-hydroperoxy-(E)-9,(E)-11-octadecadienoate	13H (E)-9 (E)-11
Methyl 9-hydroperoxy-(E)-10,(Z)-12-octadecadienoate	9H (E)-10 (Z)-12
Methyl 9-hydroperoxy-(E)-10,(E)-12-octadecadienoate	9H (E)-10 (E)-12

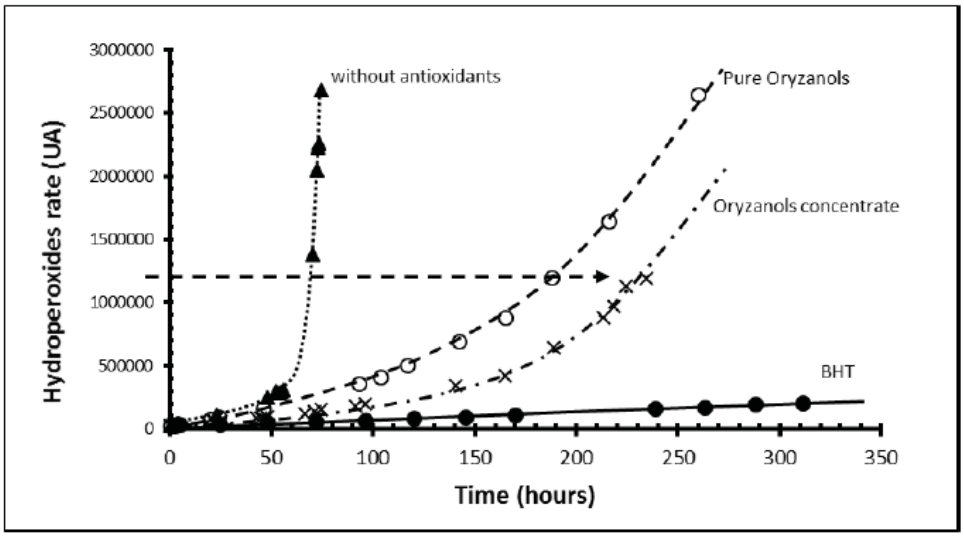

Figure 2. Total hydroperoxides rate in SFOME without antioxidants and with addition of different antioxidants during oxidation in oxygen flow at 50 °C: a) BHT, b) pure oryzanols and c) oryzanols concentrate, all in concentration 3x10-3 M (UA: arbitrary units)

Figure 2 shows the evolution of total hidroperoxides of methyl ester linoleic acid corresponding to the oxidation of SFOME at 50 °C without antioxidants and with the addition of different antioxidants (BHT, pure oryzanols or oryzanols concentrate).

The ordinates axis is showed in "arbitrary units" (UA), since the values were determined as the total area (areas of the four mentioned hydroperoxides) "normalized" with respect to methyl esters concentration (g/mL) in the sample analyzed. This normalization allowed to compare the results obtained from different analysis.

Analogous to the analysis of accelerated rancidity tests, from these graphs it was possible to determine an induction time, corresponding to the intersection point of the slopes between the initial stage and the final stage. The induction time value for SFOME without antioxidants was 64 minutes.

The SFOME with added BHT presented a slight increase in the hydroperoxide rate as a function of time, without it being possible to determine an induction period, as occurred with the sample without antioxidants. This indicates that the degree of advance of the oxidation was not sufficient to reach the acceleration phase in the formation of hydroperoxides. For the SFOME without antioxidants (Figure 2) a hydroperoxides rate of $2x10^6$ U.A. immediately after the induction period was obtained, while in the case of the SFOME additivated with BHT at a concentration $3x10^{-3}$ M (Figure 2) they never reached this rate.

The curves corresponding to the SFOME with pure oryzanols or with oryzanols concentrate (Figure 2), showed a higher increase in the hydroperoxides rate than the sample with BHT. However, even after 350 h of oxidation the sample with BHT showed no significant increase in the hydroperoxides rate. Therefore, the determination of an induction period was not possible.

Finally, to reach a hydroperoxides rate of $1.2x10^6$ U.A. took 60, 188 and 235 h for SFOME without antioxidants, pure oryzanols and oryzanols concentrate, respectively.

These results indicated that all the antioxidants protected the SFOME under the conditions of the analysis effectively and that the BHT was the one with the highest antioxidant power.

It was also observed that oryzanols concentrate protected SFOME more efficiently than pure oryzanols. This was surely because the oryzanols concentrate had small amounts of tocopherols and tocotrienols. The concentrate was analyzed and the presence of a small amount of tocopherols was confirmed.

3.2 Hydroperoxide Composition

The peaks corresponding to 13H (E)-9 (E)-11 and 9H (E)-10 (Z)-12 overlapped, making it difficult to quantify separately. This coincides with the reports made by Morales et al., 2010. For this reason, the total area corresponding to the sum of both peaks was considered.

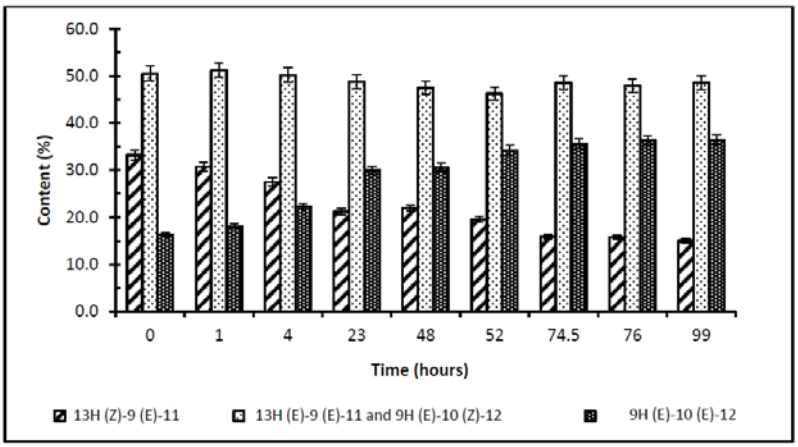

Figure 3. Content of each hydroperoxide during the oxidation of the SFOME without antioxidants

Figure 3 shows the percentage of each hydroperoxide (calculated as the percentage of the corresponding peak area with respect to the sum of areas of the four peaks) during the oxidation of SFOME without antioxidants as a function of the oxidation time at 50 °C.

The percentage of 13H (E)-9 (E)-11 and 9H (E)-10 (Z)-12 was maintained between 46 and 51 % throughout the period of oxidation studied. On the other hand, the percentage of 9H (E)-10 (E)-12 presented a continuous increase from 16 to 37 %, due to a gradual and continuous decrease of 13H (Z)-9 (E)-11 from 33 to 15 %. However, after 74.5 h the percentages did not change significantly.

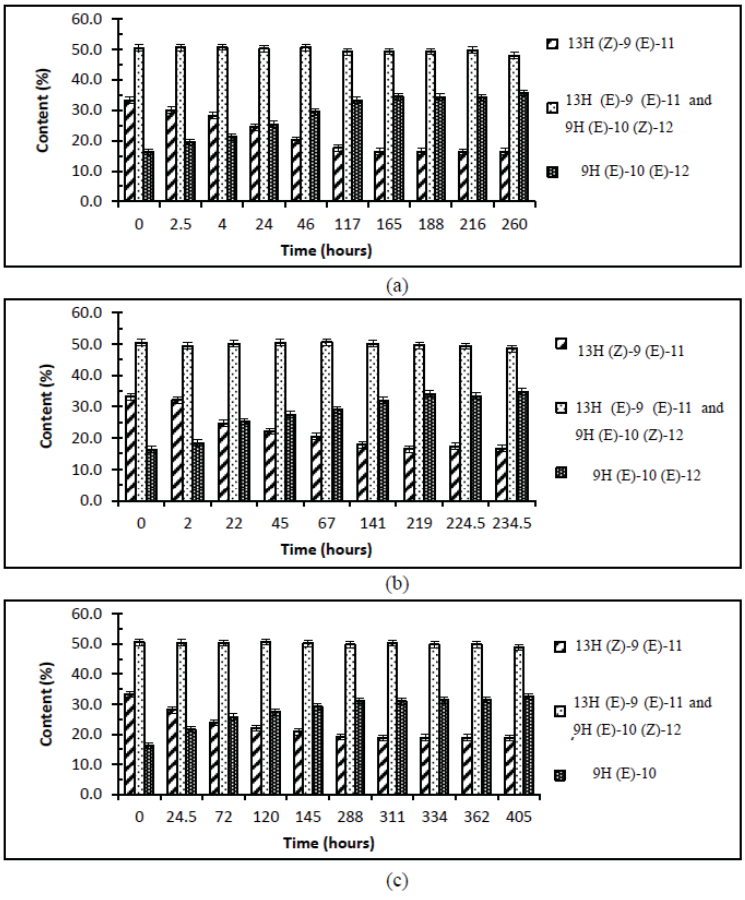

Figure 4. Content of each hydroperoxide during the oxidation of SFOME with addition of: a) pure oryzanols, b) oryzanols concentrate and c) BHT

Figure 4 shows the percentages for oxidation compounds of SFOME with the addition of different antioxidants. The behavior was similar to that of the SFOME without antioxidants: 9H (E)-10 (E)-12 increased until a maximum value and 13H (Z)-9 (E)-11 decreased until a minimum value, from which they remained constant. These values were achieved at 117, 141 and 288 hours for pure oryzanols, oryzanols concentrate and BHT, respectively.

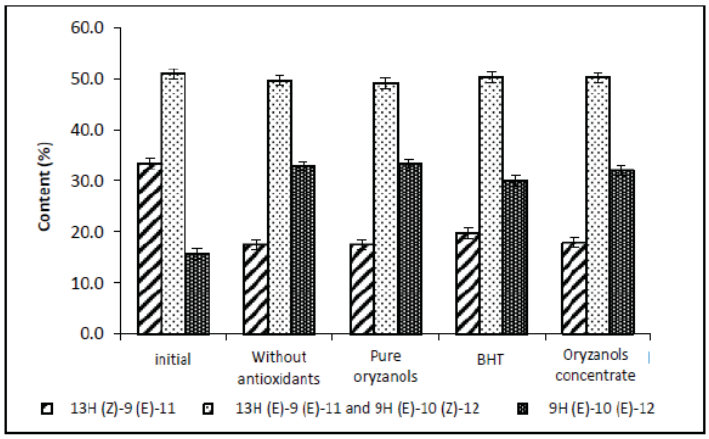

Figure 5. Content of the initial hydroperoxides and each hydroperoxide formed from SFOME for the same oxidation degree

Figure 5 compares the percentage contents of the different hydroperoxides in un-oxidized SFOME without antioxidants (initial) and oxidized SFOME (with and without antioxidants) for an oxidation time at which a similar total concentration of hydroperoxides was achieved (expressed as total area of hydroperoxides vs. concentration). In the initial sample, the percentage content of 13H (E)-9 (E)-11 and 9H (E)-10 (Z)-12 did not change after a certain time of oxidation, both in SFOME without antioxidants and with the addition of antioxidants. However, 13H (Z)-9 (E)-11 decreased by half with respect to initial SFOME, whereas 9H (E)-10 (E)-12 increased twice as much. This suggested that the oxidation mechanism favored the 9H 10-trans 12-trans formation with respect to 13H 9-cis 11-trans. On the other hand, it was observed that SFOME without antioxidants presented a similar variation in hydroperoxides percentage compared to SFOME with the addition of antioxidants.

4. Conclusions

Through this oxidation study at 50 °C it was possible to observe a sustained increase in the oxidation compounds that in some cases allowed to calculate an "apparent" induction period. This method useful for comparing the efficiency of antioxidants in the oxidation of those oils that had a certain content of linoleic acid, although it must be taken into account that it took a long time to achieve important advances in oxidation when some antioxidants were added to the methyl esters. It should also be noted that the study involved methyl esters rather than the oil itself.

The oryzanols concentrate presented a better protective effect on methyl esters than pure oryzanols. This showed that the other compounds present in the oryzanols concentrate did not affect the antioxidant power of the oryzanols in it. The increase in antioxidant power was probably due to the presence of tocopherols. However, oryzanols presented a much lower antioxidant power compared to BHT.

In addition, the hydroperoxides proportion obtained from methyl ester linoleic acid was similar in all cases and for this reason it was possible to conclude that the oxidation mechanism did not vary with respect to the different antioxidants studied.

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Challenges and Opportunities Associated with Whole Grains Use in Twin Cities Restaurants: A Food Systems Perspective

Keagan Ringling[1], Alexander Yangas[1], Melissa Riley[1], Renee Korczak[1] & Len Marquart[1]

[1]Department of Food Science and Nutrition, University of Minnesota, USA

Correspondence: Len Marquart, Department of Food Science and Nutrition, University of Minnesota, USA. E-mail: lmarquar@umn.edu

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Abstract

Increasing whole grain (WG) availability in restaurants allows consumers to make healthier choices with minimal effort while improving adherence to the Dietary Guidelines. To understand challenges associated with increasing WG availability in Twin Cities (Minneapolis & St. Paul, Minnesota, USA) restaurants, interviews and focus groups were conducted with 24 local, national, and international food system members. This report identifies food system barriers, including policy, business, and societal pressures, that limit availability of WG based foods in restaurants. Insight provided by this study allows consortium members from various sectors and disciplines to work on a precompetitive basis to include more WG in Twin Cities restaurants.

Keywords: whole grain, supply chain, systems nutrition, menu options

1. Introduction

Food environments influence diet and health. These environments are the human-built and social environments that include physical, social, economic, cultural, and political factors that impact accessibility, availability, and adequacy of food within a community or region (Rideout et al., 2015). For example, consumer food environments are characterized by the availability, variety, price and quality of foods as well as promotional signs and prominence of healthful versus non-healthful food options (Rideout et al., 2015). Restaurants provide one example of a consumer food environment that has a major influence on diet and health.

As consumers continue to rely on restaurants for food, their intake becomes dependent on available menu options. Restaurant meals have been shown to be generally less healthful as these meals provide more calories, total and saturated fat, sodium, and cholesterol per meal than a typical meal prepared at home (Scourboutakos & L'Abbe, 2012; Hearst et al., 2013). Conversely, meals away from home (AFH) can be low in several key nutrients, including fiber, calcium, and iron (Lendway & Hesse, 2014). Research indicates that AFH eating is associated with increased biomarkers of chronic disease (Kant et al., 2015). Indeed, as the reliance on AFH eating increases, it becomes clear that restaurants must play a role in offering and promoting healthier meal options.

Given the current consumer food environment, actions to improve the nutritional quality of menu options in restaurants remains prudent. Substituting WG's for refined grain products can increase the nutritive value of foods without drastically changing acceptability and organoleptic qualities of meals (Rosen et al., 2008). The most common grains in the American diet include wheat, oats, rice, maize, and rye. These grains in their whole form contain an abundance of nutrients, including vitamin E, magnesium, iron, fiber, several B vitamins, and other beneficial phytochemicals (Liu, 2007). Diets containing WG's are inversely related with obesity, insulin resistance, diabetes, and inflammation (Lutsey et al., 2007). Whole grain consumption is also associated with reduced risk of coronary artery disease, cardiovascular disease, and total mortality (Huang et al., 2015). For these reasons, the 2010 and 2015 Dietary Guidelines for Americans recommend that at least half of all grain servings should be WG (U.S. Department of Health and Human Services [USDHHS] and U.S. Department of Agriculture [USDA], 2010; 2015). More specifically, the Guidelines recommend that all adults and children over 9 years consume at least 3 servings of WG's per day. However, data from the United States Department of Agriculture (USDA) showed that Americans only consumed an average of 0.90 servings of WG's per day in 2013-2014 (Bowman et al., 2017) Results from the 2009-2010 National Health and Nutrition Examination Survey (NHANES) indicated that only 2.9% of children and adolescents (2-18 years old) and 7.7% of adults (over 18

years old) met the recommended intake of WG's (Reicks et al., 2014). Increasing the availability of WG based foods in restaurants could allow consumers to make healthier choices with minimal knowledge and effort while also improving their adherence to the Dietary Guidelines.

Currently, barriers exist within the food system and food environment that prevent the incorporation and increased availability of WG based foods in restaurants. These barriers include limited knowledge regarding WG foods, undesirable taste or texture qualities, increased preparation time, higher cost, and low availability in foodservice settings (Rosen et al., 2012)**.** Traditionally, the responsibility has fallen on consumers to manage their health by actively seeking nutritious foods when making dietary choices. However, several studies indicate that the current consumer food environment obstructs, rather than supports, healthy eating habits (Harvard School of Public Health, 2015). For this reason, our study focused on factors across the food supply that limit whole grain use in Twin Cities (Minneapolis and St. Paul) restaurants beyond consumer and restaurateur preferences. A systems perspective demonstrates that, while the ultimate decision of whether to consume WG's lies with the consumer, the circumstances which most greatly influence that decision are pre-determined long before the consumer reaches the restaurant. The findings of this study may elucidate ways to utilize the supply chain and restaurant settings to make healthier, WG menu options a more effortless and instinctive choice for consumers when eating out.

2. Methods

The Institutional Review Board (IRB) at the University of Minnesota approves this study. Individual interviews (n=24) were conducted during the summer of 2013 with a convenience sample of local, national, and international food system members that contribute to the Twin Cities restaurants. Individual interview participants included culinary (n=4), baking (n=2), milling (n=5), food supply chain (n=7), public health (n=2), and academic professionals (n=4). Participants were selected from throughout the WG food system so researchers could understand a broad a range of perspectives. Most interviews were 45 minutes to one hour in duration. Interviews were generally informal and allowed for deviation from the original interview questions. This method was chosen to understand broad themes related to different sectors and disciplines involved in the WG food system. Sessions were audiotaped and transcribed verbatim. Two investigators then independently coded transcripts to generate themes and ascribe them to the appropriate tiers of an Adapted Social-Ecological Model (ASEM) (Richard et al., 2011).

During interviews, participants were asked questions related to the following: (1) The participant's role in influencing WG consumption; (2) The influence of the grain supply chain on WG consumption in restaurants; (3) The influence of public health initiatives on WG consumption in restaurants; (4) The specific barriers preventing WG availability and consumption in restaurants; (5) Potential strategies for increasing WG availability and consumption in restaurants; and (6) Intra-sector and inter-sector collaboration between the participant and other professionals.

2.1 The Adapted Social-Ecological Model

The Adapted Social-Ecological Model (ASEM) is a five-tier modification of what is traditionally a four-tiered model (Richard et al., 2011). The Social-Ecological Model includes the following tiers: Individual, Interpersonal, Built Environment, and Policy/Society. In the ASEM, the "Built Environment" tier is subdivided into "Restaurants" and "Supply Chain." The ASEM more accurately represents the relationship between the two subcategories and the end consumer. Within the context of AFH eating, restaurants have direct influence on consumer choices, while the supply chain typically has an indirect influence through the restaurants (menu options). The ASEM demonstrates how consequences of the supply chain/restaurant relationship, which would be lost in a traditional model, have tremendous influence on consumer behavior (Richard et al., 2011). Moreover, the adoption of WG presents issues in restaurants, which would not apply upstream in the supply chain, and vice-versa. This model preserves the unique needs of each tier, while demonstrating their interconnectedness and cascading effects on the end consumer.

3. Results

Several themes were identified from participant responses. These themes have been categorized according to the applicable tiers of the ASEM. The themes and challenges in each tier of the ASEM will be addressed in ascending order, beginning at the individual level, and culminating with Policy/Societal influence. General themes related to participant beliefs about opportunities are also presented for each ASEM tier.

3.1 Individual

Interview evaluations revealed four major barriers to WG consumption in restaurants on the individual level.

These themes included: (1) health stigma, (2) sensory characteristics, (3) cost, and (4) availability, as a majority of participant responses focused on the individual level.

3.1.1 Health Stigma

Eleven respondents indicated the common perception of WG as a "health food" hinders consumption in restaurants. Although most participants acknowledged that some people seek WG when eating out, the majority is discouraged due to its reputation as "health food." "WG can easily be stigmatized as 'healthy' and healthy is death for consumer demand in many circles (Academic)." This mentality was reflected well by one quote from a public health professional who commented, "Some people go specifically to indulge themselves in restaurants, and don't want to be reminded that they're eating things that aren't necessarily the best for them…people want to indulge, and they see WG not as an indulgence, but as something that isn't as good." An academic expressed a similar sentiment, saying, "I think to a certain extent, sometimes people get saturated with the health message. And so they may have a demand, but they don't want to be told all the time that they have to be healthy."

3.1.2 Sensory Characteristics

Seven respondents indicated that unpleasant taste or textural characteristics in WG foods are a significant deterrent to their appeal with consumers. Whole grain products contain compounds that are not present in their refined counterparts. These compounds alter the taste of the final product, as well as its texture and color. Whole grain products have been described as "bitterer", "denser", "heavier" and "earthier" tasting than the refined version of the same product. Yet, there was little interest and/or motivation by respondents to want to overcome challenges and seek opportunities to develop and deliver WG foods that consumers desire and want to eat.

3.1.3 Cost

Six respondents indicated that the increased costs associated with WG foods prevent their consumption in restaurants. Whole grain products are often considered premium items due to the relatively low demand for these products and the difficulty of procuring them on a large scale. Until the demand for WG's reaches a critical mass, the supply will constantly be insufficient to meet the needs of those who require the products in greater volumes, such as restaurants. Whole grain products have a shorter shelf life than refined grain products, which limits the time it can spend in distribution centers or warehouses. Thus, most distributors only acquire the quantity necessary to meet current customer demand. This demand is often low because restaurants must pay a premium to acquire many WG products in the quantities necessary to serve their clientele. This cost is invariably passed on to the consumer who may or may not even be willing to try the dish. Restaurants will not sacrifice storage space, equipment, and profits for a product that may sit on a shelf until it expires, or worse, may alienate their customers. Rather than take this risk, a restaurateur may opt for the cheaper refined grain option that is more likely accepted by the majority.

3.1.4 Availability of Added Value Grain-Based Foods

Several respondents indicated that low availability of quality WG prevented widespread usage in restaurants. Frequently voiced opportunities included increasing the availability of popular or frequently consumed foods in restaurants that can be made with WGs that are healthier, cost-effective and desirable. These WG foods would be readily accessible to restaurant clientele and easy for chefs and food service personnel to purchase, prepare and serve in an efficient and economical manner. Children were also mentioned as a primary demographic targeted for greater exposure to WG foods.

3.2 Interpersonal

Few people communicated or initiated efforts related to WG's within the supply chain and food environment. Millers and bakers commented that a majority of their work revolves around communication related to refined grain flour and their respective baked products. Overall, participant responses about communication and collaboration were less frequently voiced relative to the use of WG's. From a chef's point of view, "The only collaboration I have about WG's is people want me to use their ingredients, so I can sell it to the customer." This may indicate a lack of support in restaurants to help chefs succeed using WG ingredients and WG foods that restaurant clientele will eat. On the other hand, it was voiced "People [Patrons] don't really have the venue to make their preferences known in restaurants." Participants suggested opportunities may exist to better understand the value and meaning of WG's relative to supply chain players, restaurant owners, managers and clientele. A deeper examination of the potential roles, functions and activities of these players might help gauge more appropriate use of WG foods in restaurants.

3.3 Restaurants

Participants reported that chefs and the general public are often intimidated by new experiences like preparing, serving, and eating WG. Other participants indicated restaurants have no immediate incentive to use WG, thus "Higher quality ingredients do not necessarily equal higher quality products." These ingredients can also be more expensive with no guaranteed reward. As a means to counter this lack of skill and hands-on experience most participants suggested chefs, cooks and restaurant staff need more training. Currently, there are few to no dollars spent teaching people how to work with WG. As a result, chefs, cooks, and restaurant staff lack foundational skills in purchasing, preparing, storing and serving these foods. Ultimately, the limited foundation through the food industry, consumers, chefs, and others inhibits whole grain adoption. Thus, a majority of participants suggested considerable benefit might result in training restaurant chefs, kitchen personnel, and serving staff in purchasing, storing, preparing and marketing popular foods made with WG.

3.3.1 Supply Chain

Study participants with primary roles within the supply chain stated, "…the supply chain is engineered to produce refined products in large quantities, unlike WG." As mentioned by a wheat breeder, "wheat is bred for optimally refined grain foods, while flour quality is based on refined grain product quality and yield, not for WG foods." From a miller's perspective, there are unique processing needs for WG. In some instances, WG milling requires capital investments in new equipment, such as additional storage silos and milling technology. It was stated strongly that academic interests and research priorities are seldom aligned with industry or consumer priorities. Thus, one food scientist indicated a tremendous need for cross-sector and cross-disciplinary training whereby faculty spend time in industry while industry scientists collaborate more intimately with universities. The intent was to increase understanding and appreciation for collaborative efforts around healthier grain-based foods. Systematically mapping barriers and opportunities in the development, delivery, and consumption of WG foods would allow for a more comprehensive look at the gaps in the supply chain, food environment, and community relative to WG use in restaurants. Additional insight from this effort could provide a means for prioritizing precompetitive and relevant research and education focus.

3.3.2 Policy / Societal:

From a policy and societal perspective white bread is ingrained in American culture. Most participants commented they engage in a greater frequency of refined grain activities compared to WG's across the sectors and disciplines within the food system. Overall, most activities were reactive (e.g. creating press releases against low carb, gluten free, etc.) rather than promoting the health attributes of grains through the inclusion of WG and fiber in frequently consumed foods.

WG's are not nearly as available through distributors in comparison to refined grains. For example, McDonald's tried to offer a whole wheat bun once, but stopped because customers preferred a white bun. From the perspective of general societal understanding of WG, one industry representative said, "whole grain definitions are too ambiguous and confusing. Consumers don't understand what WG means and neither do the chefs cooking in restaurants."

Based on participant input, opportunities may exist through a unified inter-sector collaboration by developing and implementing a comprehensive public health campaign to build positive messaging around WG foods. Focus would be encouraged through education about WG in harmony with greater availability of WG foods in the marketplace.

4. Discussion

This study examined current roles and perceptions of supply chain members, along with challenges and opportunities that each member experienced in bringing WG's from field to fork. Objectives were based on the 2010 Dietary Guidelines which asked for new food introductions and reformulation of prepared foods that deliver good taste and convenience while meeting dietary recommendations and cost constraints (USDHHS & USDA, 2010; 2015).

The food environment remains mostly devoid of viable WG choices for most Americans, as consumers fail to meet the Dietary Guidelines recommendations which state "at least half of all grain servings should be WG's" (USDHHS & USDA, 2010; 2015). Study participants consistently echoed an overarching theme, that there is little translation of agricultural food policy and dietary guidance into easy access to WG foods, which allows consumers to eat more. From a historical perspective, the grain industry is designed to proliferate the continued milling and sourcing of refined grain ingredients for baking and food manufacturing. In contrast, the WG infrastructure lacks the tools, approaches, methods, foundational knowledge, and standard business practices

necessary to carry healthful WG foods from field to consumer. The refined grain system is generally more efficient and cost effective to source, process, market and profit by selling refined grain foods that are practical, affordable and desirable for consumers. Based on this sample of participants, there appears to be a lack of shared value and meaning around WG food-related collaboration and communication strategies.

Barriers to using WG's occur throughout the supply chain from growing the grain (i.e. red wheat bred for refined grain use, lack of incentives to grow white wheat; pre-sprouting of white wheat places profits at risk), milling of grain into WG ingredients (different particle sizes result in varying functionalities, more enzymatic activity, lipids, and antioxidants which effect end-use and storage properties), baking and manufacturing into foods (lack of standard approaches and methods for WG product development; shorter shelf-life and storage), distributing and purchasing (lack of WG volume, distribution channels and cost constraints), preparing and serving in a restaurant setting (chef, production and serving staff are unfamiliar with WG ingredient storage, WG food preparation, and service) and consumer needs, wants and desires (lack of availability and accessibility of tasty added value WG foods). Until the necessary tools, approaches and WG infrastructure are established and leveraged in an intentional, synchronous and consistent manner throughout the grains supply chain and food environment, there is little motivation for players in the supply chain to support, encourage or use WG ingredients.

Restaurants are encouraged to gradually shift to incorporate more WG menu options so that AFH consumers can come closer to meeting WG recommendations. Perhaps a gradual increase in the availability of WG foods in schools and in restaurants is one approach to increasing consumption. For example, "sneaking in" better-for-you WG foods in school cafeterias and in restaurants without identifying it on the menu has the potential to be successful. Small recipe substitutions often go unnoticed, and some food and nutrition service directors already do this in hospital cafeterias. This practice is known as "stealth health", and it is a way to improve the nutritional quality of foods without customers even noticing (Food Service Director, 2012).

An example of increasing WG's in the food supply is to reformulate a popular food product such as pizza. Previous research has shown that modifications to pizza crust to include WG flour have been made without affecting acceptance among school children (Chan et al., 2008). This type of pizza is currently available for school meals but has limited availability in other food service and retail markets (Schwan's, 2013). Pizza can also be reformulated to be WG rich, lower sodium, and lower fat. Since pizza is made up of different food groups and is widely consumed and accepted; it has the potential to be a healthier staple that targets acceptable levels of WG's, vegetables, and dairy with lower fat and sodium. Pizza is just one way that foods can be modified to more closely meet Dietary Guidelines recommendations while satisfying consumer expectations for cost, taste and convenience (Jacques et al., 2013).

Efforts to gradually introduce more WG's into the marketplace provide new, cutting edge opportunities for collaboration throughout the grains community. Cross-disciplinary and cross-sector collaborations will help facilitate progress toward novel approaches to research, discovery, development, and delivery of WG based foods while collectively solving major gaps in the WG supply chain and food environment. Priorities and focus might be established on a precompetitive basis among the sectors and disciplines, related to the type of grain and food product, and place of service in the community, such as introducing WG pizza into restaurants (Tritt et al., 2015). To accomplish this system-wide goal, it requires synchronizing individual (players) and organizational contributions around a grand challenge resulting in collective impact. Adopting a culture of systems thinking while reconfiguring tools, approaches, and food design to emphasize shared value for profit and health can allow the grains community to overcome limitations in the current paradigm.

Scientists must continue to conduct research on WG's and health to solidify the knowledge base, while industry has the unique opportunity to reformulate products to make them healthier (McKeown et al., 2013). Examples of tools, techniques, and methods that might be developed include analytical methods for determining particle size, stability and rancidity in WG ingredients and foods. National and global institutions such as the International Association of Cereal Science and Technology (ICC) can play a major role in allowing these methods and techniques to be available to cereal scientists, product developers and bakers. Standard methods for using WG ingredients in grain-based foods such as bread, tortillas, pasta, cereals, and other baked products provide a precompetitive base for all bakers and manufacturers to develop and deliver better WG foods.

4.1 Study Limitations and Strengths

We acknowledge the limitations and strengths of this research. One of the limitations of this study was that a convenience sample was used to assess the barriers that exist within the food system and food environment to incorporate WG foods in restaurants. The sample size (n=24) was also small which prevents the generalization of

results on a large scale. Finally, one of the other major drawbacks to a convenience sample is the opportunity for bias to cloud the results. In terms of strengths, even though the sample was small, the individuals surveyed represented different sectors of the food system (i.e. academics, non-profit organizations, public health, government, and industry). Having perspective from these diverse disciplines is critical for developing practical solutions to deliver WG's and WG foods into the food supply.

Although the methods are not fully objective the intent of the paper was to show the 'big-picture' interrelationships amongst the roles, functions, and activities that take place throughout the whole grain food system.

5. Conclusion

Strategies to increase WG intake in the US diet should target action at each level within the food system and food environment, such as in restaurants. Restaurateurs are apprehensive in the purchase, preparation, and service of WG foods. This may be attributed to unfamiliarity, insufficient demand, shorter shelf life, and potential loss of profit. A WG/health stigma interferes with restaurant clientele selection and consumption of WG foods. Consumers are looking for WG foods that are affordable, and thus, readily available. Although incorporating WG's into restaurants may not solve all of these health problems, it is a step in the right direction, especially for young children who can establish healthy eating patterns at an early life stage. Based on this assessment, we will continue to work with a consortium of members from various sectors and disciplines to identify challenges and opportunities for the inclusion of more WG's in the Twin Cities (Minneapolis and St. Paul). The question remains: How do we create a supply chain and food environment that supports a culture of developing, delivering and increasing consumption of WG foods where interdependent stakeholders can perform their individual roles and effectively collaborate with each other? In part, added value for grain-based foods lies in the strategic use of WG flour to carefully achieve refined to WG flour ratios in most foods that are practical, healthier, affordable and desirable.

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Milk Adulteration: Detection of Bovine Milk in Caprine Dairy Products by Real Time PCR

Efstathia Tsakali[1, 2], Christina Agkastra[1], Christina Koliaki[1], Dimitrios Livanios[1], Georgios Boutris[1], Myrto Ioulia Christopoulou[1], Spiridon Koulouris[1], Stamatis Koussissis[1], Jan F. M. Van Impe[2] & Dimitra Houhoula[1]

[1]Department of Food Science and Technology, Faculty of Food Sciences, University of West Attica, Athens, Greece

[2]Department of Chemical Engineering, BioTeC+ - Chemical and Biochemical Process Technology and Control, KU Leuven, Gent, Belgium

Correspondence: Efstathia Tsakal, BioTeC+ - Chemical and Biochemical Process Technology and Control, KU Leuven, Gebroeders De Smetstraat 1, 9000 Gent, Belgium. Tel: 30-699-454-3717. E-mail: efi.tsakali@kuleuven.be

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Abstract

Milk adulteration is an international social problem. Consumption of adulterated milk may cause serious health problems and a great concern of the food industry has been raised. In this study, a method based on the polymerase chain reaction (PCR) principle was validated for detecting cow's milk in goat's dairy products. A total of 40 goat's dairy products commonly consumed in Greece, were tested. Various concentrations, from 0.01 to 90%, of cows' milk in goats' milk samples were prepared for DNA extraction and further PCR analysis. Selection of highly polymorphic regions within the cow and goat mitochondrial D-loops, showing low homology between the two species, allowed to choose specific primer pairs for detection of cow and goat DNA. After electrophoresis, cow DNA was characterised by the fragment of the size of 300 bp, goat DNA by the fragment of 444 bp. The detection limit of the PCR method was 0.01% while sensitivity and specificity of the method were both 100%. Goat dairy products samples were tested for the presence of cow DNA. Thirty six out of forty (90%) that were tested, were found to produce cow-specific PCR product in addition to goat PCR product while only two samples gave goat-specific product only. The results are disappointing in terms of the food labelling honesty but on the other hand PCR is again a quickly, easy and reliable method that could be used for extended adulteration screening.

Keywords: adulteration, milk, PCR

1. Introduction

Goat milk is a complete food, rich in proteins, vitamins, minerals and small fat molecules which makes it highly digestible compared to milk of other species (Golinelli et al, 2014). Due to its synthesis goat milk can be used as an alternative of cow and even human milk in cases of health problems such as allergy, atopy, and inflammatory diseases (Jirillo, Jirillo & Magrone, 2010). The use of goat milk and by products has beneficial effects on health maintenance, on physiological functions, and in the nutrition of children and elderly people, while it can be consumed without negative effects by people suffering from cow milk allergy (Mafra, Roxo, Ferreira & Oliveira, 2007; Ribeiro & Ribeiro, 2010; Silanikove, Leitner, Merin & Prosser, 2010; Di Pinto et al., 2017). In cases of allergies in cow milk, goat milk has been reported to resolve 30-40% of the cases (Haenlein, 2004). The recent years there is an increased demand of goat milk and goat milk-based products and their production has considerable economic importance mostly resulting from the widespread acceptance of the traditional goat cheeses (Zachar et al., 2011; Di Pinto et al., 2017). On the other hand, goat's milk small production -mostly because of natural small production per animal- and its seasonality lead to higher prices of the goat dairy products when compared to bovine ones (Golinelli et al., 2014).

Adulteration is a constantly current issue of the food industry. A food is considered adulterated if its quality is lowered or affected by the addition of substances which are injurious to health or by the removal of substances which are nutritious. Food adulteration includes not only the intentional addition or substitution or abstraction of substances which adversely affect nature, substances and quality of foods, but also their incidental contamination

during the period of growth, harvesting, storage, processing, transport and distribution. The adulteration of foods with material of other species of greater availability and/ or lower cost is a common practice (Drummond et al., 2013). In milk and dairy industry, the addition of bovine milk in dairy products of other species is a typical example (Herman, 2001). According to Commission Regulation (EC) 273/2008, a sample of milk is considered adulterated if the cows' milk casein content of the analysed sample is equal to or higher than the content of the reference sample containing 1 % cows' milk.

The most obvious problem that adulteration of milk can cause is fraud with economic aspects such as unfair competition but also include consumers' protection issues due to mislabelling or ethical, religious and cultural objections. Another important issue is the protection of the properties and the reputation of traditional P.D.O. (protected designation of origin) & PGI (protected geographical indication) cheeses as described by Commission Regulation (EC) 1151/2012 and expanded internationally via bilateral agreements between the EU and non-EU countries (Agrimonti, Pirondini, Marmiroli & Marmiroli, 2015). Whether fraudulent or unintentional (e.g. contamination during production or logistics), mislabelled products give rise to economic loss and possible dangers to public health because milk proteins from any animals (most commonly bovine) are potential allergens (van van Hengel, 2007; Di Domenico, Di Giouseppe, Wicochea-Rodriguez & Camma, 2016).

The detection of other species' milk in a sample can be complex due to genetic and non-genetic polymorphism (Recio, Perez-Rodriguez, Ramos & Amigo, 1997; Azad and Ahmed, 2016). Commission Regulation 273/2008 set isoelectric focusing of γ- caseins after plasminolysis (IEF) as the reference method for the detection of cows' milk and caseinate in cheeses from ewes 'milk, goats' milk or buffalos' milk or mixtures of ewes', goats' and buffalos' milk. This is a qualitative method which although is sensitive and accurate for the detection of cow milk in mixes it has several disadvantages which include that it is not a high-throughput method, it is not quantitative, the analysis is time consuming, it cannot discriminate goat-sheep mixtures, interpretation of the IEF profile can be equivocal and is not applicable to products made of soy milk because some weak interfering bands have been observed (Addeo et al., 1990; Mayer, Heidler & Rockenbauer, 1997; López-Calleja et al., 2007; Di Domenico et al., 2016). In parallel, several methods based on different techniques have been introduced in order to detect cow milk in goat milk of dairy products such as electrophoresis (Mayer, Burger & Kaar, 2012; Molina, Martin-Alvarez & Ramos, 1999), immunochemistry (Hurley, Ireland, Coleman & Williams, 2004; López-Calleja et al., 2007), chromatography (De Noni, Tirelli & Masotti, 1996; Ferreira & Caçote, 2003; Mayer, 2005) and mass spectrometry (Cozzolino, Passalacqua, Salemi & Garozzo, 2002). These methods, which are very specific, frequently lack in sensitivity and not always are suitable for heat treated material (Agrimonti et al., 2015). In contrast, DNA based methods are more practical, sensitive and robust (Cuollo et al., 2010; Guarino et al., 2010; Drummond et al., 2013) thus could be a valid alternative as DNA is extremely persistent during food processing and can retain sequence-specific information retrievable after an amplification reaction (PCR) (Agrimonti et al., 2015). Molecular methods for identification of animal species contribution to dairy products based upon PCR technology have been developed (Dalvit, De Marchi & Cassandro, 2007; Mafra et al., 2007; Agrimonti et al., 2015). Only a few validated real-time PCR methods have been published (Lopparelli, Cardazzo, Balzan, Giaccone & Novelli, 2007; Rentsch et al., 2013) and Di Domenico et al., 2016 demonstrated a validation report of a real-time PCR supported by the analysis of commercial samples confirmed by IEF, the official European Union reference method.

In this study, a method based on the polymerase chain reaction (PCR) principle was validated for detecting cow's milk in goat's dairy products.

2. Material and Methods

2.1 Selection and Preparation of Control Samples

Authentic milk samples from cow *(Bos taurus)* and goat *(Capra hircus)* were acquired directly from the animals locally and were kept at -18^{o}C until the analysis.

Different dilutions (mixtures) of cow milk in goat milk were prepared (0.01%, 1%, 2%, 5%, 10%, 20% and 50%). Also, pure goat milk was used as blank and cow milk as positive control.

2.2 Selection and Preparation of Food Samples

During the study period (September 2018 to March 2019) a total of 40 milk and dairy products were collected from local super markets around Athens (Greece). The products included 15 goat milk products, 15 goat cheeses and 10 yogurts from goat milk from 4 different brands.

2.3 DNA Isolation

1,5 ml of the different milk, dairy products and the control samples were centrifugated (10 minutes at 12000 x g)

to obtain a pellet. DNA extraction from these pellets was performed using the NucleoSpin Food® kit (Macherey-Nagel, GmbH & Co. KG, Germany), according to the manufacturer's instructions with a modification of adding an overnight incubation with the Lysis Buffer and the Proteinase K at 65°C instead of a 30 min incubation. The extracted DNA was quantified spectrophotometrically at 260 nm. DNA samples from sheep were used for specificity confirmation (designated specificity negative controls).

2.4 PCR Amplification

Selection of highly polymorphic regions within the cow and goat mitochondrial D-loops, showing low homology between the 2 species, allowed the choice of specific primer pairs for detection of cow and goat DNA. After electrophoresis, cow DNA was characterised by the fragment of the size of 300 bp and goat DNA by the fragment of 444bp. 50μ final volume solutions using MeltDoctor™ HRM Master Mix (Thermo Fisher Scientific™, USA) and carried out in a Veriti® 96 Well Therman Cycler (Applied Biosystems®): Initial denaturation: 94°C, 1 min; 40 cycles with the following step-cycle profile: denaturation 94°C, 30 s; annealing 60°C, 30 s; extension 72°C, 30 s; Final extension 72°C, 5 min. PCR products were separated in 2% agarose gel, stained with ethidium bromide (0.5 μg/ml) and documented under UV illumination using MiniBIS Pro device (DNR Bio-Imaging Systems Ltd., Israel).

3. Results and Discussion

The PCR assay was optimized for discriminating cow milk in goat milk and dairy products to detect adulteration with cow milk. The PCR tests used were very sensitive, specific, and reproducible. PCR tests showed a minimum detection limit of 0,01% indicating that the analytical assay unequivocally detected cow milk, even when it was present in small amounts. The sensitivity and the specificity of the method was 100%. The detection limit is very similar to that described by Bottero et al. (2003) and Feligini et al. (2005). Other authors detected amounts of cow milk as low as 1% (Maškova and Paulikova, 2006) and 0.1% (Lopez- Calleja et al., 2005; Mafra et al., 2007). To evaluate the repeatability and reproducibility of the method, 5 samples of pure goat milk were selected randomly and were contaminated with 1% cow milk and amplified in triplicate, while the procedure was repeated 3 times. The results of the PCR assay showed that the data had the same positive results.

Forty (40) samples from commercial goat milk and dairy products were analyzed by the PCR assay. In all of them, the PCR assays amplified the 300-bp fragment expected for cow derived-material, in addition to the 444-bp amplicon from goat. The former fragment indicates the addition of cow milk, considered an adulterant component, in thirtysix (36) commercial samples. This means that 90% of the products were adulterated with cow milk. In specific, all the 15 goat milk products and the 10 yogurts 100% were adulterated with cow milk while 11 out the 15 goat cheeses (73%) were adulterated with cow milk. The detected as adulterated samples (36) were then compared to the results of the electrophoresis of the control samples and the level of cow milk adulteration was found between 10-50%, with most of them (78%, 28 samples) in the range of 10 to 20% and 8 samples (22%) in the range of 20-50%. Finally, the samples that were evaluated as negative (4 samples) were also compared with the results of the electrophoresis and 2 of them could be characterized as contaminated with traces of cow milk.

Fraudulent addition of cow milk needs to be more than 10% to be economically significant. It has been reported that to test consumer perception of cheese composition, formulations may be prepared using mixtures with more than 10% (v/v) cow milk, although in a preliminary sensory test the consumers did not perceive adulteration levels less than 25% (v/v). Quality control inspections are a substantial step to guarantee unadulterated milk for consumption. Milk adulteration detection can be a difficult task as indicators of adulteration can change according to several factors (biological, climatic, agronomic etc), especially after processing, which can considerably alter milk's composition. The development of easy and cost-effective techniques for detection of milk adulteration can be a challenge, especially since these techniques must have a high degree of repeatability (Poonja *et al.,* 2016; Das *et al*., 2016).

The proposed technique is a useful screening test to detect the presence of cow milk in goat milk, even at a trace's levels. These results are consequent with those that have been obtained by the PCR procedure, by which it was also possible to detect traces amount of cow milk in goat milk and dairy products.

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Evaluation of Effects of Increasing Molar Substitution of Hydroxypropylene on Physicochemical, Functional and Morphological Properties of Starch from Water Yam (*Dioscorea Alata*)

Gibson Lucky Arueya[1] & Adenike Abiola Ojesanmi[1]

[1]Department of Food Technology, University of Ibadan, Nigeria

Correspondence: Gibson Lucky Arueya, Department of Food Technology, University of Ibadan, Nigeria. Tel: 234-805-954-6297. E-mail: arueyagibson@yahoo.com

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Abstract

Amidst rising demand for modified starch, hydroxypropylated derivatives from water yam, an underutilized tropical botanical source remains unexplored. The objective of this work therefore is to extract starch from water yam, modify same by hydroxypropylation and determine their physicochemical, functional and morphological characteristics.

Extraction of starch was carried out by blending peeled water yam previously soaked in 0.2%(w/v) $NaHSO_3$ solution and filtering the resulting slurry. The filterate was next suspended in 0.2% NaOH solution, allowed to sediment and the supernatant oven-dried (40^0C) for 24 hrs. The dried product was subjected to hydroxypropylation (HP) (propylene oxide 4-12%/100g starch) and extent of molar substitution (MS) determined. The native and Hydroxypropylated starch (HPS) were evaluated for physicochemical, functional and morphological characteristics following standard methods. Pasting properties were analysed using Rapid Visco Analyzer (RVA) and elucidation of inherent functional groups was carried out by analyzing the FTIR Spectrum.

Starch yield of 84.2% (dry wt. basis) and molar substitution (0.0024-0.05) of HPS were established. Bulk density increased (0.4988-0.6005g/cm^3) with MS. There was significant (p=0.05) increase in the degree of Whiteness (W) (42.4-63.6%). Although granule size reduction was evident (33.88-33.43µm), hydroxypropylation did not affect their morphology. There was decline in concentrations of crude protein (0.18-0.01%), ash (1.35-0.34%) and amylose (44.19-37.48%) as MS rose. In contrast, there was significant (p=0.05) increase in water (1.76-2.66g/g) and oil absorption capacities (0.72-1.42g/g), swelling power (1.54-4.19g/g) and solubility (3.17-5.84g/g) at 50^0C. Freeze thaw cycles showed marked reduction in syneresis (10.3-1.09%) as MS increased. Peak Viscosity, pasting temperature and peak time of the HPS ranges were 297.83-583.6, 81.9-86.4^{0}C, and 4.5-7.0 mins respectively. FTIR band spectra indicated the presence of hydroxypropyl substituent groups in the modified starch.

Hydroxypropylated starch (from water yam) at different molar substitution has been characterised and its properties established with strong potentials for wider applications in food systems.

Keywords: water yam starch, hydroxypropylation, propylene oxide, molar substitution

1. Introduction

Traditional sources of starch are no longer sustainable because the current industrial demand for starch is being met only by limited number of crops essentially corn, potato, wheat and tapioca (Ellis *et al*., 1998). The world starch market including their modified variants is thus dominated by starches from these sources. With increasing industrial demand for starches, there is need to explore new and alternative sources of starch. Tropical root and tuber crops such as water yam (*Dioscorea alata*) could offer this opportunity as they are rich in starch (Hoover, 2001, Wickramasinghe, 2009).

Water yam is grown widely in tropical and subtropical regions of the world. They are plants yielding tubers with a starch concentration of between 70 and 80% of dry matter. *D. alata* in particular is known for its high nutritional content (Zhang & Oates, 1999), variable shapes, the majority of which are cylindrical. The flesh of the tuber varies in color shades from white to purple (FAO, 1994).Also, the texture of its flesh is not as firm as

that of white yam and so finds far less preference in the preparation of 'pounded yam'- a popular delicacy of West Africans. Interestingly, this yam species exhibits high yield(calorific energy and protein three times more superior), multiplication ratio and better tuber storability when compared to the preferred indigenous 'English white guinea yam'(*Dioscorea* rotundata). Water yam (*Dioscorea alata*) has an added advantage for sustainable cultivation and comes in handy, especially when yam production seems to be on the decline owing to high production costs, low yields and post-harvest losses. In spite of the aforementioned beneficial characteristics, water yam seems underutilized when compared to the other varieties of yam ostensibly because it is perceived as food for the poor, playing a minor role in international trade.

In a bid to enhance the competitiveness of these potential starch sources, considerable works have been conducted on their physicochemical, functional and pasting properties as well as on their modified variants. For instance, Water yam (*Dioscorea alata*) starch was modified through alkaline and alcoholic-alkaline treatments. The results obtained showed that water absorption capacity (swelling) and solubility increased, freeze-thaw stability improved with each treatment, while a lower tendency to setback became evident (Dalahaye, 2009). Annealed purple water yam showed that annealing decreased peak viscosity, final viscosity but improved peak time (Mojiono *et al.,* 2012). Falade & Ayetigbo, (2015) also reported the effects of annealing, acid hydrolysis and citric acid modifications on physical and functional properties of starches from four yams (Dioscorea alata inclusive).The result further indicated that while starch granule sizes reduced significantly with the modifications, the annealed starch showed a better foam stability than citric modified and acid hydrolysed yam starches.

There are quite a number of previous works on hydroxypropylation of other starch sources. Hydroxypropyl derivative of pigeon pea revealed that swelling and solubility increased with molar substitution, syneresis reduced after hydroxypropylation, while increased starch paste clarity and peak viscosity were evident along with a drop in pasting temperature (Lawal, 2008).Another report on hydroxypropylation of white yam by Lawal, (2008) confirmed that hydroxypropylation enhanced the free swelling capacity (FSC), solubility, but reduced setback and retrogradation.

Wattanachant *et al*., (2001) also reported that through hydroxypropylation involving a significant increase in molar substitution, paste clarity decreased, swelling power and solubility rose compared to that of the native starch. Majzoobi *et al*., (2014) compared the characteristics of hydroxypropylated wheat starch with that of oat and found out that hydroxypropylation did not change the morphological features of oat starch while it had some effect on granular structure of wheat. Senanayake *et al.,* (2014) reported that hydroxypropylated sweet potato starch gels did not show syneresis for two weeks. Hydroxypropyl derivatives from native lima and jack bean starches showed increased viscosity progressively with molar substitution (MS), as well as improved swelling power,solubility and pasting parameters (Oladebeye *et al.*,2013). Hydroxypropylated starch of yellow millet showed the presence of slight fragmentation and a distinct groove in their central core region and increase in breakdown and setback viscosities (Olayinka *et al.*, 2015). Notably, no study on effect of hydroxypropylation modification on physico-chemical properties of native water yam (Dioscorea alata,) starch at various degree of substitution have been carried out, hence the need for this work.

It is instructive to note that many industries in developing countries like Nigeria have relied on imported maize and wheat starches for use in various applications. This importation has led to loss of large amounts of foreign currency and employment opportunities for Nigerians. Though demand for starch is ever increasing, food industries in third world countries like Nigeria often contend with challenges of increased costs, supply capacity, availability and late deliveries. There is a need therefore, to explore indigenous crops locally grown by subsistence farmers, as alternative sources of starch as well as its modified variant. Previous attempts have so far focused on cassava. Water yam just like cassava, has great potential for this purpose, yet their utilization in diversified forms has been very limited due to lack of information on its functional and morphological properties. A detailed knowledge of the characteristics of the starch would facilitate its utilization in industries; enable tailoring of the properties by chemical modification to specific applications and bringing economic benefit in the long run.

2. Materials and Methods

2.1 Isolation and Purification of Starch

Fresh tubers of water yam (Dioscorea alata, *Dagidagi*) were obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo state, Nigeria. All reagents used in this work were of analytical grade. Starch was extracted by the method of Walter *et al.,* (2000) with slight modifications. The Freshly harvested yam tubers were peeled and homogenized using a Warring commercial blender. The mixture was filtered through double layered muslin cloth, and dispersed in 0.2% NaOH solution, the starch allowed to sediment and the supernatant

decanted. The washing with distilled water continued until a clear supernatant was obtained. Starch cakes were spread thinly on the tray and oven dried at 40°C for 24hrs.

2.2 Hydroxypropylation

The method of Kaur *et al.,* (2004) was used with modifications. Water yam starch (100 g) was weighed using 500ml screw cap jars and distilled water (200 ml) was added followed by the addition of 20 g of Na_2SO_4. The slurry was mixed for 30 min and the pH adjusted to 10.5 with 1M NaOH. Propylene oxide (4, 6, 8, 10 and 12 ml) was added and the suspension was thoroughly mixed. The reaction was maintained at 40^0C in a shaking incubator for 24 h. The starch suspensions were neutralized with dilute HCl (0.1 M) and the pH was adjusted to 5.0. The slurry was centrifuged for 15 min at 10,000 rpm. The resulting starch cakes were washed with distilled water several times and oven dried at 40^0C.

2.3 Determination of Molar Substitution of Hydroxypropylated Starch

Additives was used for the determination of the hydroxypropyl groups. Starch samples (100 mg) were weighed into a 100-ml volumetric flask and sulphuric acid (25ml; 0.5M) added. The mixture was digested in a boiling water bath until a clear solution was obtained. The resulting clear solution was cooled and made up to 100ml with distilled water. An aliquot of the solution (1ml) were pipetted into 25-ml graduated test tubes, immersed in cold water and 8ml of concentrated sulphuric acid was added drop wise to the tube. After thorough shaking, the tubes were placed in boiling water bath for 20 min. The tubes were next allowed to air-cool and thereafter placed in ice bath until the solution was chilled. Ninhydrin reagent (3% Ninhydrin in 5% $Na_2S_2O_5$, 0.6ml) was added and the tubes well shaken before placing in 25 °C water bath for 1 h. The solutions were next made up to 25ml with concentrated sulphuric acid and thoroughly mixed. It was then transferred to 1-cm cells, and after 10 min, the absorbance was measured at 590nm with the starch blank used as reference. A calibration curve was prepared with an aliquot (1 ml) of standard aqueous solutions, containing 10, 20, 30, 40 and 50 mg of propylene glycol per ml. The propylene glycol concentration in the starch was calculated from the standard curve, converted to equivalent hydroxypropyl groups from each molar solution using the following equation:

$$\mathrm{MS} = \frac{162\mathrm{W}}{100 - (\mathrm{M} - 1)\,\mathrm{W}}$$

where MS, molar substitution; W, equivalent hydroxypropyl group in 100 g of starch and M, molecular weight of C_3H_6O.

2.4 Physicochemical Properties

2.4.1 Loose and Tapped Bulk Densities

Two grams of the powdered sample was placed in a 10 millilitres measuring cylinder and the volume (V_0) occupied by the sample without tapping was noted. After 100 taps on the table, the filled volume (V_{100}) was read. The bulk loose and tapped densities were calculated as the ratio of weight to volume (V_0 and V_{100}, respectively). (Emeje *et al*., 2012).

2.4.2 Powder Flowability of the Starches

The flowability of the starches was determined using the Carr index and Hausner ratio (Falade & Ayetigbo, 2015). Loose and packed bulk densities provided information on the flowability of powders. These values are used to calculate the Carr index which is a measure of the flowability and compressibility of the starches. According to Falade & Ayetigbo (2015), values (%) of 5 to 10, 12 to 16, 18 to 21, and 23 to 28 represent excellent, good, fair and poor flow properties, respectively.

The Carr index and Hausner ratio were calculated as follows:

Carr index (%) = 100 (packed bulk density-loose bulk density)/loose bulk density

Hausner ratio = packed bulk density ÷ loose bulk density

2.4.3 Colour Evaluation of Native and Hydroxypropylated Starches

The Commission Internationale de l'Eclairage (CIE) L*, a* and b* parameters was determined using a colourimeter (Chromameter CR 410 Konica Minolta, Sensing inc., Japan) having optical sensor lens at 2^o observer (Gonnet, 1999). The instrument was calibrated with a standard white tile (L* = 93.75, a* = -5.36, b* = 8.50). Multiple measurements of L*, a* and b* parameters was determined using the colourimeter on the samples. From the data obtained, deltachroma (ΔC), colour intensity (ΔE), hue angle (H*), and degree of whiteness (%W) was calculated according to the equations 1, 2, 3 and 4, respectively shown below (Gonnet, 1999).

$$\Delta C = (\Delta a^{*2} + \Delta b^{*2})^{0.5} \quad (1)$$

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5} \quad (2)$$

$$H^* = \tan^{-1} (b^*/a^*) \quad (3)$$

$$W\ (\%) = 100 - [(100 - L^*)^2 + ((a^*)^2 + (b^*)^2)]^{0.5}. \quad (4)$$

Colour measurement of both native and hydroxypropylated starches were taken using the standard white tile as reference (L* = 93.75, a* = -5.36, b* = 8.50)

2.4.4 Microscopic Examination of the Native and Hydroxypropylated Derivatives of Water Yam Starch

The size and shape of starch granules were obtained from extracted yam starch samples. A small amount of starch powder was scooped with a spatula onto a clean micro-slide (75 x 25 mm). A drop of distilled water was added and distributed thinly on the slide and covered with a slip. Starch granules were observed under a light microscope (LEICA CME, Leica Microsystems) and sizes were determined by measuring the granule diameter with an ocular micrometer fixed to the lens of the microscope. The actual sizes of the granules were calculated by multiplying their mean diameters by a factor of 2.47 μm (i. e. the factor for objective magnification that was used) which was calculated earlier using the parallax obtained between a stage micrometer (Graticules Ltd, Tonbridge, England) and the calibrations of the eye piece.. A minimum of 100 granules were selected randomly and measured for each sample. Observation was done under x 400 magnification. (AOAC, 2000)

2.5 Chemical Properties

2.5.1 pH, Moisture, Ash, Protein, Crude fibre, Crude fat and Total Carbohydrate (Nitrogen free Extract, NFE) were Determined According To Standard Methods(AOAC,2000)

2.5.2 Amylose and Amylopectin

Amylose content was determined in duplicates using the method of Hoover & Ratnayake (2002). Approximately 0.1 g (100 mg) of the starch sample was weighed into a 100 ml volumetric flask and 1 ml of 99.7-100 % (v/v) ethanol and 9 ml of 1M NaOH was carefully added and the mouth of the flask was covered with foil and the content mixed well. The sample was heated for 10 minutes in a boiling water bath to gelatinize the starch. The sample was then removed from the water bath and allowed to cool to ambient temperature. It was then filled to mark with distilled water and shaken thoroughly. About 5 ml of the mixture was then pipetted into another 100 ml volumetric flask. Acetic acid (1N, 1ml) and 2 ml of iodine solution were added, topped to mark with distilled water and shaken thoroughly, while ensuring that the flask is wrapped in aluminium foil to prevent photo-degradation of the iodine–starch complex. Absorbance (A) was then read using spectrophotometer (Spec UNICO® 1100 RS, United products and Instruments Inc.) at 620nm wavelength. A blank was prepared by following the same procedure, except that no starch sample was added in the volumetric flasks, and used to standardize the spectrophotometer at 620 nm. The amylose content was calculated as:

Amylose content (%) = 3.06 * A * 20

Amylopectin (%) = 100% - Amylose content (%)

Where A is the absorbance reading at 620nm, 3.06 is the predetermined gradient of standard amylose calibration curve, and 20 is the dilution factor.

2.6 Functional Properties of Starch

2.6.1 Least Gelation Concentration

The method of Onwuka (2005) with modification was adopted in the determination of gelation capacity; Starch dispersion (2–18%) were prepared in test tubes with distilled water (5 ml). The starch suspensions were thoroughly mixed for 5 min and heated for 30 min at 80° C in a water bath, followed by rapid cooling under running cold tap (25 °C) water for 2 h.

Least gelation concentration was determined as lowest concentration when the sample from the inverted test tube did not fall down.

2.6.2 Emulsion Capacity

Two grams of the native starch was dispersed in 25 millilitres of distilled water using a vortex mixer for 30 s. After complete dispersion, 25 ml of vegetable oil (ground nut oil) was added gradually and the mixing continued for another 30 s. The suspension was centrifuged at 1600 rpm for 5 min. The volume of oil separated from the sample was read directly from the tube. Emulsion capacity was evaluated as the amount of oil emulsified and held per gram of sample (Ihegwuagu *et al,* 2009).

2.6.3 Determination of Water Absorption Capacity (WAC)

The method of Abbey & Ibeh (1998) was adopted for determination of water absorption capacity. Starch sample (1 g) of native and each of the hydroxypropylated water yam starch treatments were weighed separately and distilled water was mixed with it to make up to 10 millilitres of dispersion. It was then centrifuged at 3500 rpm for 15 min. The supernatant was discarded and the tube with its contents reweighed as gram water absorbed per gram of sample. The gain in mass was the water absorption capacity of the flour sample.

2.6.4 Oil Absorption Capacity (OAC)

Two grams of sample was mixed with 20 millilitres of oil in a blender at high speed for 30 s. Samples were next allowed to stand at 30°C for 30 min and thereafter centrifuged at 1000 rpm for 30 min. The volume of supernatant in the graduated cylinder was noted. Density of water was taken to be 1 g/ml and that of oil determined to be 0.93 g/ml. The means of triplicate determinations were noted (Abbey & Ibeh, 1998).

2.6.5 Effect of Varying Temperatures on Swelling Power and Solubility

The method of Collado & Corke (1997) was used in this study with slight modification.Sample (0.5g) was mixed with 15 mL distilled water in centrifugal tubes. The suspension was heated at 50^{o}C, 60^{o}C, 70^{o}C and 80^{o}C and 90C° for 30 minutes. The gelatinized sample was cooled to room temperature and centrifuged (HIMAC CR 21GII, Hitachi Koki co. ltd., Japan) at 3500 rpm for 15 minutes. The supernatant was placed in a Petri dish and dried at 100^{o}C to constant weight. Solubility was expressed as the weight of dried solid based on weight of dry sample. The swelling power was represented as the ratio in weight of the wet sediment to the weight of initial dry sample. The measurement was in triplicate.

2.6.6 Starch Gel Clarity

Starch gel clarity was determined by the method of Bello-Pe´rez *et al.* (1999) by measuring transmittance of a 1% (w/w) starch paste at 650 nm, using a spectrophotometer (Spec UNICO® 1100 RS, United products and Instruments Inc.). Starch suspension in tubes was placed in a water bath at 100^{o}C for 30 minutes, vortexed every 5 minutes and left to cool to room temperature. Percentage transmittance (%T) was determined in these suspensions.

2.6.7 Pasting Properties of the Starches

Pasting properties including pasting temperature, peak viscosity, peak time, trough viscosity, breakdown viscosity, final viscosity and setback viscosity were determined. A mass of sample was weighed into a canister, and a corrected amount of distilled water added. A paddle was placed inside the canister and then centrally into the paddle coupling of the Rapid Visco Analyzer (RVA-super 4, Newport Scientific® Pty Ltd., Warriewood, NSW 2102, Australia) and a RVA Standard Profile procedure employed. The procedure is a 13 minute cycle with temperature regime as follows: An idle temperature of 50^{o}C for 1 minute, heating from 50^{o}C to 95^{o}C in 3 minutes 45s, holding at 95^{o}C for 2 minutes 30s, cooling to 50^{o}C for 3 minutes 45s, and holding at 50^{o}C for 2 minutes. The pasting parameters were calculated using the *Thermocline*® for Windows® RVA software. In order to further characterize the pasting behaviours, the viscosity breakdown ratio (BDR) was defined as the ratio of trough to peak viscosity, and used to classify the cultivars as highly (<0.5), moderately (0.5–0.8) or slightly (0.8–1.0) shear-thinning, or shear-thickening (≥1.0). Analysis was carried out in duplicate.

2.6.8 Freeze Thaw Stability

The freeze thaw stability of the starch samples were conducted according to the method described by (Kaur *et al*., 2004). Aqueous suspension of starch (5% w/w) was heated at 95°C under constant agitation for 1 h. The paste was weighed (20 g) into previously weighed centrifuge tubes and capped tightly. It was centrifuged (1,000 rpm, 10 min) to remove free water. The supernatant was decanted and tubes containing starch paste were subjected to eight freeze thaw cycles followed by centrifugation (4,000 rpm, 30 min). Alternate freezing and thawing was performed by freezing for 24 h at -18°C and thawing for 4h at 30°C. The percent water separated after each freeze thaw cycles was measured in terms of syneresis.

$$\text{Syneresis\%} = \frac{W_{H_2O} \text{ X } 100}{Ws}$$

2.6.9 FT-IR Spectroscopy

The IR spectra of starches were run as KBr pellets on FTIR System (Spectrum Two PerkinElmer, England) in the frequency range 350–40000 cm^{-1} or more according to method of Gbenga *et al.,*(2014) with slight modifications. Two milligrams of the samples were grounded and mixed uniformly with pure KBr powder. This mixture was

next placed in an evacuable KBr die in a 13 mm clear disk and then pressed in a hydraulic press to form a KBr pellet using the same amount of pressure (10,000–15,000 lbs) for all samples to avoid polymorphic changes. The pelletized sample was placed in a cell holder (Universal Demountable Cell) and then inserted into the FTIR equipment and scanned at a range of 350–4000 nm or more. The spectrum and suspected compound was seen displayed and recorded on the computer screen.

2.7 Statistical Analysis

All analysis were carried out in triplicate except otherwise stated. One-way analysis of variance was performed to determine significant differences ($P < 0.05$) between means and Duncan's multiple range test was used to separate means using SPSS version 20.

3. Results and Discussion

3.1 Extraction and Yield of Native Water Yam Starch

The yield obtained from the water yam extraction was 84.2% (dry weight basis). The yield is greater than values obtained by Verwimp *et al,.* (2004) for rye starch (42.2%) and Chavan *et al*. (2010) for horse gram starch (22 to 31%) but less than values obtained by Nadiha *et al.* (2010) for sago, potato and corn starches, which were 93.6%, 93.4% and 96.5% respectively. Starch yields of 49.3 and 59.3% for pigeon pea and mung bean starches, respectively had been reported by Mwetta, (2009). Similarly, Barimalaa et al. (2005) reported starch yields of 47.9, 45.52 and 42.75 g/100 g seed from four different varieties of bambara groundnut. Banigo & Mepba (2008) reported starch yields of 45.9-47.1 and 54.6% for African yam bean and cowpea starches respectively. The major component of the tubers is starch, which can amount up to 85% of total dry weight of the tuber such as in *Dioscorea alata* (Huang *et al.,* 2006).Variation in the starch content is related to the maturity stage, variety, different climatic and agronomic conditions (Rahman *et al.,* 1999).The high yield most probably is due to the granular size which allows for easy extraction of the starch granule.

3.2 Molar Substitution of Hydroxypropylated Water Yam Starch

The molar substitution (MS) of the hydroxypropyl group into granular starches was used to measure the reaction efficiencies of hydroxypropylation and cross-linking. The MS of the starch with 4–12% propylene oxide are as shown in table 1. The hydroxypropyl groups and MS reflecting the effect of hydroxypropylation were in the range 0.087–1.823% and 0.002–0.05 respectively. It was observed that there were progressive increases in MS as the volume of propylene oxide added to the reaction medium rose; the highest MS attained being 0.05. Corresponding increases were also noted in molar substitution (MS) with the addition of propylene oxide during the hydroxypropylation of white yam (Lawal, 2008). This observation is consistent with increases in the MS of hydroxypropyl finger millet starch as the volume of propylene oxide added to the reaction mixture increased (Lawal, 2008). A similar observation was also reached with the hydroxypropylation of canna and maize starches (Chuenkamol *et al.,* 2007). Higher concentration of propylene oxide resulted in greater rate of collision between the starch alkoxide and the reagent in the proximity of starch granule (Lawal, 2009) causing higher degree of substitution.The reaction efficiency of hydroxypropylation depends on the diffusion or penetration of the alkaline catalyst and the etherifying agent into the starch granules and the probability of reactivity between the starch alcoholate nucleophile and the propylene oxide molecule (Vorwerg *et al,* 2004).Higher concentration of propylene oxide enhanced the probability of its reactivity with the starch alcoholate nucleophile. This observation is consistent with previous reports on hydroxypropylation of wheat starch (Choi & Kerr, 2003).Also in the succinylation of acha starch, the results indicated that % succinylation increased with increase in concentration of succinic anhydride (Arueya & Oyewale, 2014).The substitution reaction by etherification initially requires starch activation by an alkaline agent to facilitate the O–H nucleophillic bond, in addition to forming the starch O. This reactive starch type (O.) reacts with a substituting molecule, the propylene oxide, resulting in a bimolecular substitution and hydroxypropyl starch. Recommendations regarding the permitted substitution for hydroxypropylated starches, limit the value of the degree of substitution to 0.2 for use in food formulations (Dias *et al.,* 1997).

Studies by Pal *et al*. (2000) with corn and amaranto hydroxypropylated starches indicated that recommendations do not consider the specificity of each starch. The highest MS reported here is 0.17 and that is within the limit allowed for the use of hydroxypropylated starch in food applications (Dias *et al.,* 1997), which translates to an upper limit of MS= 0.2 i.e. 7.0 g/100 g i.e. MS = 0.2 Native starch has distinct morphological, functional and physical chemistry characteristics, which would require a molar substitution limit for each type of starch. Significant difference among these values indicated the effects of the volume of propylene oxide used for the substitution reaction. The hydroxyl groups of starch can be reactive and substituted by a range of functional groups for modifications (Mweta, 2009). Substitution degree is an important parameter in the evaluation of the

etherification process, identifying the number of substituting group moles per glucose unit, where this definition only applies to polymeric substitution (Mweta, 2009).Yam starches from several species have been subjected to hydroxypropylation (Lawal *et al.,* 2008b; Odeku & Picker-Freyer, 2009) with a concomitant enhancement in their hydrophilic properties.

Table 1. Conditions of synthesis and the resulting molar substitution of hydroxypropylated water yam starch

Sample	Starch (g)	Na2SO4(g)	Propylene oxide (ml)	Temperature (°C)	pH	Time(hr)	MS
4% HPS	100	20	4	40	10.5	24	0.0024
6% HPS	100	20	6	40	10.5	24	0.021
8% HPS	100	20	8	40	10.5	24	0.024
10% HPS	100	20	10	40	10.5	24	0.032
12% HPS	100	20	12	40	10.5	24	0.05

3.3 Physical Properties of Water Yam and Starch Extract (Native and Hydroxypropylated)

3.3.1 Dimensions of the Water Yam Tubers

The tubers were more uniform (Figure 1) in geometry (length, width), weight and shapes. The length of the water yam tubers ranged from 197.35mm to 227.06mm. Techi *et al*, (2012) reported length of 44.07cm for some cultivars of water yam. The width of the water yam ranged from 148.03mm to 160.8mm.The Shapes were regular gourd and consistent. Small variances were though observed in the dimensions.

Figure 1. Water yam tubers

3.3.2 Granule Morphology of the Native and Hydroxypropylated Water Yam Starch

The granule morphology and size distribution of native and hydroxypropylated water yam starches reflected a size distribution pattern (Table 2).Native water yam starch granules showed various shapes: triangular, round, oblong, oval and oval-triangular with the last three being predominant. Few round shapes were observed and similar to other studies [Tetchi *et al*., (2012), Otegbayo *et al.* (2011), Rugchati &Thanacharoenchanaphas (2010)]

The Native water yam starch gave size distribution range of 8.06-72.5µm with mean (33.882µm), median (34.1µm), mode (30.24µm) dimensions. Naknaen (2014) also reported that the shape of jackfruit native starch appeared irregular, round to bell or semi-oval with an average size of 7.66 µm. For the 4%HPS, the interval size distribution is 8.58µm - 62.99µm with mean, median and mode dimension to be 28.36µm, 29.31µm, and 29.71µm respectively. The size distribution range of 4%HPS is similar to that of native starch with the upper range lower than that of native starch. The highest size (62.99µm) at this concentration is evidently reduced when compared to the native starch. Similar trend was observed with the values of mean, median, and mode indicating reduction in sizes with a subsequent reduction in the percentage of small and large granules. The hydroxypropylated granules at 4%HPS still maintained their granular form without fragmentation. In contrast, Schmitz *et al.,* (2009) reported that hydroxypropylated cassava starch exhibited natural granule alteration from the native form. Recently, Perera *et al*. (1997) using X-ray diffraction showed that hydroxypropylation caused

profound changes in physical arrangement of starch chains within the amorphous and crystalline domains of starch granules, Changes in the morphology of starch granules was reported in the alkaline treatment of sago, potato and corn starches(Nadiha, 2010). Based on the morphology and granule size, yam starch granule is closely related to potato starch. In terms of size distribution, yam starch has distribution pattern different from tapioca and potato associated with symmetrical distribution (Hossen, *et al.*, 2011).

Variations in amylose and amylopectin structures and their relative amounts in a granule play an important role in controlling starch granule size and shape. Activity of the enzyme granule bound starch synthase (GBSS) during growth may also affect starch granule morphology (Blennow *et al.,* 2002). The membranes and the physical characteristics of the plastids have been linked to a particular morphology of starch granules during development (Jane *et al.,* 1994; Lindebooma *et al.,* 2004).

Among samples of 6% HPS, the percentage of small and large granules increased by 1.55% and 8.64% respectively. There is no fragmentation or change in shape of the granules observed. This is in agreement with findings on hydroxypropylation of jackfruit (Naknaen, 2014) where the modified granules were still intact, free of fragmentation. However, the presence of traces of degradation was observed on some starch granules, particularly at higher level of propylene oxide (HP_{40}-HP_{50}).Also oxidation of native acha starch did not affect the shape, appearance and structural arrangement of the starch granules (Isah *et al.,* 2015) but contrast with Singh *et al,.* (2006) where the presence of small protruberances and fragmentation on the surface of potato starch granules were observed. Physico-chemical properties, such as transparency of the starch paste, enzymatic digestibility, amylose content and swelling power have been significantly correlated with the average granule size of the starches separated from different potato cultivars (Kaur *et al.,* 2007a, b). It is obvious from a cursory look at Table 2 that the level of modification influenced the size and size distribution of the water yam starch granules. Paradoxically, while Chuenkamol *et al.,* (2007) reported surface alteration of canna starch granules on hydroxypropylation, it was not the case with potato starch as its morphology remained intact (Kaur *et al.,* 2004).

Granule diameter is an important parameter in particle-particle interactions, mixing and homogeneity in food product formulation (Riley *et al.*, 2008).

Generally, small and medium sized starch granules have been reported to have varied utilization in the food industry (Omojola *et al*, 2010) which makes both the native and modified starch desirable both in the food and other industrial applications. Granule size and size distribution of starch are unique properties of starch that have an influence on the functionality of the starches. Granule shape of starches does not influence their functional properties but can be used to identify the source of the starch. Granule size affects starch functionality being a contributing factor to its extractability (ease of starch extraction and sedimentation), pasting properties swelling power, gelatinization temperature, and viscosity (Sanguanpong *et al,* 2004). Recent works have proved the importance of understanding the starch granule morphology, such as the influence of the process in starch granule structure. Perera & Hoover (1999) observed the structure of defatted potato starch granules treated with humid heat. Marcon (2004) used microstructure examination to reveal the effect of the cassava starch sour improved fermentation on several origins of starch granule structure. Kaur *et al.* (2004) developed studies on hydroxypropylated potato starch and the influence of this process on native potato starch microstructure.

Table 2. Granule morphology and size distribution of native and hydroxypropylated water yam starch

Sample	Predominant shapes	Interval size Distribution (μm)	Mean (μm)	Median (μm)	Mode (μm)	Small Granule (%)	Large granule (%)
Native	Round, elongated oval, ellipsoid, triangle	8.06-72.5	33.88±1.3	34.10	30.24	25.02	42.81
4% HPS	Round, elongated oval, ellipsoid, triangle	8.58-62.99	28.36±1.2	29.31	29.71	20.38	34.92
6% HPS	Round, elongated oval, ellipsoid, triangle	6.46-73.64	32.63±1.3	30.99	27.39	21.55	43.56
8% HPS	Round, elongated oval, ellipsoid, triangle	8.58-72.5	31.16±1.2	30.24	29.31	21.32	38.90
10% HPS	Round, elongated oval, ellipsoid, triangle	6.46-82.28	30.69±1.5	28.23	48.53	18.04	38.90
12% HPS	Round, elongated oval, ellipsoid, triangle	10.75-58	33.43±1.1	33.83	29.71	24.26	43.53

Means in columns are significantly different at 5% level ($P< 0.05$).Small granules (1-10μm), Large granules (>25μm)

3.3.3 Loose and Packed Bulk Densities of Native and Hydroxypropylated Water Yam Starch

Table 3 shows the loose and tapped bulk densities, Hausner ratio, Carr index, compressibility and flowability of the starches. Loose bulk density ranges from 0.5-0.6005 in the order of 4% to 12% for the hydroxypropylated starch while that of native starch is 0.4988.Packed bulk density showed similar trend. This followed the pattern of decrease in amylose content and it was also observed that hydroxypropylation has effect on the particle size of the granules.

Hydroxypropylation increased both the loose and packed bulk densities and this was reflected in the particle sizes. Thus, 12%HPS had the highest loose and packed densities. Starch with the highest granule size gives an indication of the greater ease of dispersability and reduction of paste thickness (Udensi & Eke, 2000). Low bulk density of starch is a good physical attribute when determining transportation and storability since the products can be easily carried and distributed to locations where they are required (Agunbiade & Sanni, 2003). Low bulk density is advantageous for the infant food as both calorie and nutrient intake are enhanced per feed (Onimawo & Egbekun, 1998). High bulk density is a good physical attribute when determining mixing quality of particulate matter (Lewis, 1990). The bulk density of the cereal starch was less than the potato starch (0.98 g/mL) (Singh et al., 2009). The modified water yam starches had higher bulk densities than the native starch. Bulk density of foods increases with increase in starch content (Bhattacharya & Prakash, 1994). High bulk density of protein material is important in relation to its packaging (Okezie & Bello, 1988). The results of the bulk density of the starch samples also showed that the native starch from the water yam will be good for developing foods that requires more protein while the hydroxypropylated starches will be good for energy foods.

Bulk density is an important parameter for determining industrial requirements for packaging, material handling and utilization in wet processes in the food industry (Muazu *et al*., 2011) Starch flours with high bulk densities such as 4% HPS to 12% HPS could be used as thickeners (Ocloo *et al*., 2010). The parameters of Carr's index and Hausner ratio predict the flow and compressibility of powders (Muazu *et al*., 2011). In literature, Hausner ratio above 1.2 and Carr's index above 23% do not indicate good flow or good compressibility (Iheagwara, 2013).

Carr index and Hausner ratio for the native and hydroxypropylated water yam starch was in order of: native water yam starch (24.0, 1.2531) < 8% HPS, (31.0, 1.2333) < 6%HPS(33.0,1.3571),< 4%HPS(33.3,1.333) < 12%HPS (35.2,1.4167) <10%HPS(35.8,1.4583).

The Carr index provides an indirect measure of material fluidity, and the higher its value, the more cohesive the substance (Riley *et al.*, 2008). The 4-12% HPS samples were indicated less suitable values in flowability but good for compressibility. Ostensibly, hydroxypropylation improved compressibility but not flowability of water yam starch. This differs from the results reported by Olorunsola *et al.,* (2011) for native and acid hydrolysed sweet potato starch. The lower the Carr index of a material, the lower the porosity, the better the flowability, but the poorer the compressibility. Therefore, the improvement or otherwise of compressibility and flowability of hydroxypropylated water yam starches depend on the increase in molar substitution.

Table 3. Densities and flow characteristics of native and hydroxypropylated water yam starch

Sample	Loose bulk density	Packed bulk density	Hausner ratio	Carr index (%)	Compressibility	Flowability
Native	0.4988±0.0	0.6187±0.1	1.2531±0.1	24.0	Fair	Poor
4% HPS	0.5±0.0	0.6667±0.1	1.3333±0.1	33.3	Good	Very poor
6% HPS	0.5130±0.0	0.6897±0.1	1.3571±0.1	33.0	Good	Very poor
8% HPS	0.5266±0.0	0.6867±0.1	1.2333±0.1	31.2	Fair	Very poor
10% HPS	0.5826±0.0	0.7692±0.1	1.4583±0.1	35.8	Excellent	Very poor
12% HPS	0.6005±0.0	0.8±0.0	1.4167±0.1	35.2	Excellent	Very poor

Means in columns are significantly different at 5% level (P< 0.05).Carr index of 5-10,12-16,18-21,23-28 represent excellent, good, fair, and poor flowability respectively. Flowability is conversely related to compressibility.

3.3.4 Colour Evaluation of Native and Hydroxypropylated Water Yam Starch

The colour of starch due to the presence of polyphenolic compounds, ascorbic acid and carotene has impact on its quality. Table 4 shows the result from the colour evaluation of native and hydroxypropylated water yam starches. L*(lightness) ranged from 83.0 for the native starch to 93.2 for the 12%HPS, a*(Red/green) ranged from 0.6 to 0.0 for native and 12%HPS respectively and b*(yellow/blue) ranged from 1.5 for native water yam starch to 3.0 for 12%HPS.L* followed the order of native <4%HPS <6%HPS <8%HPS <10%HPS <12%HPS.

Evidently, the 12%HPS sample had the highest value of lightness (93.2) followed by 10%HPS (91.9), 8%HPS (91.6), 6%HPS (90.8), 4%HPS (88.5).The result showed significant increase in lightness, L* and b* for all the hydroxypropylated water yam starches but a* magnitude reduced significantly compared to the native starch. These changes can be attributed to some level of removal of extraneous matter like proteins, fat, fibre, latex, salts, sugars, pigments during hydroxypropylation. Starch isolate obtained by Shimelis *et al.* (2006) from improved bean (*Phaseolus vulgaris* L.) was deemed pure and of acceptable colour extraction following treatment with NaOH and $NaNO_3$ solution, during which compounds such as polyphenols, ascorbic acid and carotene were eliminated.The residual polyphenols, ascorbic acid and carotene carried over to the final starch product may have been responsible for the relatively lower lightness values (81.26-88.42) (Shimelis *et al.,*2006). The observed colour values suggest that 12 % HPS is more yellowish in colour than other hydroxypropylated water yam starches. The presence of inherent pigments in the starch if carried over to the final product reduces the quality hence acceptability of starch product (Galvez & Resurreccion, 1992).

Table 4. Surface colour of native and hydroxypropylated water yam starch

Sample	L*	a*	b*	H*	ΔC	ΔE	W (%)	C*	E*
Native	83.0±0.0	0.6±0.0	1.5±0.0	66.8±0.2	9.2±0.0	14.19±0.0	44.2±0.0	1.6±0.0	10.87±0.0
4% HPS	88.5±0.1	0.4±0.0	2.9±0.0	82.6±0.0	8.0±0.0	9.6±0.0	53.29±0.1	2.9±0.0	6.02±0.1
6% HPS	90.8±0.0	0.2±0.0	3.9±0.0	86.9±0.0	7.2±0.0	7.8±0.0	57.86±0.0	3.9±0.0	4.91±0.0
8% HPS	91.6±0.0	0.07±0.0	2..5±0.0	88.3±0.1	8.1±0.0	8.4±0.0	59.84±0.1	2.5±0.0	3.3±0.0
10% HPS	91.9±0.0	0.03±0.0	3.3±0.2	89.5±0.0	7.5±0.1	7.7±0.1	60.6±0.1	3.3±0.2	3.7±0.2
12% HPS	93.2±0.0	0.01±0.0	3.0±0.0	89.8±0.0	7.7±0.0	7.7±0.0	63.6±0.1	3.0±0.0	3.02±0.0

Means of columns are significantly different at 5% level (P< 0.05)

3.4 Chemical Properties of Native and Hydroxypropylated Water Yam Starch

3.4.1 Proximate Composition

Table 5 shows the proximate composition of the native and hydroxypropylated water yam starches. The range of the moisture content of the native water yam starch was 10.47 while those of the hydroxypropylated starches ranged from 12.01% to 14.01% .The moisture content for the hydroxypropylated water yam starches are higher than that of its native counterpart but it reduces as the volume of propylene oxide increases. This is at variance with the moisture content of saba banana starch which was 11.4%, whereas the moisture content of hydroxypropylated banana starches ranged between 7.3 - 10.8 %.(Jau-Shya, Lee *et al*, 2011).High moisture content promotes microbial deterioration and also enhances discolouration of starches.This observation is similar to that of annealed African yam bean starch, bambara groundnut starch and mucuna bean starch which was reported to have higher moisture than those of their native starches (Adebowale *et al.*, 2009; Adebowale & Lawal, 2002; Adebowale & Lawal, 2003).The lower moisture content of the hydroxypropylated starches at high concentration of propylene oxide indicates decreased susceptibility to microbial attack, less ability to interact with foods that are moisture sensitive and high ability to absorb water ,although the moisture content of modified starches were within official limit 4% to 12% (Olayemi, 2008). High moisture may lead to activation of enzymes and proliferation of micro-organisms.

As shown in the result, the crude protein decreased as the volume of propylene oxide increased i.e. increase in degree of substitution decreased the crude protein content Protein content of yam starches varied greatly among different reports, with most of the reports showing a value of <0.2%.The discrepancy between different studies is most likely attributable to isolation methods and crop genetics. Lower protein content is a reflection of a more efficient extraction method for purifying the starch (Baldwin, 2001).Protein content of the native water yam starch is also low compared to other native leguminous starches, an indication of the absence of endosperm proteins and non-starch lipids. Notably, the nitrogen content of isolated starch represents the endosperm storage proteins, lysophospholipids and proteins located inside starch granules (Chavan *et al.,* 2010). This could also be a function of the cultivar (Bartova *et al*., 2006). The amount of protein is usually considered as an index of purity of legume and cereal starches.It has been reported that yam starches have higher CP than sweet potato starch (Ali *et al.*, 2012) and cassava starch (Nuwamanya *et al.* 2010). Interestingly, Adebowale *et al.* (2009) reported reduced crude protein for annealed African yam bean starch. Most proteins precipitate out of solutions when adjusted to their iso-electric point, that is when pH equals pKa (Belitz *et al.*, 2009) and acid-thinned hybrid maize starch has also been found to have lower crude protein than its native starch (Lawal *et al.*, 2005). Hydroxypropylated starches had lower protein than native starch probably as a result of the high oxidative and denaturing property it possesses on proteins. The denatured protein bodies eventually precipitate out, and are

decanted, thus leading to a reduction of proteins in the starch.

Crude fat of the starches ranged from 0.14% (12%HPS) to 1.06% (native starch) following the order: Native starch > 4%HPS (0.73) > 6%HPS (0.61) >8%HPS (0.50) > 10%HPS (0.32) >12%HPS (0.14).Lipids-starch interaction may influence functional, thermal and retrogradation properties of starches (Singh & Kaur, 2009). Similar values have been reported for Chinese yam starches (Odeku & Picker-Freyer, 2007); and *D. cayenensis-rotundata* starch (Zuluaga *et al.*, 2007).The modification (hydroxypropylation) significantly reduced the crude fat of the native starch.

Ash content of the native starch was 1.35 which is higher compared to its hydroxypropylated water yam starches. This is at variance with the findings of Schmitz *et al.,* (2006) that hydroxypropylated starch ash content of cassava was superior to the native starch. However, the ash content is still a bit similar to the ash content (0.30 ± 0.011%) of sweetsop and soursop starches (Nwokocha & Williams, 2009), lower than that of *treculia africana* starch (Nwokocha & Ogunmola, 2005) and cocoyam starch and within the same value as that of cassava starch (Nwokocha *et al.,* 2009). Hydroxypropylated ash content ranged from 0.22 (12%HPS) to 0.34 (10%HPS) to 0.45 (8%HPS), 0.66 (6%HPS) to 0.89 (4%HPS),a decrease in ash content was observed as the molar substitution increased. Ash content for native water yam starch (1.35%) was superior to the modified starches. The ash content which measures the bulk of the residue left after burning, it is also often indicative of the metallic content, all starches contain mineral ions. The ash content is the composite of all inorganic minerals in the starches

Crude fibre was not detected in the native and hydroxypropylated starches, this is similar to other observations (Odeku & Picker-freyer, 2007, Adebowale *et al.,* 2002; Olayinka *et al*., 2011). However, Rugchati & Thanacharoenchanaphas, 2010 reported low crude fibre for water yam starch.

Nitrogen free extract, NFE represents all the carbohydrate components of the starch, and it ranges from 84.957 to 87.622%.The highest carbohydrate content in this study was 87.622% (12%HPS) with no significance difference *(P>.05*) within the varieties.

Table 5. Chemical composition of native and hydroxypropylated water yam starc

Sample	Moisture %	Ash content %	Crude fat	Crude protein	NFE %	Amylose %	Amylopectin %
Native	10.47±0.0	1.35±0.0	1.06±0.07	0.183±0.1	86.937	44.186±0.1	55.814
4% HPS	14.01±0.1	0.89±0.0	0.73±0.05	0.093±0.0	84.957	41.942±0.7	58.058
6% HPS	13.70±0.2	0.66±0.0	0.61±0.04	0.092±0.0	85.508	40.841±0.3	59.159
8% HPS	13.1±0.1	0.45±0.0	0.50±0.07	0.009±0.0	86.371	40.208±0.1	59.792
10% HPS	12.39±0.3	0.34±0.0	0.32±0.04	0.009±0.0	87.321	37.475±0.1	62.525
12% HPS	12.01±0.0	0.22±0.0	0.14±0.04	0.008±0.0	87.622	36.252±0.0	63.296

Means in columns are significantly different at 5% level (P< 0.05)

3.4.2 Amylose and Amylopectin Content of Native and Hydroxypropylated Water Yam Starch

Table 5 above also showed the amylose and amylopectin content of the native and hydroxypropylated water yam starch ,the amylose content ranged from 44.186% for the native starch,41.942% 94%HPS),40.841% (6%HPS),40.208% (8%HPS), 37.475% (10%HPS), 43.166% (12%HPS).The amylose content of the hydroxypropylated starches varied significantly (*P<0.05*) and 12%HPS was found to have the lowest (36.112%) while 4%HPS had the highest (41.942%). The amylose content of the native and the hydroxypropylated water yam starches are significantly different. Amylose content decreased with hydroxypropylation, an observation comparable to those from alkaline treatment of potato, sago and corn starches (Nadiha *et al*,2010).The reduction of apparent amylose content of hydroxypropylated starches (Table 5) could be attributed to the disruption of the amorphous region containing amylose chains (Karim *et al.,* 2007). In addition, the propylene oxide appears to affect more of the amylose rather than the amylopectin molecules and/or regions of the granules (Lai *et al.,* 2004). Lai *et al.* (2004) further suggested that the ions in propylene oxide solution diffuse into the amylose-rich amorphous regions of the granules, break intermolecular bonds, and cause the granules to swell to a higher degree with a concomitantly higher exudation of amylose. The amorphous regions in different starches might differ in terms of dimension and molecular arrangement (Wang & Wang, 2001). Amylose content of yam starch is reported to be between 14-30% depending on the species (Moorthy, 2002). Oke *et al.* (2013) reported comparable amylose contents of between 27.47-41.90% for ten water yam cultivars' starches. In a similar development, Otegbayo *et al.* (2011) observed amylose content for native water and white yam starches as 28.99-33.11% and 27.45-32.80% respectively, along with a validated value (27.44-33.03%) for 18 white yam

cultivars' starches. Differences in amylose/amylopectin ratio of starches might also occur because of the activity of the enzymes involved in starch biosynthesis of various starches (Krossmann & Lloyd, 2000), genetic variations, climatic conditions, cultivation practices and botanical source (Odeku & Picker-Freyer, 2007). The amylose content of the native starch was 41.942% which is higher than those reported by Hoover & Senanayake (1996) for oat (22.2 to 22.5%), Sandhu & Singh (2007) for corn (16.9 to 21.3%), and Chavan *et al.,(*2010) for Horse Gram (34.00 to 36.30%) starches. The amylose content of starch determines crystallinity and thus affects solubility (Yuan *et al.,* 2007), factors important in determining the applicability of the starch. Amylose is essentially a linear polymer of 1,4-linked α-D-glucopyranose units constituting about 15-20% of the starch contrasting sharply with amylopectin, a highly branched polymer of 1,4 and 1,6 linked D-glucopyranosyl units (80-85%.of the starch). It is also a measure of the tackiness of the starch. Amylose content in yam starches also varied considerably according to various reports.(Farhat *et al*.,2009) have obtained the following values for amylose content in starches of different Dioscorea species – *D. alata* – 25%, *D. rotundata* and *D. cayensis* – 23.8% and *D. dumetorum* -12.6%. A value of 29.7% was reported for the amylose content of *D. abyssinica* starch from Ethiopia, while Asaoka & Blanshad, (1991) obtained 24.1% for *D. ballophylla* starch. Only very little variation in the amylose content was observed with age of the crop for *D. esculenta*, *D. alata* and *D. rotundata* starches.

As shown in Table 5, the value of amylose content for native water yam starch is higher than those of hydroxypropylated starches, a fact confirmed by Perera *et al.* (1997) who observed decreased amylose leaching for hydroxypropylated potato starch. It has been reported that amylose is derivatized to a greater extent than amylopectin (Shi & BeMiller, 2002; Kavitha & BeMiller, 1998) and that derivatization increased the amylose leaching. Thus, the reduction in amylose content of hydroxypropylated water yam starches may have been due to some amylose leaching during the hydroxypropylation process, as well as partial hydrolytic effect of the mild heat treatment which may have led to lysis of some glucosidic bonds in the amylose chains found in the amorphous region of the starch granules.

Environmental factors and agronomic practices can greatly affect the amylose content of yam starches (Huang *et al*., 2006; P´erez *et al*., 2011; Akinoso & Abiodun, 2013; Otegbayo *et al.,* 2014). Drought decreased the amylose content of starch from *D. dumetorum* (Otegbayo *et al.,* 2014). The harvesting year was found to influence the amylose content of starch from *D. trifida* (P´erez *et al.,* 2011). Harvesting month (from 7 to 11 months since planting) had little effect on the amylose content of starch from *D. dumetorum* (Akinoso & Abiodun 2013). In contrast, starch from *D. alata* harvested at the 260th day (about 9 months) after planting had higher amylose content than when harvested earlier (Hung et al., 2006). This discrepancy may be attributed to the differences in the harvesting period, yam genotype, and some environmental conditions. With a 3-month postharvest storage, the amylose content of starch from tubers of *D. Alata decreased* (Brunnschweiler, *et al.,* 2005). Amylose is a major component of starch and plays an essential role in the properties and uses of starch (Srichuwong & Jane 2007). Functional properties of starches depend on the amylose content to a large extent (Awokoya *et al.*, 2011). Great variation in the amylose content of diverse genotypes of the same species and between diverse species has been observed. Amylose contents of genotypes from various yam species ranged from 1.4% (P´erez et al., 2011) to 50% (Rolland-Sabat´e *et al*., 2003). Apparent amylose content from 43 genotypes in 5 species ranged from 15.1% to 27.1% (Otegbayo *et al.,* 2014). Among the most studied species, *D. dumetorum* and *D. esculenta* tend to have lower amounts of amylase (< 20%) compared to *D. alata*, *D. rotundata*, *D. cayenensis-rotundata*, and *D. cayenensis* (>20%) (Amani *et al*., 2004; Otegbayo *et al*., 2014). Amylose content in distal and proximal regions of tubers of *D. alata* is rather similar (Brunnschweiler *et al.*, 2005), indicating an even distribution of amylose through the whole tubers. These environmental and human factors suggest possible manipulation of the amylose content of starch to further artificially diversify the variation.

Hydroxypropylation of water yam starch reduced ash content, protein, fat, amylose and pH. These reductions were probably due to degradations of starch during the chemical modification process. The chemical composition is a simple and convenient way of illustrating the purity of the starch extracts, whereby higher starch and lower contents of other components (protein, fat, ash, and fibre) are highly desirable. The protein, fat, ash and fibre contents of hydroxypropylated Water yam starch were lower than the native starch. The results indicate that pure starch could be obtained from water yam through hydroxypropylation. The result also implied that native water yam starch is less pure compared to the hydroxypropylated water yam starches.Report of many other investigators also indicated that the amount of protein, fat, ash and fibre are usually considered as an index of purity of legume starch (Lii & Chang, 1981; Galvez & Resurreccion, 1993). Reduction in ash, protein and amylose contents are due to structural disintegration that took place during chemical modification processes (hydroxypropylation).

The ash content is significantly correlated (r = -0.959) with crude fat (CF).Higher crude fat in the starches relate

significantly with higher protein contents (r = 0.929) and lower NFE (r = -0.448). Moisture content significantly correlated (r = -0.896) with nitrogen free extract. pH and amylose were not significantly correlated to the other proximate components of the starch. However, Amylose and Amylopectin have a perfect negative correlation (r= - 1.000).

3.5 Functional Properties of Native and Hydroxypropylated Water Yam Starches

3.5.1 Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC)

Water and Oil absorption capacities of the hydroxypropylated water yam starches increased with the rise in molar substitution (Table 6).The WAC of the native and hydroxypropylated water yam starches ranged from 1.763g/g for native starch to 2.659g/g (12%HPS) in the order of 12%HPS (2.659G/g) > 10%HPS (2.630g/g) > 8%HPS (2.259g/g) > 6%HPS (2.207g/g) > 4%HPS (2.062g/g) > native starch (1.763g/g). WAC for native water yam starch is lower than the hydroxypropylated starches and there is significant difference in the WAC among the hydroxypropylated starches. Improvement in water and oil absorption was a result of introduction of hydroxypropyl groups to the starch molecules, which facilitated a more enhanced holding capacity. The increase in Water absorption capacity could also be due to some structural re-allignment exposing the hydrophilic end hence increased water absorption (Altan *et al*., 2009). Hydroxypropylation of banana starch has also been associated with increased water binding capacity, (Waliszewski *et al*., 2003). A similar pattern of result has been noted with acetylated sweet potato starches **(**Das AB *et al.,* 2010). The ability of the starches to absorb water is a function of hydrophilic groups (-OH, -COOH, inter-glucose oxygen atoms) available for binding with water (Otegbayo *et al.*, 2011),amylose to amylopectin ratio, temperature, presence of phosphate-esters or protein, crystallinity and botanical source (Noda *et al.*, 2008; Chen *et al*., 2003; Osundahunsi *et al*., 2003; Walter *et al.*, 2000). High WAC is an indication of a loose association of starch polymers in the granules of the starches, while low WAC presumes close association of starch polymers in the starch granules (Shimelis *et al.*, 2006).The increase in WAC on hydroxypropylation of water yam starch suggests weakened associative forces between the starch polymers in its granules ostensibly owing to the introduction of hydroxypropyl groups onto the starch polymers (Liu *et al*., (1999). Loosened starch structure due to the inhibition of inter-chain association facilitates the hydration of the starch granule arising from increased WAC. It has also been reported that there was increase in WAC of annealed African yam bean starch (Adebowale *et al.*, 2009) and red sorghum starch (Adebowale *et al*., 2005).

Water absorption capacity of starch/flour is useful indicator of whether protein can be incorporated with the aqueous food formulations, especially, those involving dough handing (Osungbaro *et al*., 2010). Interactions of protein with water, plays a significant role in functional property of hydration, swelling power solubility, and gelation (Etudaiye *et al.,* 2009). The high water absorption capacity of the flours suggests possible use in soup formulations (Olaofe *et al*., 1998). WAC is an important property in canned foods, confectioneries, pasta, and other cereal-based foods and hydroxypropylation evidently increased the WAC of the native water yam starch.

Oil absorption capacity ranged from 0.467g/g (native starch) to 1.417g/g 912%HPS), with significant variation among the hydroxypropylated starches (Table 6). The oil absorption capacity of the hydroxypropylated water yam starches increased marginally compared with native starch.The hydroxypropylated starches had similar oil absorption capacity as cassava (1.57g/g; Gunaratne, (2006) and lesser yam (*Dioscorea esculenta*) (1.9g/g; Ukpabi, 2010) but higher than raw fluted pumpkin flour (0.37g/g; Giami & Bekebain, 1992). According to Lahl & Braun (1994), lipid binding is dependent on the surface availability of hydrophobic amino acids. Oil absorption capacity is important as oil acts as flavor retainer and gives soft texture to food,thus improving mouth-feel (Aremu *et al*., 2006,Ubbor & Akobundu, 2009;). Since the flours had good oil absorption capacity it suggests the presence of good lipophilic constituents and therefore may be suitable for production of sausage, soups and cakes (Aremu *et al*., 2006). Hydroxypropylation improved the oil absorption capacity probably as a result of higher density of lipophilic residues on the surface of its granules (Morrison *et al.*, 1993). Citric acid modification slightly reduced the OAC of native white, water and bitter yam starches as against increase in OAC of citric-treated white yam flour (Akubor, 2013). Oil absorption capacity is an important property in fried foods, cooked foods, soups, pasta, and confectioneries.

The pH values is in the order of native (8.177) > 12%HPS (6.877) >10%HPS (6.58) >8%HPS (6.510) >6%HPS (6.423) >4%HPS (6.143) 6.877 < for the native and hydroxypropylated starches. The highest pH was displayed by native water yam starch (8.177) while 4%HPS starch gave the lowest (6.143) and the highest was 12%HPS(6.877) among the hydroxypropylated water yam starches. pH value is one of the physico-chemical properties of starch important to application.Similar observation has been reported for improved haricot bean by Shimelis et al., (2006).The pH values of the hydroxypropylated starches were found to be slightly lower than that

of the native starch obviously because of the reaction with propylene oxide but it still falls within the pH range of 3 – 9 obtained for most starches used in the pharmaceutical, cosmetics and food industries.

Table 6. Water absorption capacity (WAC) and Oil absorption capacity (OAC) of native and hydroxypropylated water yam starch

Sample	WAC (g/g)	OAC (g/g)	pH
Native	1.763±0.1	0.467±0.0	8.177±0.1
4% HPS	2.062±0.1	0.718±0.0	6.143±0.0
6% HPS	2.207±0.0	0.868±0.0	6.423±0.1
8% HPS	2.259±0.0	1.13±0.0	6.510±0.0
10% HPS	2.630±0.0	1.355±0.0	6.58±0.0
12% HPS	2.659±0.0	1.417±0.0	6.877±0.1

Means in columns are significantly different at 5% level (P< 0.05)

3.5.2 Least Gelation Capacity (LGC) of Native and Hydroxypropylated Water Yam Starch

Modifying the native water yam starch resulted in lower LGC (2%), thus enhancing their gelling capacity. This occurs with increase in molar substitution (Table 7), a phenomenon at variance with the report of Sung-Gil Choi (2001) on hydroxypropylation of wheat starch where gel hardness dipped significantly with increasing hydroxypropylation. This though compared favourably with the observation of Choi *et al* (1997) that of hydroxypropylation of high amylose corn starch which gave paste viscosity that was much less than that of native corn starch. The native water yam starch apparently developed soft viscous gels at lower concentrations, which became progressively hard as concentration increased.The gel network may have been closely linked to formation of intermittent hydrogen cross bonds among amylose and re-association of amylose molecules at random intervals (Vaclavik & Christian, 2008). Hydroxypropylated water yam starches exhibited least gelation concentration at 2%,a characteristic desired for thickener in food system, it could also be a useful replacement in viscous food formulation such as soups and baked foods. Gelatinization affects digestibility and texture of starch containing foods (Mweta, 2009).

Table 7. Least gelation concentration (LGC) of native and hydroxypropylated water yam starch

Concentration (%)	Native	4% HPS	6% HPS	8% HPS	10% HPS	12% HPS
2	V,SG(-)	V,SG(+)	HG(+)	HG(+)	F,HG(+)	F,HG(+)
4	V,SG(+)	HG(+)	HG(+)	F,HG(+)	F,VHG(+)	F,VHG(+)
6	V,FG(+)	HG(+)	F,HG(+)	F,HG(+)	F,VHG(+)	F,VHG(+)
8	HG(+)	F,HG(+)	F,HG(+)	F,VHG(+)	F,VHG(+)	F,VHG(+)
10	F,HG(+)	F,HG(+)	F,HG(+)	F,VHG(+)	F,VHG(+)	F,VHG(+)
12	F,HG(+)	F,HG(+)	F,HG(+)	F,VHG(+)	F,VHG(+)	F,VHG(+)
14	F,HG(+)	F,HG(+)	F,VHG(+)	F,VHG(+)	F,VHG(+)	F,VHG(+)
16	F,HG(+)	F,VHG(+)	F,VHG(+)	F,VHG(+)	F,VHG(+)	F,VHG(+)
18	F,HG(+)	F.VHG(+)	F,VHG(+)	F,VHG(+)	F,VHG(+)	F,VHG(+)
LGC (%)	6	4	2	2	2	2

V-viscous gel, F-firm gel, SG-soft gel, HG-hard gel, VGH-very hard gel, (+)-solid gel,(-)- no solid gel

3.5.3 Swelling Power and Solubility of Native and Hydroxypropylated Starch

Swelling power and solubility of the native and hydroxypropylated water yam starches are temperature dependent with swelling power increasing as the temperature rose from 50°C to 90°C (Table 8).The swelling power and solubility properties of the native and modified starches werelong been recognised as temperature and pH-dependent (Adebowale *et al.*, 2005, Adebowale *et al.*, 2009, Olayinka *et al.*, 2011). Therefore, the increases in swelling power of hydroxypropylated starches can be attributed to the disruption of the amorphous region in the granule, which presumably reduced the restraining effect of amylose, thus allowing the granule to swell more freely (Karim *et al.,* 2007).Liu *et al*. (1999) reported that hydroxypropylation increased the swelling power of starch with the soluble starch leaching out of the swollen granules on cooking. Swelling and solubility of starch vary with botanical source (Peroni *et al*., 2006). At 50°C and 60°C there was no significant change in the swelling power of the native starch probably because gelatinisation had not occurred, swelling power ranged from 2.045g/g to 2.085g/g, the intermolecular micellar binding forces were still strong (Lorenz, 1990) and the crystalline regions of the starch granules were largely intact. Hydroxypropylated starches had the most

favourable results for swelling power. The principal property of etherified starch is high water retention (Schmitz *et al.,* 2005).Native starch presented inferior values for swelling power compared to hydroxypropylated water yam starches. This is in agreement with Kaur *et al*. (2004), who studied hydroxypropylated potato starch in comparison with native starch's physical chemistry properties. The values for the hydroxypropylated starches ranged from 2.814g/g (4%HPS) to 4.187g/g(12%HPS) and 3.151g/g (4%HPS) to 7.307g/g (12%HPS).The native starch had the lowest Swelling Power at 50 °C and 60°C while 12%HPS had the highest Swelling Power (SP). The high value for 12%HPS could be as result of highest volume of propylene oxide used for the modification which contributed to the highest MS value. Increased MS values of hydroxypropylated starch derivatives are positively correlated with solubility and swelling power (Gunaratne & Corke, 2007;Lawal, 2009). The swelling power (35.5-52.8 g/g) and solubility (8.19-10.7%) values of the hydroxypropylated rice starches were higher than those of native rice starch (26.6 g/g and 7.78%) and increased with an increase in MS (Chun & Yoo, 2007).

As the temperature increased from 70°C to 80°C,there was an increase in swelling power of the hydroxypropylated starches in this order: 4%HPS < 6%HPS < 8%HPS < 10%HPS <12%HPS and is proportional to their individual molar substitution. The implication of this is that for each temperature there was a rise in the swelling power of the starches for every 2% increase in propylene oxide. Swelling power (SP) and solubility can be used to assess the extent of interaction between starch chains, within the amorphous and crystalline domains of the starch granule (Ratnayake, *et al.,* 2002).

It can be inferred that these changes are attributed to the relative ease at which inter-molecular bonds are interrupted and its rise associated in entropy (Odeku & Picker-freyer, 2007), increased mobility of starch molecules resulting in increased percolation of water (Lawal *et al*., 2005), and overriding high energy required to break such bonds at the higher temperature of 80^{o}C. As a result, the crystalline regions of the starches become relatively accessible for water molecules to bind, thus effectively swelling. Starch ether production and starch hydroxypropylation improve the starch's water retention capacity, because of intermolecular bond established between the water molecules and the starch macromolecule new sites. The native water yam starch had significantly lower SP than their hydroxypropylated water yam starches at 80^{o}C. The restricted swelling of native starch may be due to increased crystallinity as a result of preferential hydrolysis of amorphous regions (amylose), and less importantly, crystalline regions (amylopectin), as reported for cassava starch (Chatakanonda *et al.*, 2011), hybrid maize starch (Lawal *et al.*, 2005) and sweet potato starch (Olorunsola *et al.*, 2011, Mweta, 2009).

At 90°C, swelling power ranged from 6.143g/g (native starch) to 10.904g/g (12%HPS) within the temperature range studied (50–90°C), SP were temperature dependent and highest SP in each case was obtained at 90 °C. Progressive increases were observed as the temperature increased and it was observed that hydroxypropylation improved the SP of the native water yam starch at all temperatures. This is similar to the observation on hydroxypropylation of finger millet (Lawal, 2009). Increases in swelling power after hydroxypropylation were facilitated by introduction of the bulky hydroxypropyl groups into the starch molecules. These caused repulsion among the molecules, thereby facilitating water percolation into the granules. It is also reasonable to conclude that the introduction of the hydrophilic hydroxypropyl groups improved water percolation into the starch and this improved swelling remarkably. Similar increases in swelling capacity following hydroxypropylation have been reported for wheat starch, banana and potato starch (Hung & Morita, 2005; Waliszewski *et al.,* 2003).

Among the hydroxypropylated starches 12%HPS had the highest SP value which could be attributed to its highest value for MS as earlier mentioned, lowest value for protein, lipids, and the amylose content. High swelling and low WBC has been linked to starches with low amylose content since amylose reinforces internal network within granules thus restricting swelling (Tester & Morrison 1990). Swelling power has also been reported to increase with increasing long chains of amylopectin and decreasing amylose content (Sasaki & Matsuki, 1998). Amylose in the presence of lipids forms insoluble complexes limiting swelling and solubility of the starch (Tester & Karkalas, 1996). It is widely believed that lipid complexed with amylose restricts granule swelling (Tester & Qi, 2004).Among the other factors that have been shown to influence granular swelling are granular size (Vasanthan & Bhatty, 1996), crystallinity ,temperature,amount of phosphate linked to amylopectin (Jane *et al.,* 1996), amylose content (Tester & Morrison, 1990a), protein (Han & Hamaker, 2002; Han *et al.,* 2002).

The swelling power and solubility of the hydroxypropylated starches were higher than the native starch. Progressive increase in swelling power and solubility were observed as the MS increased among the hydroxypropylated starches. (Naknaen, 2014, Lawal, 2008), Liu *et al.,* (1999) reported that hydroxypropylation increased the swelling power of starch and the soluble starch which leach out of the swollen granules on cooking. This is in agreement with Oladebeye *et al.,* (2013) in the hydroxypropylation of legume starches, who reported

that the values of swelling power, solubility of the native starches were significantly different from the hydroxypropyl derivatives. Hydroxypropylation apparently enhanced the free swelling capacity (FSC) and solubility as reported by Lawal *et al.,* (2008) in the hydroxypropylation of white yam. . Hydroxypropylation resulted in a significant increase in swelling of granules, especially between 70°C and 90°C, since the hydroxypropyl groups replaced the original –OH groups and allowed easier access of water molecules into the starch granules (Mweta,2009).At all temperatures, the swelling of hydroxypropylated starches were higher than the native water yam starch. Swelling and solubility provide evidence of non-covalent bonding between starch molecules and therefore allow comparison of relative bond strength at specific temperatures (Moorthy, 2002). The extent of interaction between starch chains within the amorphous and crystalline domains is influenced by the amylose to amylopectin ratio, and by the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching and conformation (Hoover, 2001). When the starch molecules are heated in excess water, their crystalline structure is disrupted and water molecules become linked to the exposed hydroxyl groups of amylose and amylopectin by hydrogen bonding, which causes an increase in granule swelling and solubility (Singh, 2004).

Solubilities of native and hydroxypropylated starches were also temperature dependent (Table 8). At 50°C to 60°C when gelatinization had probably not fully occurred, solubility ranged from 3.172g/ (native starch) to 5.841g/g (12%HPS) and 3.908g/g(native starch) to 6.037g/g 912%HPS) respectively. However at 70°C, when partial gelatinization may have occurred and the starches had imbibed water considerably, solubility generally increased in all starches, ranging from 4.20g/g native starch to 7.206g/g (12%HPS). This may be attributed to structural reorganization, which tends to weaken the starch granules following hydroxypropylation and ranked in this order: 12%HPS > 10%HPS >8%HPS > 6%HPS >4%HPS >native starch. Solubility also increased as the level of modification increased from 4%HPS to 12%HPS. It is reasonable that increase in starch hyrophilicity after hydroxypropylation facilitated water percolation into the starch granules. Increase in water percolation enhanced leaching of amylose granules largely from the amorphous region of the starches.

Solubility of the hydroxypropylated water yam starches increased significantly at 80°C and 90^{o}C (in the order of native starch < 4%HPS < 6%HPS < 8%HPS <10%HPS <12%HPS). At this time full gelatinization had occurred probably owing to increased levels of total soluble sugars (reducing and non-reducing) resulting emanating from partial hydrolysis (Manuel, 1996). Slight reduction in the polymeric nature of the amorphous and crystalline regions of the starches during hydroxypropylation may also have provided better access for water molecules to penetrate the almost-impregnable amorphous and crystalline regions of the native starches, thereby enhancing solubility. In addition, inter- and intra molecular hydrogen bonds in the starch chains were disrupted, the granular structure of the starches were weakened and the motional freedom of starch chains increased as the temperature increased. This facilitated increases in solubility of the starch as observed. The extent of swelling is positively related to the solubility, i.e., the higher the swelling power, the greater the solubility. The increases in swelling after hydroxypropylation could be put to advantage in starch applications such as food thickening and preparation of hydrogels. Thus, swelling and solubility of starch is temperature dependent, increasing with increasing temperature due to weakening of internal associative forces maintaining the granular structure (Peroni *et al*., 2006).Starch swelling occurs concomitantly with loss of birefringence and precedes solubilisation (Singh *et al.,* 2004). Solubility of native and hydroxypropylated water yam starches were temperature dependent (Table 8). Solubility also increased as the level of modification increased from 4%HPS -12%HPS. It is reasonable that increase in starch hyrophilicity after hydroxypropylation facilitated water percolation into the starch granules and the increase in water percolation enhanced leaching of amylose granules largely from the amorphous region of the starches. In addition, inter and intra molecular hydrogen bonds in the starch chains are disrupted, hence the granular structure is weakened and the motional freedom of starch chains increases as the temperature increases thus improving solubility (Lawal, 2009).Similar result was reported by Lawal, (2009) in the hydroxypropylation of finger millet where solubility were temperature dependent and solubility increased as the level of modification increased. The solubility of the starches exhibited similar pattern to that of swelling power and differed significant ($P<0.05$) among the hydroxypropylated starches.

All the hydroxypropylated starch derivatives exhibited greater solubility and swelling power than native starch. It is commonly known that incorporation of bulky, hydrophilic hydroxypropyl groups into starch molecules within granules prevents inter-chain association among starch molecules, leading to destabilization of starch granule structures. The loosened granules facilitate hydration, and thus, enhance granule swelling, which further results in an increase in leaching of hydroxypropylated starch molecules from swollen granules (Gunaratne & Corke, 2007; Lawal, 2009, Singh *et al.,* 2007).

Table 8. Swelling Power and Solubility of native and hydroxypropylated water yam starch

Sample	Swelling Power (g/g)					Solubility (g/g)				
	50° C	60°C	70°C	80°C	90°C	50° C	60°C	70°C	80°C	90°C
Native	1.545±0.0	3.585±0.1	5.579±0.0	6.05±0.1	6.143±0.1	3.172±0.0	3.908±0.1	4.201±0.1	5.23±0.2	4.863±0.1
4% HPS	1.814±0.0	3.751±0.1	6.278±0.1	7.314±0.1	7.898±0.3	3.841±0.0	4.037±0.1	4.814±0.0	5.862±0.1	5.048±0.1
6% HPS	2.904±0.0	3.692±0.1	6.924±0.1	7.863±0.1	8.051±0.1	4.171±0.0	4.695±0.0	5.100±0.1	6.143±0.1	5.926±0.1
8% HPS	3.140±0.1	3.952±0.3	7.729±0.0	9.478±0.6	10.035±0.1	4.870±0.0	5.206±0.0	5.763±0.2	8.667±0.1	6.720±0.0
10% HPS	3.960±0.1	5.242±0.2	8.224±0.4	9.852±0.1	10.362±0.2	5.276±0.1	5.909±0.1	6.202±0.2	9.271±0.2	8.166±0.0
12% HPS	4.187±0.0	7.307±0.1	9.594±0.1	10.193±0.0	10.904±0.4	5.841±0.2	6.037±0.1	7.206±0.2	9.796±0.0	8.901±0.1

Means in columns are significantly different at 5% level (P< 0.05)

3.5.4 Starch Gel Clarity (SGC)

Gel clarity ranged from 8.8% (native starch) to 44.5% (12%HPS) as inferred from their transmittance (Fig.2).The clarity increased with increase in MS having the 4%HPS with the lowest value for clarity among the hydroxypropylated starches while the native starch had the lowest value compared to the modified starches. In literature, light transmittance of potato starch, corn and amaranth starch increased after hydroxypropylation (Pal *et al.,* 2000, Kaur *et al.,* 2004 and Singh *et al.,* 2004a) and this suggests reduction in paste turbidity. In addition, paste clarity of normal and waxy maize starches also increased after hydroxypropylation (Liu *et al.,* 1999). Substitution of the hydroxyl groups with hydroxypropyl groups on the starch molecules prevented reformation of inter and intra-molecular bonds after paste formation. These development improved paste clarity and prevented turbidity of the starch paste. Improved paste clarity is a useful property in the manufacture of some foods like jellies, sausages and fruit pastes, which require transparency (Jyothi, *et al.,* 2005). The chemical substitution of the –OH groups on the starch molecules by hydroxypropyl moieties hampers the formation of an ordered structure following gelatinization , and thus retards retrogradation, resulting in a more fluid paste with improved long-term clarity (Lawal, 2004). The high retention of water entering the starch granule results in a greater swelling power and favours the clarity of pastes and gels. Reduced swelling of native water yam starch is mainly responsible for its reduced paste clarity; a conclusion that is in agreement with similar studies; Zheng *et al.,* 1999 , Morikawa & Nishinari, 2000a; Reddy & Seib, 2000 and Kaur *et al*., 2006,. Starches used to thicken fruit pies are preferably transparent while those in salad dressings are opaque.

As shown in figure 2, native water yam starch had the lowest clarity just like other functional properties of starch, varies with its source (Tetchi *et al*., 2007b; Singh *et al*., 2003).There is a great variation in gel turbidity after gelatinization of starch, as well as the changing pattern among diverse species and within the same species during storage (Amani *et al.,* 2005; Otegbayo *et al.,* 2014).Compared with potato and cassava starches, yam starches from diverse species had higher gel turbidity (Amani *et al.,* 2005).Potato starches have higher paste clarity (96%T) than corn (31%T), wheat (28%T) and rice (24%).Mweta, (2009).compared clarity of potato, tapioca, wheat, and corn starch pastes among others. They reported higher paste clarity for potato (96%T), than for tapioca (73%T), wheat (62%T) and corn (41%T) starches. Tetchi *et al*. (2007b) also found that potato starch pastes were more transparent (79%T) than cassava (47%T), sweet potato (17%T) and cocoyam (16%T) starch pastes. These differences in paste clarity have been attributed to differences in chemical composition such as phosphate and amylose. Potato starch pastes have higher paste clarity than cereal starches due to high content of phosphate monoesters as opposed to higher contents of phospholipids in cereal starches. Phospholipids present in starches form complexes with amylose and long chain fractions of amylopectin resulting in limited swelling and hence lower light transmittance. On the other hand, phosphate monoesters covalently bond to amylopectin fraction and due to repulsions between phosphate groups on adjacent amylopectin groups swelling is enhanced and hence light transmittance (Singh *et al*., 2003). Amylose reorganisation forms aggregates that reduce light transmittance of starch pastes (Tetchi *et al*., 2007b). High amylose starches reassociate more readily than amylopectin starches thereby resulting in more opacity (Bultosa *et al*., 2002). Paste clarity was related to amylose content, because native water yam starch presented the most opaque gel and the highest amylose content. This observation compares favourably with native and hydroxypropylated cassava starch (Schmitz *et al.*, 2005) Paste clarity is valued in starch applications such as food additives because it enhances consumer acceptability of the product, particularly those products that could stay on shelf for a long time. Examples of such products are jellies, sausages, and fruit pastes. It is reasonable that inter- and intra-molecular repulsion among the bulky hydroxypropyl groups is responsible for the enhanced paste clarity after modification. In previous work, it was also reported that carboxymethylation of starch improved starch paste clarity (Lawal *et al.,*

2007). Similarly, light transmittance of potato starch, corn amaranth and cassava starches increased after hydroxypropylation. (Pal *et al.,* 2002; Kaur *et al.,* 2004; Jyothi *et al.,* 2007).

Starch gel clarity seems to be directly related to the solubility (especially at 80°C and 90°C) of the starches where the amylose content of starches has a bearing on its ability to solubilise in water. Similar observation was made by Takizawa *et al*., (2004) for starches of potato, sweet potato and cassava, whose paste clarity increased with oxidative modification and related to their solubility. There were significant differences in gel clarity between native and hydroxypropylated water yam starches. Water yam starch has good gel clarity, though, comparatively pale to that of cassava starch (Betancur-Ancona *et al.*, 2003). The clarity of a starch gel directly influences the sheen and colour of products that contain them as thickener (Betancur-Ancona *et. al.*, 2003).

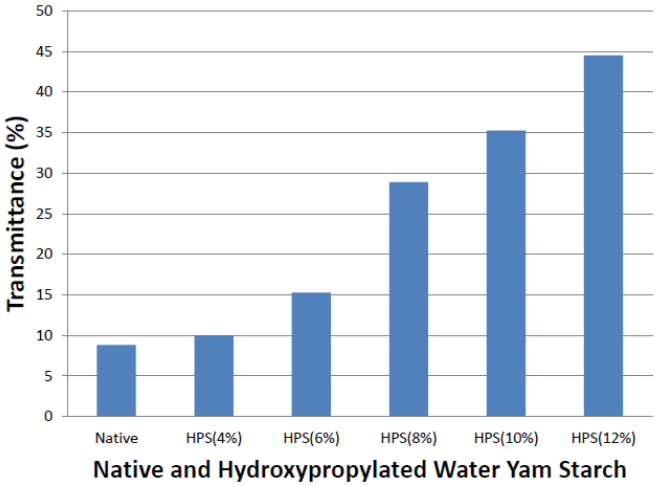

Figure 2. Starch gel clarity of native and hydroxypropylated water yam starch

3.5.5 Emulsion Capacity (EC) and Relative Occluded Volume (ROV)

Emulsion capacity of the native and hydroxypropylated water yam starches ranged from 16.91% (native starch 12%HPS) to 51.13% (native starch) as shown in Table 9. The 12%HPS had the highest emulsion capacity and relative occluded volume-functional properties which reduced with increase in molar substitution .This reflects a potential to form better oil-water interface, and hence better hydrophobicity (Adebowale *et al.,* 2009). This is in agreement with the report from oxidation of acha starch by Isah *et al.,* (2015) that oxidation improved the water and oil absorption capacity of the native acha starch and also the emulsion capacity was significantly improved .The emulsion capacity were in the order of 12%HPS (51.13%) >10%HPS (47.59%) >8%HPS (33.702%) > 6%HPS (27.71%) > 4%HPS (16.962%) > native starch (16.909%). There was significant difference ($P<0.05$) in the emulsion capacity of the starches. The 4% concentration of propylene oxide did not have a significant effect on the emulsion capacity of the native starch until when the concentration increased to 6%.The higher the concentration the better the emulsion capacity. High emulsion characteristic are an indication that the flour sample is an excellent emulsifier in various foods (Berger, 1997). Emulsion capacity of the hydroxypropylated water yam starches were superior to that of native starch and increased as modification level increased.

Oil absorption capacity of the starches correlated significantly with water absorption capacity ($r =0.979$, $P <0.01$) which is an indication that the starches generally had hydrophilic and hydrophobic groups that can absorb water or oil onto its structure matrices. OAC is strongly significantly correlated ($r = -0.849$, $P< 0.05$) with LGC as well as WAC ($r = -0.840$).At all temperature ,solubility is significantly correlated ($r =0.991,0.984, 0.964,0.967$, and 0.974) to WAC as well as OAC ($r = 0.968,0.963,0.947,0.957,0.91$). Starches in suspension form gel at elevated temperatures as a result of amylose fractions leaching, and swelling brought about by amylopectin fractions, thus leading to increasing ability of the starches to absorb water (Mweta, 2009). Gel clarity of starches is high when starch suspensions are fully gelatinized, which involves amylose solubilization and amylopectin swelling, hence the high significant relationships of starch gel clarity (SGC) and SP at 80°C and 90°C ($r = 0.986,0.983$, $P<0.01$); and SGC and solubility at 80°C and 90°C ($r = 0.986, 0.983$). There was strong significant correlation between OAC and the ability of the starch to form emulsion ($r = 0.967$, $P< 0.01$), WAC ($r =0.957$) and SGC ($r =0.977$) but insignificant to LGC of the starches.

A strong correlation was observed at swelling power of 50°C (r =0.983) with emulsion capacity when full gelatinization had not taken place, this implies that most starch emulsions are unstable at higher temperatures. Swelling power and solubility of the starches correlated significantly regardless of the temperatures effects and all there is strong association between the functional properties which were all significantly correlated positively except least gelation concentration.

Table 9. Emulsion capacity and Relative occluded volume of native and hydroxypropylated water yam starch

Sample	Emulsion capacity (%)	Relative occluded volume (v/v)
Native	16.909±0.8	0.175±0.0
4% HPS	16.962±0.3	0.203±0.0
6% HPS	27.71±0.3	0.215±0.0
8% HPS	33.702±0.3	0.311±0.0
10% HPS	47.59±0.18	0.380±0.0
12% HPS	51.13±0.7	1.046±0.0

Means in columns are significantly different at 5% level (P< 0.05)

3.5.6 Pasting Properties of Native and Hydroxypropylated Starch

Real-time viscosity measurements at constant shear and regulated temperature change rates undergone in a heating, holding and cooling pattern/profile for starches can reveal shear, stress and other tensile properties of a starch paste. The pasting properties of the native and hydroxypropylated water yam starches and their significant differences are presented in Table 10.The pasting profile is as shown in figure 3. Agronomic practices have a great influence on the pasting properties of starch. Harvesting time has been linked to the pasting of yam starch (Huang *et al.,* 2006; P´erez *et al.,* 2011; Akinoso & Abiodun, 2013). Peak viscosity of the native water yam starch was 583.63RVU while peak viscosity for the hydroxypropylated starches ranked in the order of 8%HPS (571.17RVU) > 12%HPS (505.29RVU) >4%HPS (454.54RVU) >10%HPS (450.92RVU) >6%HPS (297.83RVU). Peak Viscosity for the native starch was the highest (583.63RVU), this is higher than values from Ji *et al.,* (2003) who reported PV in the range between 152 and 222 RVU for selected corn lines. A report from Oke *et al.* (2013) gave values for Peak Viscosity and Final Viscosity, Pasting Temperature as 400.92RVU and 504.08RVU, 80.38-86.15^{o}C, respectively for native water yam starches, the difference may be attributed to the cultivar. Peak viscosity of starches from *D. alata*, *D. rotundata*, *D. cayenensis*, and *D. Dumetorum* (1 genotype for each) ranged from 2028 to 3893 mPa (Farhat *et al.,*1999). PV of starches from 27 genotypes of *D. Rotundata* ranged from 177RVU to 524RVU (Otegbayo *et* al *D. Dumetorum* (1 genotype for each) ranged from 2028 to 3893 mPa (Farhat *et al.,* 1999). PV of starches from 27 genotypes of *D. Rotundata* ranged from *al.,* 2014).Among the hydroxypropylated starches 8%HPS had the highest peak viscosity, all the hydroxypropylated starches had lower Peak Viscosity compared to the native starch and they are all significantly different. The higher peak viscosity exhibited by native starch in this study was due to higher granule rigidity and integrity, associated with the presence of amylose (Karim *et al.,* 2007).Variations in Peak Viscosity was observed for the modified starches and this in agreement with Kaur *et al.*, (2004), who reported that hydroxypropylated starches present variations in PV property because of a granular associative force reduction. This is at variance with Choi *et al*., (1997) who reported that peak viscosity of hydroxypropylated corn starch increased with increase in the degree of substitution.The reduction in viscosity of hydroxypropylated starch upon cooling is related to its amylose content. However, this justification does not apply with regard to the modified starches, which show low viscosity upon cooling and low amylose content in comparison with native starch (Table 5). This performance may have occurred because of the increase in associative forces within the native starch granules that resulted in decreased penetration of water (Schmitz et al., 2005). This is in contrast with cassava starch (Chatakanonda *et al.*, 2011, Jyothi *et al*., 2011), fermented cassava starch (Gomes *et al*., 2004b) and the increasing peak viscosity of starch may be linked to increasing granule size (Huang *et al.,* 2006). This reduction may be due to hydroxypropylation treatment tightening the connections among the molecules of starch, making them to assume a more stable could be because the amorphous region (likely containing amylose) was not disrupted by treatment, thus the granular structure was not weakened. Consequently, when shear was applied to the starches during pasting, the granules could attain their maximum swelling capacity, resulting in increase of the peak viscosity.The decrease in peak viscosity observed in hydroxypropylated water yam starch could be related to the presence/absence of surface proteins (Han & Hamaker, 2002). The protein on the surface of the starch granule would be partially removed when treated with propylene oxide (Han & Hamaker, 2002).This premise is supported by our protein determination, which showed that the protein content for hydroxypropylated

starches decreased significantly compared to that of native corn starch (Table 5). This native starch may find application in foods requiring high viscosity. Native starch and hydroxypropylated starches showed significantly distinct viscosity characteristics, which determine their possible industrial applications.

The native water yam starch had the highest trough viscosity (508.96RVU) and breakdown viscosity of 896cp, significant differences were observed among the starches (native and hydroxypropylated. An increase in breakdown viscosity was observed at 8%HPS (312.75RVU) to 12%HPS (115.67RVU). Trough viscosity (TV) is the lowest viscosity attainable in the isothermal phase of the viscosity profile; while the breakdown viscosity (BV) is the deduction of trough viscosity from peak viscosity, as a measure of the susceptibility of a starch paste to disintegrate and lose viscosity during the high temperature holding phase (Han & Hamaker, 2002).Breakdown is a measure of the response of starch pastes to shear-thinning during the holding period at 95 °C (Lai *et al.,* 2004). Thus, breakdown viscosity indicates the tendency of starch to resist shear force during heating (Karim *et al*., 2007). Shi & BeMiller (2000), and Pal *et al.,* (2002) reported that hydroxypropylated corn starch showed increased shear breakdown because the susceptibility of swollen starch granules to prolonged stirring was increased by the modification. This is in agreement with Han *et al.,* (2004) for hydroxypropylated waxy rice and corn starches which showed lesser BV compared to the native starches. The reason is unclear, but we presume that the absence of amylose in the waxy starches tested might have produced different shear-thinning results (Han *et al.,* 2004).Among the hydroxypropylated starches, only 8%HPS was highly shear thinning while 6%HPS was slightly shear thinning. Others (native 10%HPS and 12%HPS) are moderately shear thinning. Degree of shear thinning is a property that may be harnessed in enhancing heat transfer in canned foods and as suspension aids. The 6%HPS had the lowest breakdown viscosity, hence the higher the breakdown in viscosity, the lower the ability of the sample to withstand heating and shear stress during cooking (Adebowale et al., 2005). Breakdown values were significantly higher ($p \leq 0.05$) for 8-12%HPS starches which is an indication that the granules were not strong and did not resist breakdown under shear and heat, a result also noted by Singh *et al.,* (2006). Low breakdown coupled with high viscosity is a desirable property of starch because its paste has a non-cohesive texture suitable for many food and industrial applications.

The final viscosity (FV) of the starches ranged from 811.63RVU to 303.92RVU, with the lowest values observed in 8%HPS and the highest found in native starch. The final viscosity (FV) is the viscosity after holding cooked starch at the cooling phase. It is a crucial parameter used to define the quality of starch-based foods as it indicates the ability of the material to form a viscous paste or gel after cooking and cooling and its resistance to shear force during stirring (Adebowale *et al*., 2008). According to commonly observed pasting properties of hydroxypropylated starches (Chuenkamol *et al.,* 2007; Gunaratne & Corke, 2007; Jyothi *et al.,* 2007; Lawal, 2009; Liu *et al.,* 1999), the enhanced hydration and swelling of starch granules via starch hydroxypropylation result in rapid viscosity development and the increased peak viscosity (corresponding to the increase in breakdown viscosity) of the starch paste. Also, bulky substituent restricts inter-chain association, leading to reduced final viscosity (associated with decreased setback viscosity) (Chung *et al.,* 2010)

The setback viscosity (SV) is the increase in viscosity of the starch paste during the cooling phase of pasting. It's the deduction of trough viscosity from final viscosity and represents the ability of the swollen solubilised granules to re-associate, a feature more commonly linked to amylose than amylopectin. Setback reflects the retrogradation tendency of starch upon cooling (Zaidul *et al*., 2003) i.e. increase in viscosity of starch pastes during cooling indicates propensity of the various granules (swollen intact and broken granules, colloidal dispersions, and dissolved molecules) to retrograde as temperature of the paste decreases (Adebowale *et al*., 2009).

The SV among the hydroxypropylated starches ranked in the order of 4%HPS > native >12%HPS >6%HPS >10%HPS >8%HPS.Setback viscosity reduced after hydroxypropylation, this is in agreement with the report on hydroxypropylation of white yam, *Dioscorea rotundata* (Lawal *et al.,*2008).The native water yam starch showed a higher tendency to regain viscosity during cooling than the native starches do, thus having significantly higher SV, and may be more susceptible to retrogradation as the swollen solubilised starch which were earlier leached out tend to re-associate again, re-establishing a highly ordered network of increased intermolecular bonding between the molecules. Similar discoveries were made for African yam bean (Adebowale *et. al.*, 2009); fermented cassava starch (Gomes *et al.*, 2004b); and red sorghum starch (Adebowale *et al*., 2005). The setback is more pronounced at lower concentrations (4%HPS) than higher concentrations (6-12%HPS) water yam starch. The bulky hydroxypropyl groups introduced during the modification prevented the structural realignment of leached amylose molecules after gelatinization (Lawal, 2008), thus minimizing retrogradation. This is compares favourably with findings of Lee & Loh, (2011) in the hydroxypropylation of babana starch, during which decrease of setback values in hydroxypropylated starch was observed with

increasing of MS. For the hydroxypropylated water yam starches, it is as a result of steric effects imposed by the bulky hydroxypropyl groups which inhibit proper alignment of starch chains during chain aggregation and crystallization (Perera & Hoover, 1999), thus facilitating lower setback values and tendency to retrograde (Betancur-Ancona *et al*., 1997; Yu *et al.*, 2005).It may also be as a result of weakening of intermolecular interactions between amylose molecules thus hindering realignment of the molecules. Besides, the presence of low molecular weight sugars tend to bind up more moisture (necessary for furnishing hydrogen and hydroxyl ions) that would have been otherwise available for amylose molecules to form strong re-associating hydrogen bonds between one another (Yu *et al.*, 2005).Setback of the native water yam starch was found to decrease when starches were hydroxypropylated (Wattanachant *et al.,* 2002).Pasting temperature was recorded when the rate of change of viscosity above the set point was first achieved (Jin-song, 2008), which indicates the onset gelatinization temperature of the starch. Pasting temperature decreased with increase in MS, it ranked as native (84°C) >4%HPS (84.1°C) >6%HPS (81.3°C) >8%HPS (79.5°C) >10%HPS (81.95°C) >12%HPS (81.93°C) and they were all significantly different. Hydroxypropylation reduced the pasting temperature, this is in agreement with Choi, et *al.,* (1997) in the hydroxypropylation of corn starch, Lee and Yoo (2011) (banana starch hydroxypropylation) and Phisut Naknaen (2014) in the hydroxypropylation of jackfruit seed starch. The high pasting temperature of native starch indicated its higher resistance towards swelling. Seetharaman *et al.,* (2001) reported pasting temperatures in the range of 74.9–84.7°C for Argentinian corn landraces. Hydroxypropyl groups are hydrophilic in nature and when introduced into starch granules, weaken the internal bond structure holding the granule together, leading to an increase in accessibility of starch granules to water. This reduction in bond strength is reflected in starch pasting temperature .Peak time reduced significantly after hydroxypropylation except at 6%HPS that a sharp increase was observed. The time required to cook the starch paste to the peak viscosity, the peak time (PkT), ranged from 7mins (native starch) to 4.467 minutes which implies that they are not difficult to cook to peak viscosity. Hydroxypropylated starches have hydrophilic surface activity, making their surface easily accessible to hot liquor, a factor attested to by their high solubility.

Pasting temperature of the starches correlated significantly with peak time ($r = 0.953$, $P<0.01$) similar to that reported for starches of lesser yam, water yam, elephant yam, new cocoyam, *kudzu*, sweet potato, sago, taro, arrow root and cassava (Srichuwong *et al.*, 2005).Trough viscosity and final viscosity were significantly correlated ($r = 0.938$).Breakdown viscosity was positively correlated with the pasting temperature ($r = 0.900$). Kaur *et. al.,* (2006) reported negative correlation of Breakdown viscosity with Pasting Temperature.

Table 10. Pasting properties of native and hydroxypropylated water yam starch

Sample	Peak Viscosity PV (RVU)	Trough Viscosity TV(RVU)	Breakdown Viscosity BV(RVU)	Final Viscosity FV(RVU)	Setback Viscosity SV(RVU)	Peak Time(mins)	Pasting Temperature (°C)	Breakdown Ratio
Native	583.63±15.03	508.96±13.49	74.67±1.53	811.63±7.37	302.67±6.13	5.6±0.0	84±1.13	0.87(MST)
4% HPS	454.54±7.48	410±3.42	44.54±4.07	750.67±8.37	340.67±4.95	5.53±0.0	84.1±0.0	0.90(SST)
6% HPS	297.83±11.55	284.54±10.90	13.29±0.65	466.38±11.61	181.83±0.71	7±0.0	86.4±0.1	0.96(SST)
8% HPS	571.17±2.83	258.42±0.71	97.67±2.12	303.92±7.07	45.5±6.36	4.46±0.0	79.5±0.6	0.45(HST)
10% HPS	450.921±10.14	352.83±1.53	97.67±8.01	483.71±3.36	130.46±1.24	5±0.1	81.95±0.6	0.78(MST)
12% HPS	505.29±9.02	389.63±13.73	115.67±4.71	578.17±1.53	188.5417±12.20	5.13±0.1	81.93±0.6	0.77(MST)

Means in columns are significantly different at 5% level ($P< 0.05$). HST-highly shear thinning, (BDR<0.5), MST-moderately shear thinning (BDR of 0.5-0.8), SST-slightly shear thinning (BDR OF 0.8-1.0).

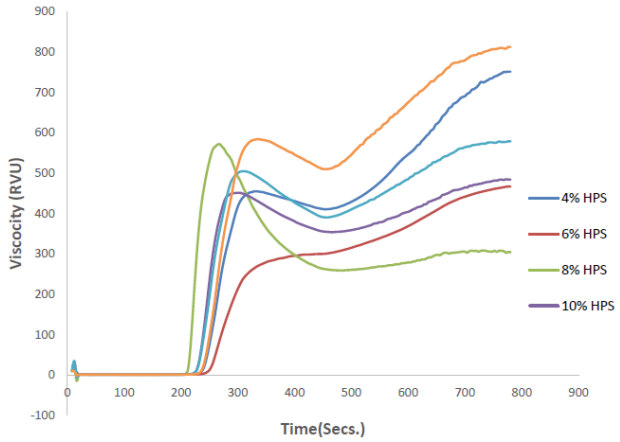

Figure 3. Pasting profile of native and hydroxypropylated water yam starch

3.5.7 Freeze Thaw Stability (FTS) of Native and Hydroxypropylated Water Yam Starch

Percentage syneresis increased progressively as the number of freeze thaw cycles rose during which native starch gel (spongylike in texture) exuded a lot of water (Figure 4). Comparatively, the gels from hydroxypropylated water yam starches were soft and elastic, discharging less water. Noteworthy is the fact that syneresis was not observed in hydroxypropylated starch until the third cycle for 4-6%HPS (syneresis of 4.28% and 3.39% respectively) and fourth cycle for 8-12%HPS (1.09%, 1.30% and 1.13% respectively). Also, syneresis apparently reduced as the MS of the hydroxypropylated starches increased with the greatest reduction being at 8%HPS. The amount of water exuded by the frozen starches differed significantly ($P \leq 0.05$).This is similar to what was established from the hydroxypropylation of finger millet (Lawal, 2009) and syneresis where in the former became higher with storage duration (Lawal, 2008). Syneresis in freeze–thawed gels is attributed to the increase of molecular association between starch chains at reduced temperature, exuding water from the gel structure. This compares favourably with the observation by Afolabi & Owolabi (2005) on hydrothermal treatment of finger millet starch. They noted a rise in hydrophilic and hydrophobic tendencies with increasing level of hydrothermal treatment while acid and enzymatic modifications culminated in decrease in syneresis (Lawal, 2009). The Drastic reduction in diminished syneresis following modification could also be attributed to the impediment of inter-chain bonding between the starch molecules by the incorporated hydroxypropyl groups. In addition, the hydrophilic nature of the hydroxypropyl groups enhanced water-holding ability of the starch pastes, thereby limiting the amount of water exuded. Schmitz *et al.,* (2005) came to a similar conclusion during which in the hydroxypropylation of cassava starch during which the lowest loss of water in the freezing–thawing cycles was obtained.. Hydroxypropylation effectively retards the gelling and retrogradation tendencies of starch gels, increases water holding capacity, and improves the freeze-thaw stability or cold storage stability (Hoover *et al.,* 1988; Liu *et al.,* 1999). Freeze-thaw stability expressed as percentage syneresis is also used as an indicator of starch retrogradation.Retrogradation property is a fundamental characteristic of starch utilisation in the food industry, where it is used as a thickening agent in sauces, allowing for unaltered transparency during storage (Schmitz, 2004).When starch gel is stored for a long time under refrigerated or frozen conditions, there is a gradual increase in rigidity as crystallites begin to form, and phase separation between polymer and solvent occurs. Considering the amount of water released in all the cycles, gels from 8-12%HPS exhibited lowest tendency to syneresis compared to 4-6%HPS and native starches. This result indicates that hydroxypropylated starch at high concentration of propylene oxide (8-12%HPS) is more stable to freeze-thawing than lower concentration (4-6%HPS) and hence would be better suited for use in frozen products than others (Mweta *et. al.,* 2008). This is in agreement with the findings of Lee & Yoo, (2011) who reported that increase in propylene oxide during hydroxypropylation substantially improved the freeze-thaw stability of Saba banana starch.. The fall in syneresis for 8-12%HPS can be attributed to reduction in the inter chain bonding between the starch molecules (Lawal, 2009). Among all the starches, 8-12%HPS would be most suitable as they presented the highest free-thaw stability

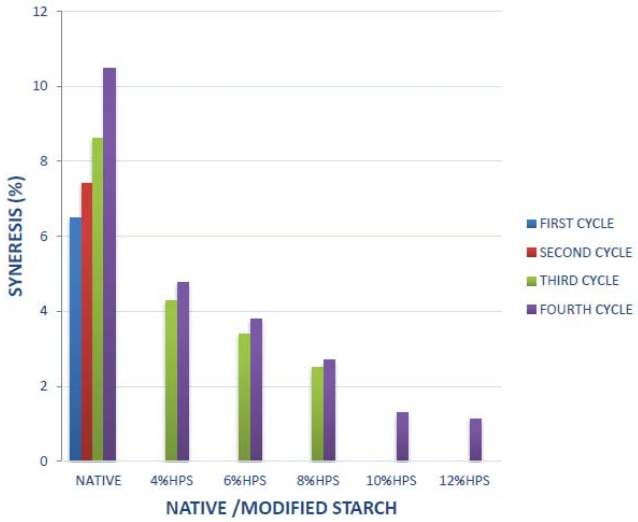

Figure 4. Freeze thaw stability of native and hydroxypropylated water yam starch

3.5.8 Fourier Transforms Infrared (FTIR) Spectroscopy Analysis

The FT-IR spectra of the native and hydroxypropylated yam starch indicated bands corresponding to the stretching of the principal groups. This compares favourably with the findings of some research works (Mano *et al*., 2003,Xu *et al*., 2007, Guerra *et al*., 2008).Among the FTIR spectra of hydroxypropyl starches, 12%HPS showed a stronger peak band at 3645.7 cm^{-1} than the native and other hydroxypropylated starches.

The FTIR spectra of 4%HPS had shown characteristic peaks between 3228.78-3645.7 cm^{-1} indicating C-H aromatic stretch, 1645.58 -1646cm (C=C stretch), 929.45-932.45 -cm (C-H stretch), 2922 cm (C-H aliphatic stretch). The observed absorption band at 1004-1155 cm^{-1}, is a C-O stretch which indicates the presence of alkyl ethers group. it demonstrated the peak at around 1413 cm^{-1} as the O-H bend (Mano *et al*., 2003).The FTIR spectra of all hydroxypropylated starch samples reflects the typical absorption of the starch backbone as well as the additional peaks. New peaks occurred at 1050.7 cm^{-1} and 1260 cm^{-1} indicating the substitution of alkyl ether group on the starch molecular chains. The frequency of C=O is much lower from the value found for the parent carboxylic acid possibly due to the resonance effect. During the ionization, the formation of COO- group would exhibit resonance effect between the two C-O bonds. Consequently, the characteristic carbonyl absorption vanished and are replaced by two bands - 1645 cm^{-1} and 1456 cm^{-1} and between 1413 cm^{-1} and 1301 cm^{-1}, corresponding to the anti-symmetrical and symmetrical vibrations of the COO- structure**.** The FTIR spectra of water yam starch and hydroxypropyl starch was found at 1004 cm^{-1}. The spectra also showed that the absorption at 1371.5 cm^{-1} could be due to the overlapping of CH2 and O-H in-plane bending. The absorption band of O-H stretching is reduced in intensity, shifting to 3645.7cm^{-1}. This shift in the O-H group could be attributable to the interaction of O-H group with the carboxylic group. The reduction in intensity of this band may also correspond to partly substituted O-H group with the hydroxypropyl moeity during the etherification process. Fang *et al*., (2002) observed similar changes of the O-H vibration during the modification of potato starches.

4. Conclusion and Recommendation

Generally, the results obtained in this work indicate that hydroxypropyl water yam starch has very good physicochemical, morphological and functional parameters that could be utilized and thereby position this under-utilized starch source for good starch market globally.

Extensive work can still be carried out on the degree of retrogradation, texture profile analysis (TPA) during storage of the water yam starch and gelatinisation using differential scanning calorimetry (DSC).Sorption isotherm behaviour of the starch to determine the safe storage temperature could also be determined and the concept of plasticization of water using nuclear magnetic resonance (NMR) spectroscopy. In addition, use of x-ray diffractograms (XRD), scanning electron microscopy (SEM), for the native water yam starch to show the structure pattern of the starch and to determine the surface properties and crystallinity is also an additional knowledge to widen its food application.

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Anti-hemolytic, Anti-lipid Peroxidation and Antioxidant Properties of Three Plants Locally Used to Treat Metabolic Disorders: *Allium sativum, Persea americana* and *Citrus sinensis*

Boris G. K. Azantsa[1], Mary-Ann A. Mbong[1], Guy RN Takuissu[1], Rosane S. Matsinkou[1], Imelda L. N. Djuikoo[1], Janvier F. Youovop[1], Judith L. Ngondi[1] & Julius E. Oben[1]

[1]Laboratory of Nutrition and Nutritional Biochemistry, Department of Biochemistry, University Of Yaounde 1, p.o. Box: 812 Yaounde, Cameroon

Correspondence: Dr. Azantsa K. G. Boris, Laboratory of Nutrition and Nutritional Biochemistry, Department of Biochemistry, University Of Yaounde 1, PO. Box: 812 Yaounde, Cameroon. Tel: +237-677-920-184. E-mail: borisazantsa@yahoo.fr

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Abstract

Citrus sinensis, *Persea americana* and *Allium sativum* are good sources of large number of various bioactive substances including antioxidants. Antioxidants play an essential role in the prevention and treatment of type 2 diabetes and other metabolic diseases. This study was conducted to evaluate and compare the anti- lipid peroxidation, anti-hemolytic and antioxidant capacity of aqueous extracts of the three plants *in vitro*. Polyphenolic and flavonoids content of aqueous extracts (EA) of plants were determined. The anti-lipid peroxidation was evaluated on homogenates of rat liver. Anti-haemolytic effects were assessed using erythrocyte system model induced free radicals from human red blood cells. Total antioxidant capacity and antiradical or scavenging capacity were evaluated on biological free radical systems (OH, $NO^{\cdot}$) and synthetic radicals (DPPH and $ABTS^{+}$). Results showed that, different extracts possess variable amounts of polyphenolic compounds. They exhibited significant anti-hemolytic and anti-lipid peroxidation activities. Aqueous extracts of *Citrus sinensis* are powerful scavenger of $OH^{\cdot}$ (IC_{50}=1.05x10^{-3}mg/ml) and $NO^{\cdot}$ (IC_{50}= 1.29x10^{-3} mg/ml) free radicals while *Persea americana* seed extract possesses the highest anti-lipid peroxidation capacity (59.72%) and the best scavenging capacity against DPPH and ABTS. Red blood cells were highly protected (at 97.87%, $p<0.05$) by *Allium sativum* extract at 1mg/mL and *Citrus sinensis* (87.7%, $p<0.05$). Therefore, anti-hemolytic, anti-lipid peroxidation effects of the aqueous extracts of the three plants can justify their use and efficacy in alternative treatment of metabolic diseases.

Keywords: anti-hemolytic, anti-lipid peroxidation, antioxidant, plant extracts, diabetes mellitus

1. Introduction

Type II diabetes mellitus (T2DM) is a metabolic disorder characterized by prolonged hyperglycemia, insulin resistance and/or reduced insulin sensitivity (Kooti, Maryam, Zahra, Damoon & Majid, 2016). It is associated to several complications including macro and micro-angiopathies. T2DM constitutes a risk factor for cardiovascular diseases. A recent report estimates at 500 million its worldwide prevalence; comparable in high and low-income countries (Kaiser, Nicole & Wouter, 2018). Its diagnosis includes measurement of fasting blood glucose and glycated haemoglobin (HbA1c) (Aggarwal et al., 2013). In T2DM patients, hemorheological parameters are often disturbed due to excess glucose concentration in the blood. This affects haematocrit, plasma proteins, erythrocyte aggregation and deformability (Youn et al., 2017). All these disturbances affect blood viscosity of diabetic patients compared to healthy patients (Young, Michael, Mooney & Daniel, 2008). Erythrocytes or red blood cells (RBC) play a key role in the metabolism as well as in oxygen (O_2) and drug transportation. Attacks of polyunsaturated fatty acid of lipids and proteins found in erythrocyte membrane are responsible for hemolysis and can justify the greater osmotic fragility observed in diabetic patients. The osmotic fragility has been proven to be positively correlated to glycated hemoglobin (Kung, Tseng & Wang, 2009; Ebrahimzadeh, Nabavi & Nabavi, 2009; Afsar et al., 2016). Hyperglycaemia in T2DM causes changes in the shape of RBC and provokes a mild corrosion, making them to aggregate, leading to enhanced viscosity and retarded motility (Alshalhi et al., 2018; Leal, Merel & Giel, 2018). Cell membranes of RBC are made up of

proteins and lipids, mainly phospholipids. Phospholipids ensure its biological function, but glycation of membrane proteins alters its function. Such alterations occur when the generation of ROS resulting from hyperglycaemia creates an oxidative stress (Rodrigo, Bächle, Araya, Prat & Passalacqua, 2007, Omale & Alewo, 2014). Lipid peroxidation also disturbs activities of membrane bound enzymes by changing phospholipids and fatty acid compositions, decreasing $Na+/K^{+}$-ATPase activity (Rodrigo et al., 2007; Viskupicova et al., 2015). *In vitro*, an increased concentration of glucose causes hemolysis, eryptosis, and calcium accumulation. It intensifies lipid peroxidation and loss of activities of erythrocyte enzymes like glutathione-s-transferase and reductase (Viskupicova et al., 2015; Asmat, Khan & Ismail, 2016).

There are various side effects associated to different classes of prescribed drugs; varying from nausea, diarrhea, vomiting and hypoglycemia. Medicinal plants offer less side effects, are more affordable and effective (Kooti et al., 2016). *Allium sativium bulb*, *Persea americana* seed and stem bark of *Citrus sinenis* have been intensively used to treat diabetes (Bordoloi, & Dutta, 2014). Their effects on the reduction of glycaemia solely or combined have been reported (Azantsa et al., 2017; Azantsa et al., 2018), with proven safety and efficacy (Yasir, Das, & Kharya, 2010). *Persea americana* mill (Lauraceae) is a tree plant also called avocado or alligator pear (Ranade & Thiagarajan, 2015). It is recommended for anaemia, exhaustion, hyper-cholesterolemia, hypertension, gastritis, and gastro-duodenal ulcer (Antia, Okokon & Okon, 2005; Kumala, Utami, & Sari, 2013). The methanolic extracts obtained from the seeds demonstrated high *in vitro* DPPH scavenging activity (Vinha, Moreira, & Barreira, 2013; Dreher & Davenport, 2013).

Allium sativum also called garlic, is a member of the Liliaceae family. It is a cultivated food and spice highly exploited throughout the world. *Allium sativum* consumption has significant effects on lowering blood pressure, prevention of atherosclerosis, reduction of serum cholesterol and triglyceride, blood glucose and inhibition of platelet aggregation (Eidi et al., 2006; Chan et al., 2013). *Allium sativum* scavenges ROS by increasing the cellular antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase, and enhancing glutathione levels (Borek, 2001; Bayan, Koulivand & Gorji, 2014). Ethanolic extracts of *Allium sativum L.* bulbs possess antioxidant potential against hydroxyl peroxide and reducing power ability on DPPH radicals (Narendhirakannan & Rajeswari, 2010; Huzaifa, Labaran, Bello, & Olatunde, 2014).

Citrus sinensis L. that belongs to the Rutaceae family is one of the most cultivated plants in the world. It is a source of food and vitamins for humans (Jensen et al., 2008; Etebu, & Nwauzoma, 2014). The leaves' extracts of *Citrus sinensis* possess antioxidant activities (Azantsa et al., 2017a). If the antioxidant properties of some extracts of these plants have been reported, no comparative studies exist on the efficacy of their aqueous extracts. This study aims at comparing the anti-hemolytic effect of the three plants based on radical-induced erythrocyte lyses; to assess the anti-lipid peroxidation effects; to determine the antioxidant and scavenging capacity against free radicals *in vitro*.

2. Method

2.1 Chemicals and Reagents

Folin-ciocalteu reagent, catechin, ethanol, aluminium chloride ($AlCl_3$), potassium acetate (CH_3COOK), quercetin, hydrogen chloride (HCl), sodium chloride (NaCl), ferric sulfate ($FeSo_4$), thiobarbituric acid (TBA), ascorbic acid, sulfanilic acid, sodium nitroprusside, sulphuric acid, naphtylethylene diamine dichloride and copper sulfate ($CuSO_4$) were purchased from Sigma (USA). Human blood sample was purchased from CHU-hospital, Yaounde, Cameroon.

2.1 Collection of Plants and Preparation of Aqueous Extracts

Persea americana seeds and stem *b*ark of *Citrus sinensis* were harvested in march 2017 in Mendong -Yaounde (City capital of Cameroon). *Allium sativum* cloves were bought from Mokolo market, Yaounde, Cameroon. *Citrus sinensis* L (voucher N° 25859/SRF), *P.americana mill* (voucher N° 31940 HNC), *Allium sativum (*voucher N° 44810HNC) were identified at the National Herbarium.

The collected plant parts were rinsed with distilled water. The peels of *Allium sativum* bulb and *Persea americana* seeds were removed from the flesh. Parts were cut into small pieces and shade dried in open air till constant weight. Dried materials were ground to obtain powder. One hundreds (100) g of each powder was mixed with 800 ml distilled water. The mixture was vigorously shaken and kept for 24 hours, to enhance proper dissolution and extraction of the bioactive compounds in the samples. Each solution was shaken vigorously and filtered with Whatmann filter paper (Whatmann Int. Ltd., Maidstone, U.K) at room temperature. Filtrates obtained were evaporated in a rotary evaporator (BUCHI Rotavapor R-114, Switzerland) at 45 ºC until the extracts became dry. The aqueous extracts obtained were stored in polyethylene bags to avoid moisture.

2.3 Determination of Total Polyphenol Content

The total phenolic content of each extract was determined spectrophotometrically, using the Folin-Ciocalteu method as described by Singleton and Rossi (1965). Briefly, to 30 µL of the extract, 1 mL of the Folin- ciocalteu reagent (diluted 10 times with distilled water) was added. Thirty minutes after incubation at 25 °C, absorbance was read at 750 nm with a spectrophotometer. Catechin was used as the standard at different concentrations (0 – 1000 µg/mL) for the calibration curve. Analyses were performed in triplicate and the content of phenolic compounds in extracts was expressed in µg equivalent catechin/mg of extract.

2.3 Determination of Flavonoids Content

Total flavonoids content was evaluated using the method described by Aiyegoro and Okoh (2010). To 1 mL of extract (1 mg/mL) prepared in ethanol, 1 mL of aluminium chloride, 1 mL of potassium acetate and 5.6 mL of distilled water were added. The mixture was incubated at 25 °C for 30 minutes. Absorbance of the reaction mixture was measured at 420 nm with the spectrophotometer. Quercetin was used as standard at different concentrations (0 – 1000 µg/mL) for the calibration curve. The analyses were performed in triplicate and the amount of flavonoids in extracts was calculated and expressed in µg equivalent quercetin/mg of extract obtained from the calibration curve.

2.4 Ant-lipid Peroxidation Effects of Citrus sinensis, Allium sativum and Persea americana Extracts

Anti-lipid peroxidation of plant extracts was assessed as described by Prasanth, Shasidhara & Sridhara (2000). Firstly, a liver was isolated, dissected from a Wistar rat and washed in NaCl (0.9%); then cut into pieces, ground and mixed in 10 % (w/v) of phosphate buffer 0.1 M, pH 7.4. Three hundred (300) µL of extract prepared at various concentrations (250, 500, 750 and 1000 µg/mL) were added to 500 µL of liver homogenate. The mixture was incubated with 100 µL of NaCl and the lipid peroxidation was initiated by addition of 100 µL of a ferric sulfate (15 mM) solution. The mixture was incubated at 37 °C for 30 min. One (1) ml of equal volume of TBA (1%)/Hcl (10 %) was added to the solution followed by the addition of 1 ml of ascorbic acid (6 mM). The final mixture was heated at 80 °C for 20 min in a hot water bath. After 30 min, the tubes were cooled in water for 10 min and centrifuged at 800 g for 15 min. Absorbance was read at 532 nm against the blank consisting of reagents. The percentage inhibition of lipid peroxidation was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.5 Anti-hemolytic Activity of Citrus sinensis, Allium sativum and Persea americana Extracts

Anti-hemolytic activity of plant extracts was assessed as described by Arbos, Claro, Borges, Santos & Weffort-Santos (2008) with modifications. Measurement was done based on radical- induced erythrocyte lyses using an extract concentration of 1mg/mL only. A human blood sample was purchased from blood bank of the University Teaching Hospital (CHU), Yaounde, Cameroon. Three milliter (3mL) of blood was collected from the sample and centrifuged, the supernatant was discarded and red blood cell suspension used for anti-hemolytic assay. A volume of 0.2 mL of extract was mixed with 1 mL of NaCl (0.9 %). To the mixture, was added 0.1 mL of red blood cell suspension, then incubated at room temperature for 30 min. Then, 0.1 mL of CuSO4 (0.1 M) was added to induce haemolysis via oxidative degradation of the cell membrane. After 30 min of incubation, absorbance was read at 532 nm against a blank (1.3 mL of NaCl and 0.1 mL of red blood cell suspension). Complete haemolysis (control) was achieved by mixing 0.2mL of NaCl (0.9 %), 0.1 mL of the RBC suspension and 1.1 mL of distilled water. The level of protection of RBC cell membrane by extracts was calculated using the formula:

$$\text{Protection level(\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.6 Determination of Radical Scavenging Capacities

The radical scavenging capacities (RSC) of different crude extracts were evaluated against DPPH•, $ABTS^{+}$, NO• radicals as previously described by Azantsa et al., (2017a).

2.6.1 DPPH• Scavenging Assay

DPPH•-RSC assay was based on measurement of the loss of DPPH• color after reaction with test compounds. The DPPH• scavenging activity was measured as reported by Katalinié, Milos, Modun, Musi & Boban (2004). Fifty microliters (50 µL) of the extract at different concentrations (2.5, 5.0, 7.5 and 10 mg/mL) were introduced in 1.950 mL of an ethanolic solution of DPPH. After 30 minutes of incubation in the dark, the absorbance was

measured at 515 nm against the blank consisting of reagent. Tests were done in triplicate. RSC was expressed as percentage inhibition and calculated by the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.6.2 $ABTS^+$ Scavenging Assay

The ABTS-RSC was measured according to a modified procedure reported by Re *et al*. (1999). One hundred microliters (100 µL) of the extract at different concentrations (2.5, 5, 7.5 and 10 mg/mL) was introduced in test tubes, followed by 1000 µL of $ABTS^+$ reagent, then incubated for 30 minutes in the dark. The absorbance was then read at 734 nm against the blank consisting of reagents. Tests were carried out in triplicates. RSC was calculated by the following formula:

$$\text{Inhibition(\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.6.3 Nitric Oxide (NO•) Scavenging Assay

NO•-RSC was evaluated by measuring the accumulation of nitrite (formed by the reaction of $NO^{\cdot}$ with oxygen).The scavenging activity of extracts on nitric oxide was evaluated using the method of Sreejayan and Rao (1997). Two (2) ml of sodium nitroprusside (10mM) dissolved in phosphate buffer saline (pH 7.4) was mixed with 1mL of extract at various concentrations (0.25-1mg/mL). The mixture was then incubated at 25°C. After 15min incubation, 0.5 mL of the incubated solution was pipetted and mixed with 1mL sulfanilic acid (0.33% in 20% acetic acid) at 25^0 C for 5 minutes, followed by the addition of 1mL of naphthylethylenediamine dichloride (0.1% w/v). The mixture was then incubated at room temperature for 30 min and its absorbance read at 540nm. Test were carried out in triplicate. RSC was calculated by the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.7 Antioxidant Activities

2.7.1 Total Antioxidant Capacity (TAC)

This reduction mechanism was evaluated using the method described by Prieto, Pineda & Aguilar (1999).The extract (0.2 mL) prepared in ethanol was mixed with 2 mL of the reagent (0.6 M of sulphuric acid, 28 mM of sodium phosphate and 4 mM of ammonium molybdate). All the tubes were sealed and incubated at 95 °C for 90 min. After cooling the tubes, the absorbance of the solutions were measured at 695 nm against the blank containing 2mL of the reagent and 0.2 mL of ethanol. The total antioxidant capacity was expressed in milligram equivalents of ascorbic acid per gram of dry materials (mg EAA/ g dry materials).

2.7.2 Determination of Inhibitory Concentration 50 (IC_{50})

The concentration (in the final reaction media) that causes a decrease of initial absorbance (control) by 50% is defined as IC_{50}. The IC_{50} values for all RSC were determined by regression of the inhibition values.

2.8 Statistical Analyses

Data were expressed as the averages of triplicate analyses and as mean ± standard deviation and as percentages. The statistical package for social science (SPSS) 20.0 (*Chicago-Illinois Inc*.) was used. One-way ANOVA followed by post-hoc Tukey's test was performed to compare variable amongst groups. Chi square test was used to compare percentages. Results with a $p < 0.05$ were considered significant. Using GraphPad Prism 6.0 (*GraphPad Prism INC., CA, USA)*, logarithm of the extract concentration was plotted against RSC to obtain a nonlinear regression curve –fitting and a variable slope to determine IC_{50}.

3. Results

3.1 Determination of Total Polyphenols and Flavonoids Content

Polyphenols were higher in *Allium sativum* and *Citrus sinensis* than *Persea americana* seed. There were more flavonoids in *Allium sativum* and *Persea americana* than *Citrus sinensis* (Table 1).

Table 1. Total Polyphenolic and flavonoids content of different extracts

Plants extracts	Bark of *Citrus sinensis*	*Allium sativum* bulbs	*Persea americana seeds*
Polyphenols (µg d'EC/mL)	208.89 ± 25.24 [a]	211.11 ± 15.40[a]	1082.22 ± 17.33[b]
Flavonoids (µg d'EQue/mL)	0.85 ± 0.05[a]	3.70 ± 0.31[b]	2.09 ± 0.08[c]

Results are expressed as Mean ± Standard Deviation; EQu: equivalent quercetin; EC: equivalent catechin. EA: aqueous extract; Values with different letters a,b, c assigned are significantly different ($p<0.05$).

3.2 Anti-lipid Peroxidation of Citrus sinensis, Allium sativum and Persea americana

All the extracts protected liver homogenates against lipid peroxides. *A. sativum* and *Citrus sinensis* showed moderate inhibitory effects on lipid peroxidation. Extracts from *P. americana* showed the highest ($p<0.05$) inhibition with increasing concentrations (from 53.37 to 59.88%) (Figure 1).

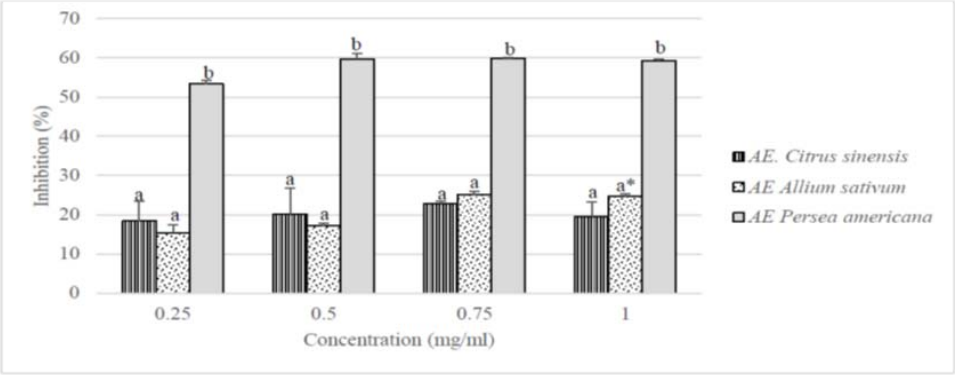

Figure 1. Inhibitory activity of extracts on lipid peroxidation

Graph bar with different letters a,b, c assigned are significantly different ($p<0.05$); *: $p<0.05$ compared to the lowest concentration

3.3 Anti-hemolytic Activity of Citrus sinensis, Allium sativum and Persea americana extracts

Protective effect of *Allium sativum* against red blood cell lysis (anti-hemolytic activity) was higher ($p<0.05$) than *Persea americana* and bark of *C. sinensis* extract. *Allium sativum* protected RBC at 97.7%, while *Citrus sinensis* protected at 87.7% (Figure 2).

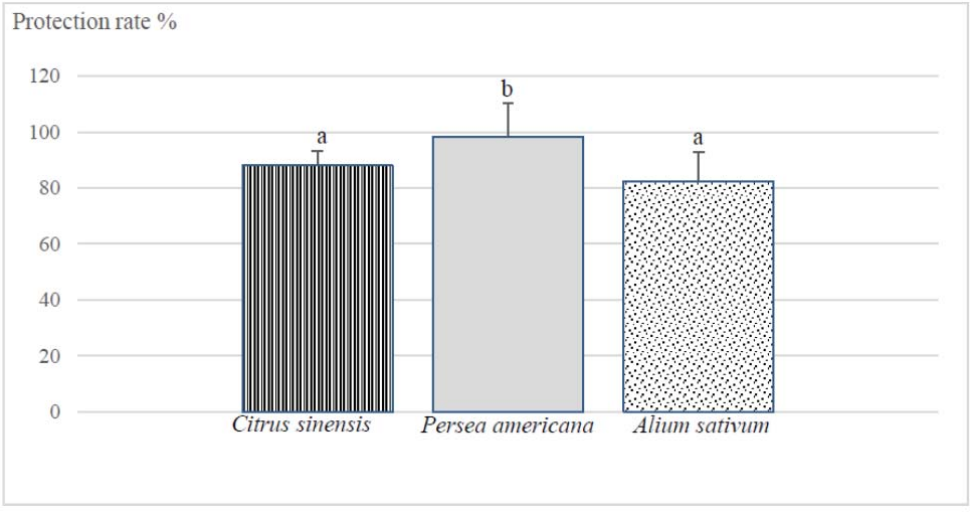

Figure 2. Anti-hemolytic activity of extracts

Results are expressed as Mean ± SD. Experiment done in triplicate. Bar graphs with different letters a, b represent significant difference ($p<0.05$) amongst extracts of the different plant.

3.4 Scavenging Effects of Extracts on DPPH Radical

All extracts showed inhibition of DPPH free radical in a dose dependent manner. *Persea americana* displayed the best values from 47.48% at the lowest concentration to 80% at the highest concentration of extracts. They were followed by *Citrus sinensis* and the lowest percentage of inhibition was observed with *Allium sativum* (Figure 3).

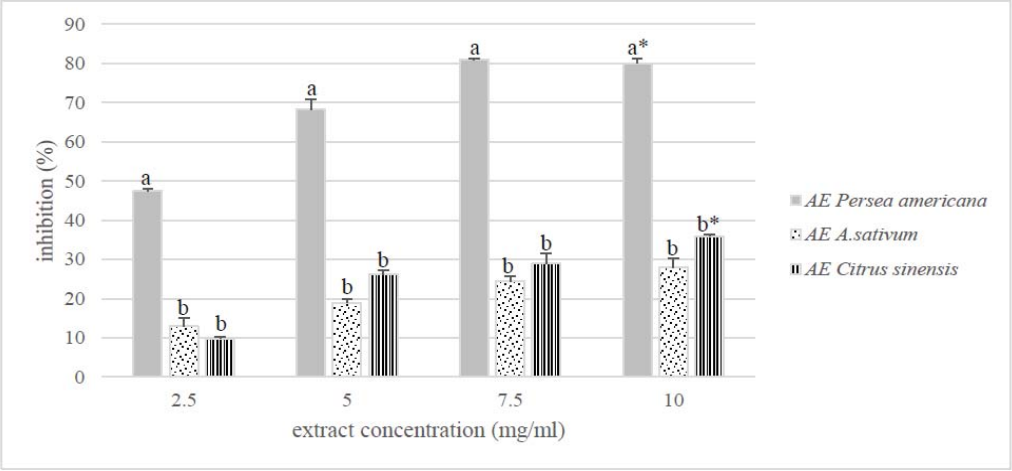

Figure 3. Inhibition of DPPH radical by aqueous extracts

Results are expressed as Mean ± SD. Experiment done in triplicate. Bar graphs with different letters a, b represent significant difference ($p<0.05$) amongst extracts of the different plants

Inhibitory concentration 50 of all the extracts revealed that 2.30mg/ml of *Persea americana* was able to produce 50% inhibition of DPPH radicals generated (Table 2).

3.5 Scavenging Effect of Extracts on ABTS Radical

Only two extracts inhibited $ABTS^+$ radical. *Persea americana* was very active on $ABTS^+$ free radicals scavenging up to 97% at the lowest dose of 2.5mg/ml. However, *Citrus sinensis (AE C s)* displayed null inhibition percentage at the tested concentrations (Figure 4).

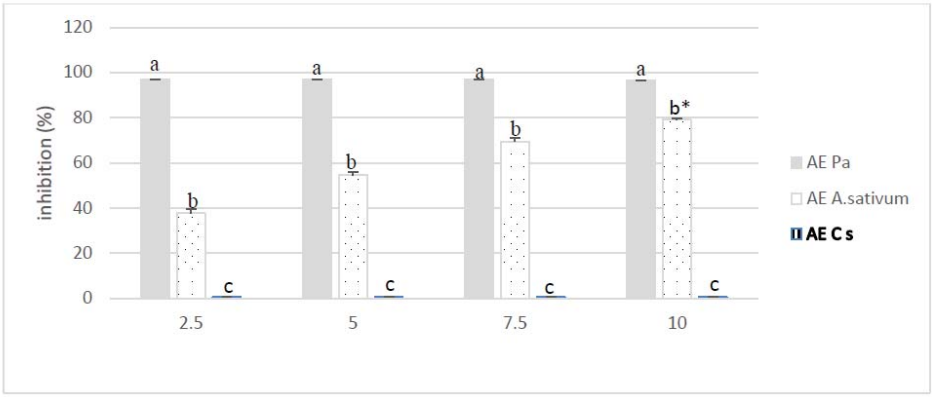

Figure 4. Scavenging effect of extracts on ABTS radical

Results are expressed as Mean ± SD. Experiment done in triplicate. Bar graphs with different letters a,b, c represent significant difference ($p<0.05$) amongst extracts of the different plants; *: $p<0.05$ compared to the lowest concentration.

Concentration of extracts capable of scavenging 50 % of the ABTS free radicals were found with AE of *P. americana*, with the lowest $IC_{50}=1.02 \times 10^{-3}$ mg/mL compared to AE of *Allium sativum* (IC_{50} = of 60.10 mg/mL).

3.6 NO Scavenging Activity of Extracts

The extracts of *A. sativum* and *Persea americana* demonstrated higher inhibition against NO radical, as shown in Figure 5. The results expressed as percentage of inhibition varied as follows: 43.19 (0.25 mg/mL) to 55.54% (1 mg/mL) for aqueous extract.

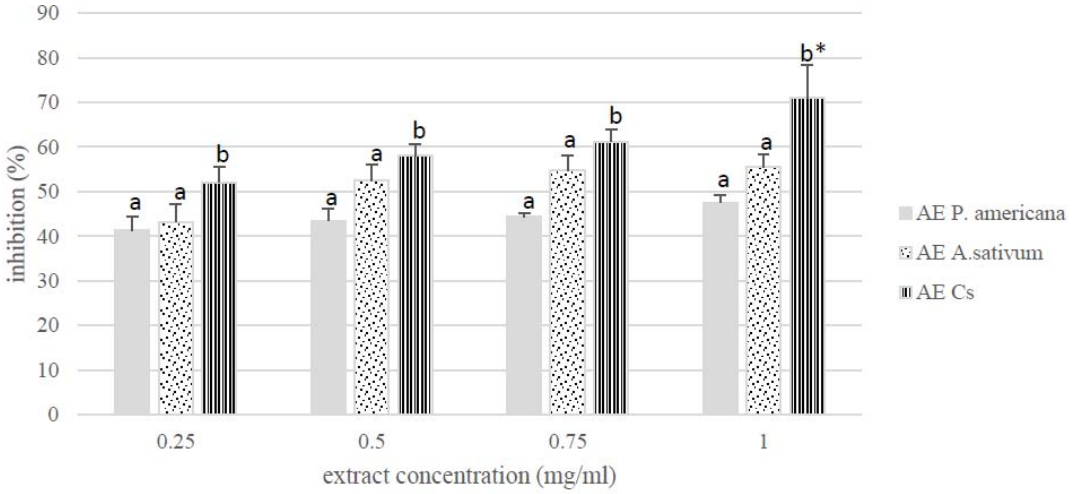

Figure 5. NO scavenging activity of different extracts

Results are expressed as Mean ± SD. Bar graphs with different letters a,b, c represent significant difference ($p<0.05$) amongst extracts of the different plants;*: $p<0.05$ compared to the lowest concentration. AECs: Aqueous extracts of *Citrus sinensis*

The results expressed as percentage of inhibition varies as follows: 41.27 (0.25 mg/mL) to 47.54% (1 mg/mL) for aqueous extract. Compared to the other extracts, *Allium sativum* had the lowest IC_{50} (0.14 mg/mL) compared to other extracts (Table 2).

3.7 $OH^{\cdot}$ Scavenging Activity of Extracts

Citrus sinensis was able to scavenge OH radical at 45%. Percentage inhibitions were higher at lower dose < 0.75mg/ml and lower at higher dose except for *P. americana* (Figure 6). Aqueous extracts of *P. americana seed* demonstrated highest ability to scavenge OH radical *in vitro.* Values varied from 44.3% (0.5mg/mL) to 40.9% (0.5 mg/mL).

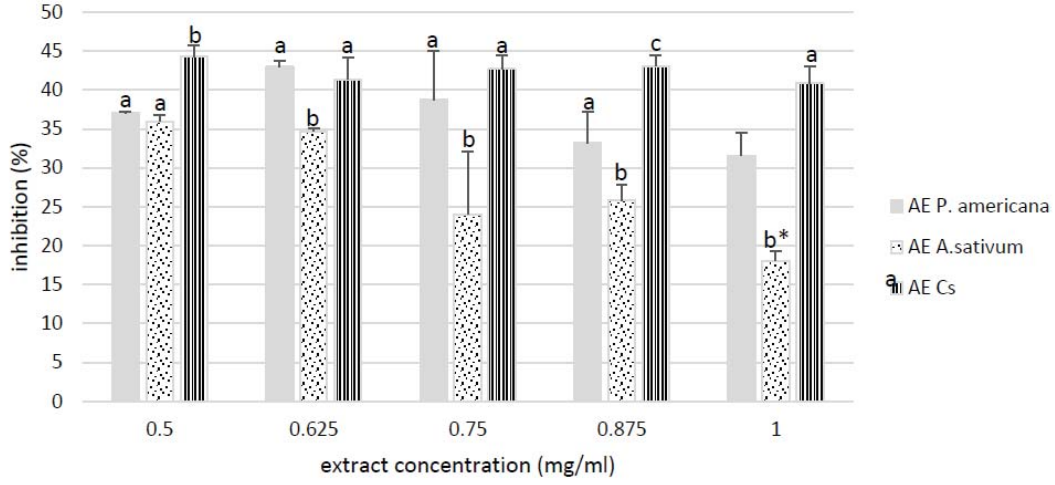

Figure 6. scavenging activities of extracts on $OH^{\cdot}$ Radicals

Results are expressed as Mean ± SD. Bar graphs with different letters a,b, c represent significant difference ($p<0.05$) amongst extracts of the different plants;*: $p<0.05$ compared to the lowest concentration; AECs: Aqueous extracts of *Citrus sinensis*

Concentration of extracts capable of scavenging 50 % of free radicals are summarized in Table 2. It appears that *Allium sativum* displayed the lowest IC_{50} = 4.57 x 10^{-4} mg/mL against OH radicals and the AE of *P. americana* (IC_{50} = 6.27 x 10^{-4} mg/mL), the poorer on NO radicals. (Table 2).

Table 2. Inhibitory concentration (IC_{50}) values for assays

Extracts / Radicals	Inhibitory Concentration- IC50 (mg/ml)		
	Allium sativum	Persea americana	Citrus sinensis
DPPH	124.80	2.30	8.22
ABTS	60.10	1.02 x 10^{-3}	0
NOo	0.14	6.12 x 10^{4}	1.05 x 10^{-3}
OHo	4.57 x 10^{-4}	6.27 x 10^{-4}	1.29x10^{-3}

3.8 Determination of the Total Antioxidant Capacity of Extracts

Total antioxidant capacity increases with increasing concentrations in all extracts, varying from 0.7-1.5 respectively for lower and higher dose (1mg/mL). At all doses, *Persea americana* and *Allium sativum* were higher than *Citrus sinensis*. Extracts of *A. sativum* showed the highest total antioxidant capacity with increasing concentrations of extracts. The results expressed as equivalent ascorbic acid varies as follows: 0.70 (0.5 mg/ml) to 1.51 µg EAA/mL (1 mg/ml) for aqueous extract and 1.56 µg EAA/mL (1 mg/ml) for aqueous extract.

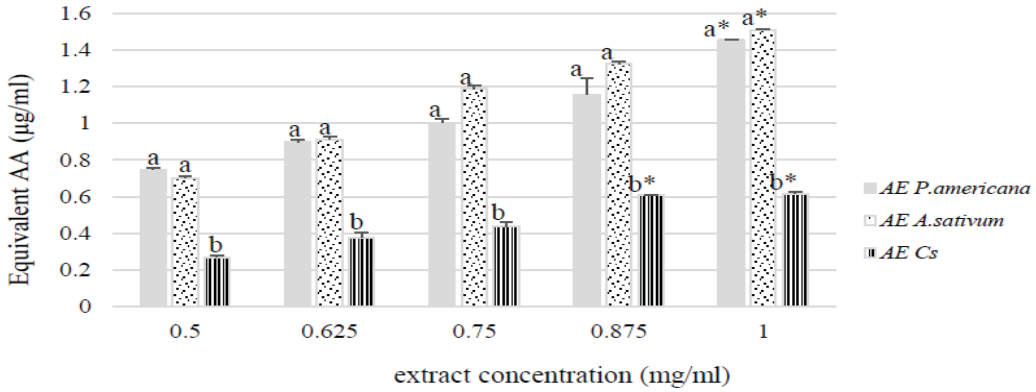

Figure 7. Total antioxidant capacity of *plant extracts*

Results are expressed as Mean ± SD. Bar graphs with different letters a,b, c represent significant difference ($p<0.05$) amongst extracts of the different plants;*: $p<0.05$ compared to the lowest concentration. AECs: Aqueous extracts Citrus sinensis

4. Discussion

Plants, herbs and spices have been reported to have curative effects on type 2 diabetes and metabolic syndrome (Kuate, Nouemsi, Biapa, Azantsa & Wan, 2015; Kooti et al., 2016, Matsinkou, Dakam, Azantsa, Ngondi and Oben, 2017). Hypoglycemic, anti-obesity and anti-oxidant capacities of *Persea americana*, *Allium sativum* and *Citrus sinensis* have been reported (Eyo, Ozougwu & Echi, 2011; Smitha, Jyoti & Chaithra, 2016). Toxicity studies have proven them safe as well (Yasir et al., 2010). However, mechanisms by which they act are not yet fully understood. Free radicals are generated in T2DM due to hyperglycemia. Prolonged hyperglycemia leads of depletion of enzymatic and non-enzymatic antioxidants (Weseler and Bast, 2010). Reactive oxygen and nitrogen species (RONS) attack various organs and alter their function. Oxidative damages on membrane of red blood cells, used as a suitable and simplest model system for elucidation of redox mechanisms in this study showed that *Allium sativum* extracts provide greatest protection (97.7%), preventing lysis and hemolysis compared to *Persea americana* seed and *Citrus sinensis* (Figure 2). Because of the absence of nuclei, ribosomes and mitochondria coupled to lack of protein synthesis and mitochondria based oxidative reactions in mammalian RBC, the protection rate observed reflects direct interaction with the membrane (Youn et al., 2017; Zohra & Atik, 2014). Hemolysis which is therefore the result of necrosis and destruction of bilayer membrane due to deformability of its shape (Youn et al., 2017) caused by hyperglycaemia sometimes interferes with glycosylated hemoglobin (Hb1Ac) levels, very important in the diagnosis. *Allium sativum* protects cell membranes, preventing alteration of lipids and proteins. ROS cause lipid peroxidation and protein alterations of functions

which contribute towards Diabetes Mellitus and its complications including neuropathy, retinopathy, stroke, endothelial dysfunctions, insulin resistance and dyslipidemia (Phillips, Cataneo, Cheema,& Greenberg, 2004; Asfandiyarova, Kolcheva, Ryazantsev, & Ryazantsev, 2007). Furthermore, ROS and RNS interfere with physiological processes in cells (Wesler & Bast, 2010; Omale and Alewo, 2014). *Persea americana* seeds were capable to scavenge and inhibit lipid peroxidation more than *Allium sativum* and *Citrus sinensis* (Figure 1) preserving cells integrity. The anti-lipid peroxidation effect observed with *Persea americana* can be attributed to polyphenol content which was higher in *P. americana* compared to *Citrus sinensis* stem bark and *Allium* (Table 1). Evidences reported strong positive correlations between polyphenols and total antioxidant capacity. Also, strong negative correlations have been proven between DPPH radical and polyphenol contents of plants (Vaya et al., 2003; Ramchoun et al, 2015; Azantsa et al., 2017a). The efficacy of plants resides in their scavenging ability. *Citrus sinensis* extracts and *Persea americana* scavenged OH radicals than NO radicals (Figure 3). *Allium sativum* extracts scavenged ABTS radical than *Citrus sinensis* that showed no activity on ABTS at the measured concentrations of extracts (Figure 4). However, all the extracts displayed high total antioxidant capacity with highest values observed with *Allium sativum* and *Persea Americana* (Figure 7). Antioxidants from all plants reduced lipid peroxidation and inhibited production of free radicals OH and NO, DPPH, ABTS as earlier demonstrated by El-Bahr (2013). Action of phenolic compounds are based on redox ability of their phenolic hydroxyl groups (Velioglu, Mazza, Gao, & Oomah 1998; Ramchoun et al, 2015). Different plants showed variable inhibition levels depending on free radical sources. Polyphenolic compounds and flavonoids found in extracts (Table 1) play a crucial role in the protection of RBC from oxidative damages, preventing hemolysis through scavenging action of free radicals, ROS and RNS (Khalili, Mohammad, & Yaghoub, 2014; Audomkasok, Warapom, Sukanya & Voravuth, 2014).

5. Conclusions

Aqueous extracts of C. sinensis, A. sativum and *P. americana* possess total antioxidant capacity and can scavenge free radicals at various extends. The aqueous extract *of Citrus sinensis* stem bark is a powerful scavenger of biological free radical OH and NO. *Persea americana* seeds powerfully prevent lipid peroxidation and are efficient against ABTS and DPPH radicals. *Allium sativum* extract provided highest protection against hemolysis from radical induced red blood cells lysis. Given that different extracts seem to be efficient on different targets, a formulation based on *C. sinensis, A. sativum* and *P. americana* could be envisaged to manage diabetes and metabolic syndrome.

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Next Generation Sequencing (NGS) for the Determination of Fish Flesh Microbiota

Theofania Tsironi[1], Vladimiros Lougovois[2], Vassiliki Nefeli Simou[2], Afrodite Mexi[2], Stamatios Koussissis[2], Efstathia Tsakali[2, 3], Spiridon Andreas Papatheodorou[2], Valentini Stefanou[2], Jan Van Impe[3] & Dimitra Houhoula[2]

[1]Laboratory of Food Chemistry and Technology, School of Chemical Engineering, National Technical University of Athens, Athens, Greece

[2]Department of Food Science and Technology, Faculty of Food Sciences, University of West Attica, Athens, Greece

[3]Department of Chemical Engineering, BioTeC+ - Chemical and Biochemical Process Technology and Control, KU Leuven, Gent, Belgium

Correspondence: Efstathia Tsakali, BioTeC+ - Chemical and Biochemical Process Technology and Control, KU Leuven, Gebroeders De Smetstraat 1, 9000 Gent, Belgium. Tel: 30-699-454-3717. E-mail: efi.tsakali@kuleuven.be

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Abstract

The objective of the study is the assessment of the microbial ecology and safety of fish in Greece using next-generation sequencing (NGS) and the correlation of the species of microbial flora with the production of histamine. Fourteen different fish species were obtained from local fish stores (Greece) within 1 day from capture. The initial microbiota in fish flesh was determined using NGS. The main pathogenic bacterial species identified in the tested fish samples included *Vibrio* spp., *Clostridium* spp., *Staphylococcus*, *Flavobacterium* and *Janthinobacterium* representing both native freshwater habitats and contaminants arising from different sources, including sewage and direct contamination by wild animals, livestock, and feed. The initial spoilage microbiota of fish consisted of several psychrotrophic Gram-negative bacteria, such as *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Shewanella*, *Psychrobacter*, *Lactobacillus*, *Brochothrix* and *Photobacterium*. The results of the study show the potential of the application and the usefulness of NGS for the determination of microbial flora associated with food-borne diseases and spoilage in fish products. Histamine formation correlated with the valid reads (concentration and number of bacteria) and slightly with the genus of the identified microorganisms.

Keywords: fish, next-generation sequencing, spoilage bacteria, pathogens, 16S rRNA, histamine

1. Introduction

Food borne diseases are reported as an important threat for public health and the European economy. Overall, more than 250 different food borne diseases have been reported, with the majority being bacterial infections. Several bacterial illnesses might be attributed to seafood consumption that has been contaminated either at source or during processing and/or retail display. Such illness cases may be related to infection with bacteria or the ingestion of toxins having been produced in the food product prior to consumption. Along with human non-pathogenic bacteria and natural microflora relevant to the aquatic environment, pathogenic bacteria are often isolated from fish. According to the European Food Safety Authority and the literature, pathogens such as *Campylobacter, Salmonella, Yersinia, E. coli*, and *Listeria monocytogenes* are responsible for significant foodborne outbreaks globally (Leisner & Gram, 1999; Novoslavskij et al., 2016). Fish has been reported as the most commonly implicated food category in outbreaks. Approximately 260.000 people get sick daily in the United States after consumption of contaminated fish (Barrett et al., 2015). According to FAO (2018), disease or illness outbreaks that were attributed to the consumption of fish and shellfish in the EU in the period 1983-1992 ranged between 1.9% (United Kingdom, Scotland) to 12.4% (Denmark) of total food-borne outbreaks. In the case that food source has been identified, the range of fish and shellfish outbreaks ranged between 4.4% (United Kingdom, England/Wales) to 16.1% (Finland). However, not all pathogens are connected with fish and fish product related foodborne outbreaks.

At the same time, an increasing consumer demand for high quality fish products has been reported globally. However, fresh fish is a significantly perishable food product due to its composition and its spoilage is attributed mainly to microbial activity. Hygiene practices and temperature during handling, transportation and storage are the most important factors that determine fresh fish safety and quality up to the consumer level. *Pseudomonas* spp. is reported as the dominant spoilage microorganism in the case of aerobically stored fresh, chilled fish (Giuffrida et al., 2013). *Pseudomonas spp*. growth has also been reported as an adequate quality indicator for the determination of shelf life of Mediterranean fish during aerobic storage, such as gilthead seabream (*Sparus aurata*) (Tsironi & Taoukis, 2010; Tsironi & Taoukis, 2012 and 2014). However, changes in storage conditions, including temperature and packaging, results in significant modifications in the spoilage mechanisms and determine the dominant spoilage bacteria of the fish product. For example, the microflora of modified atmosphere packaged fish is dominated mainly by various Gram-positive microorganisms, mainly lactic acid bacteria as these microorganisms show higher resistance to CO_2 (Sivertsvik et al., 2002). A codominance of lactic acid bacteria and *Brochothrix thermosphacta* in modified atmosphere packaged (40% CO_2) gilthead seabream has been reported by Drosinos et al. (1997), while Dalgaard et al. (1997) reported considerable contribution of *Photobacterium phosphoreum* in the spoilage of chilled modified atmosphere packaged cod, trout and tuna. Although microbial analysis of fish and fish products has been mostly implemented using conventional culture methods, recently novel molecular tools have been applied in order to most accurately evaluate the spoilage and pathogenic bacteria present in these products (Özdemir & Arslan, 2019; Kaktcham et al., 2019) .

Consumption of seafood products, mainly scombroid fish, is associated with histamine fish poisoning (HFP). Histamine is formed in fish by the activity of microorganisms which have the ability to produce the enzyme histidine decarboxylase. The histidine decarboxylases produced by these bacteria catalyse the conversion of free histidine, naturally present at high levels in the muscle of some fish, to histamine. The species that are reported to most likely to produce histamine are *Morganella morganii, Morganella psychrotolerans, Photobacterium damselae, Photobacterium phosphoreum, Raoultella planticola, Hafnia alvei* and *Klebsiella spp.* (Dalgaard et al., 2008; EFSA, 2011; Veciana-Nogues et al., 2004). The incriminated fish in most cases contain high histamine levels, which is attributed to bacterial activity as a result of inappropriate handling, processing and/or storage conditions. In general, histamine has been implicated, at least partly, as a major causative agent. In healthy individuals HFP may be reported after the consumption of a dose of at least 50 mg histamine by fish and fish products. If we estimate a single serving size of 250 g as a high consumption level, a limit of 200 mg/kg has been determined (FAO/WHO, 2013). According to the US regulations seafood is safe for consumption when histamine concentration is below 50 mg/kg. The value considered in the EU is 100 mg/kg. Along with the current trend to develop novel diagnostic tools for food pathogens and outbreak related toxins, rapid and cost effective methods are also developed for the evaluation of the potential of a specific fish product to be implicated to HFP. Hasan et al. (2019) developed a rapid tool for the electrochemical sensing of histamine in fish based on magnetic molecularly imprinted polymer.

Because of recent crises in food quality and safety, food monitoring is regarded as one of the top priorities of the EU-Commission. The White Paper on Food Safety reinforces the need for controls “from the farm to the fork”, which includes: (*i*) official controls, (*ii*) raised food safety standards according to the microbiological criteria of the Codex Alimentarius, and (*iii*) improved detection methods and laboratory quality control. The introduction of NGS represents an important, fundamental technological advance in the biological sciences since the development of the polymerase chain reaction (PCR) in the mid-1980s. It has provided powerful new tools for the determination and study of non-culturable or poorly characterized organisms and emerging pathogens and it thus can enable rapid and open-ended profiling of genotypic and diagnostic markers for virulence and antimicrobial resistance (Peters et al., 2004; Diaz-Sanchez et al., 2013). Few applications on NGS have been reported recently for the determination of microbial flora in food products, i.e., dairy products (Ribani et al., 2018), fish (red drum) (Silbande et al., 2018) and shrimp (Yang et al., 2017).

The objective of the study is the assessment of the microbial ecology and safety of fish in Greece by NGS and the correlation of the species of microbial flora with the production of histamine.

2. Materials and Methods

2.1 Raw Materials and Preparation of Samples

A representative number of fish samples was obtained from local fish stores (Greece) and transported to the Department of Food Science and Technology (University of West Attica, Greece) within 2 hours. 14 different species were studied in total, i.e., (1) Atlantic salmon (*Salmo salar*), (2) Albacore tuna (*Thunus alalunga*), (3) European anchovy (*Engraulis encrasicolus*), (4) Chub mackerel (*Scomber japonicus*), (5) Atlantic mackerel

(*Scomber scombrus*), (6) European pilchard (*Sardina pilchardus*), (7) Grey mullet (*Mugil cephalus*), (8) European hake (*Merluccius merluccius*), (9) Gilthead seabream (*Sparus aurata*), (10) European sea bass (*Dicentrarchus labrax*), (11) Picarel (*Spicara smaris*), (12) Comber (*Serranus cabrilla*), (13) Dentex (*Dentex macrophthalmus*) and (14) Striped red mullet (*Mullus surmuletus*). Samples were transported directly to the laboratory in polystyrene boxes with appropriate quantity of flaked ice (0°C).

Minced fish flesh was inoculated with the reference strain *Morganella morganii* ATCC 25830 and placed in sterile plastic tubes at 37°C for 24 h. This procedure activated the culture used as the inoculum.

Inoculated and Control samples were stored at controlled isothermal conditions of 0 and 10ºC in high-precision (±0.2°C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan). Temperature in the incubators was constantly monitored with electronic, programmable miniature dataloggers (COX TRACER ®, Belmont, NC). Samples were taken at days 0, 4 and 8 for microbiological analysis and determination of histamine concentration.

2.2 DNA Extraction and Next Generation Sequencing

Fish samples were aseptically homogenized in a laminar flow hood. DNA extraction was performed using the NucleoSpin Tissue kit (Macherey-Nagel, GmbH & Co. KG, Germany) according to the manufacturer's instructions, with the addition of a Proteinase K overnight incubation step at 65^{o} C. Extracted DNA was quantified using a spectrophotometer at 260nm and 280nm. After DNA extraction, 16S rRNA genes were amplified using domain-level bacterial primers that contain sequencing adapters and unique, sample-specific sequences.

Multiple samples barcoded and sequenced simultaneously on a single Ion PGM 318 chip resulted in sufficient number of reads. The number of total reads per sample was between $2.5x10^6$ and $4.5x10^6$. Approximately 50-65% of these reads passed stringency filters and of these, 60-75% mapped to the databases.

The primers included in the kit are used to amplify 16S variable regions from samples. After generating amplicons, the Ion Plus™ Fragment Library Kit was used to ligate barcoded adapters and synthesize libraries. Barcoded libraries from all 14 samples were pooled and templated on the OneTouch2™ system followed by 400bp sequencing on the Ion PGM. Automated analysis, annotation and taxonomic assignment occurs via the Ion Reporter Software pipeline. Classification of reads is through alignment to either the curated MicroSEQ ID or curated Green genes databases.

2.3 Microbiological Analysis

For microbiological enumeration, a representative sample (10 g) was transferred to a sterile stomacher bag with 90 mL sterilized Ringer solution (Merck, Darmstadt, Germany) and was homogenized for 60 s with a Stomacher (BagMixer ® interscience, France). Samples (0.1 mL) of 10-fold serial dilutions of fish homogenates were spread on the surface of the appropriate media in Petri dishes for enumeration of different spoilage bacteria. Total aerobic viable count was enumerated on Plate Count Agar (PCA, Merck, Darmstadt, Germany) after incubation at 25ºC for 72 h. *Pseudomonas* spp. were isolated and enumerated on Cetrimide Agar (CFC, Merck, Darmstadt, Germany) after incubation at 25ºC for 48 h. *Morganella morganii* was isolated and enumerated on blood agar after incubation at 37°C for 24 h. For *Enterobacteriaceae* spp. isolation and enumeration the pour-plate method was used, on Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany) after incubation at 25ºC for 48 h.

2.4 Evaluation of Histamine Concentration

Veratox® for Histamine test kit (AOAC-RI #070703 approved method, Product No. 9505, Neogen, USA) was used for the quantitative analysis of histamine in fish flesh. The test is a competitive direct ELISA. The range of quantitation was from 2.5 mg/kg to 50 mg/kg.

The color intensity in the microtiter wells was measured photometrically using the Spectrostar Nano plate reader (BMG labtech, Ortenberg, Germany). The MARS Data Analysis software v.3.01 R2 (BMG labtech, Ortenberg, Germany) was used to evaluate the results.

3. Results and Discussion

In the present study the dominant initial microbiota of 14 fish species obtained from the Greek fish market were evaluated. 16S rRNA gene sequence analysis gave information at both species and strain levels.

The main pathogenic bacterial species identified in the tested fish samples included *Vibrio* spp., *Clostridium* spp., *Staphylococcus, Flavobacterium* and *Janthinobacterium* representing both native freshwater habitats and contaminants arising from different sources, including sewage and direct contamination by wild animals,

livestock, and feed. The initial spoilage microbiota of fish consisted of various psychrotrophic Gram-negative bacteria, mainly *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Shewanella, Psychrobacter*, *Lactobacillus, Brochothrix* and *Photobacterium.*

Several researchers have studied the spoilage microbiota of iced fish caught from the Mediterranean area by using a classical approach and concluded that *Pseudomonas* spp. and *Shewanella* spp. are the most predominant spoilage microorganisms grown on plates. The results of the present study are in agreement with previous studies investigating the initial spoilage microbial flora of fish (Gram & Dalgaard, 2002; Koutsoumanis et al., 2002; Tsironi & Taoukis, 2012). *Psychrobacter* spp., which was identified in most of the studied species, was first reported as part of the initial microbiota of fish from Greek waters by a recent study by Parlapani et al. (2015) in sea bream using 16S rRNA gene analysis. A high prevalence of *Staphylococcus* spp. was also observed, as also reported by Chaillou et al. (2014). Coliforms were not detected at all tested storage temperatures. The microbial load (i.e., total viable count.) in Control fish and samples inoculated with *Morganella morganii* during storage at 0 and 10°C is illustrated in Figures 1-4. It is evident that the microbial count is in all cases higher for the samples stored at 10°C compared to the respective samples stored at 0°C.

Histamine concentration in Control fish and in samples contaminated with *Morganella morganni,* stored isothermally at 0 or 10°C, is presented in Figure 5. Histamine levels were higher for samples stored at 10°C compared to the respective samples stored at 0°C. According to FAO/WHO (2013), histamine formation in fish is dependent on the time/temperature conditions during handling, and therefore time/temperature control is an important issue from harvesting to the time of consumption. The absence or negligible production of histamine in some of the tested samples may be attributed to either a low free-histidine content in the flesh or the absence of histamine-producing bacteria such as *Morganella morganii*, *Raoultella* spp. and *Photobacterium phosphoreum* in the fish flesh microflora (Kanki et al., 2004; Özoğul, 2004; Silbande et al., 2018). In the European Union (EU) and Codex, fish such as *Scombridae*, *Clupeidae*, *Engraulidae*, *Coryphaenidae*, *Pomatomidae* and *Scomberesocidae* are identified as scombrotoxin hazards (FAO/WHO, 2013). Histamine formation correlated with the valid reads (indicating the concentration and number of bacteria) and slightly with the genus of the identified microorganisms, as indicated in Tables 1 and 2. The activity of *Morganella morganii* in the contaminated samples indicated a synergistic effect with the natural microflora for the formation of histamine during isothermal storage of fish, especially at 10°C.

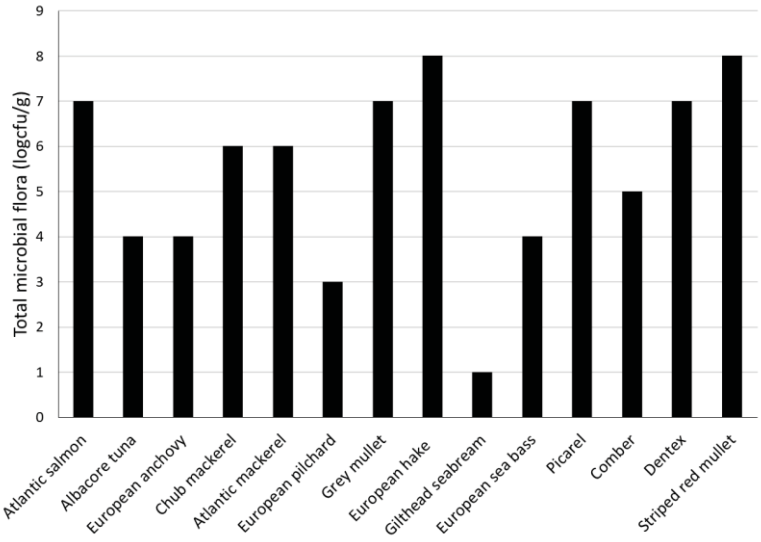

Figure 1. Mean value (log cfu/g) of total microbial flora in Control fish stored isothermally at 0°C, for 0-8 days, enumerated using culture techniques

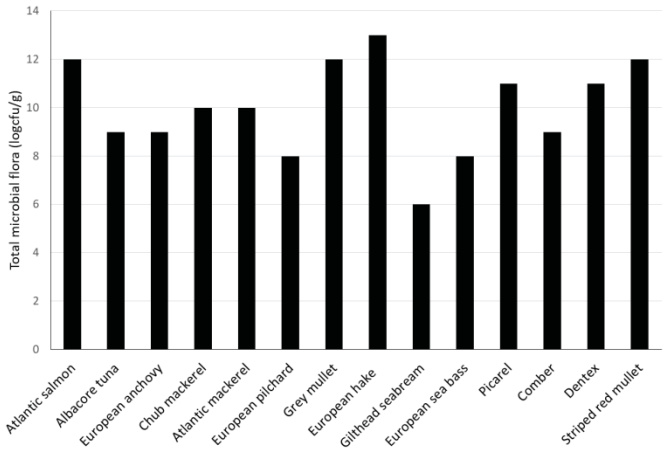

Figure 2. Mean value (log cfu/g) of total microbial flora in Control fish stored isothermally at 10°C, for 0-8 days enumerated using culture techniques

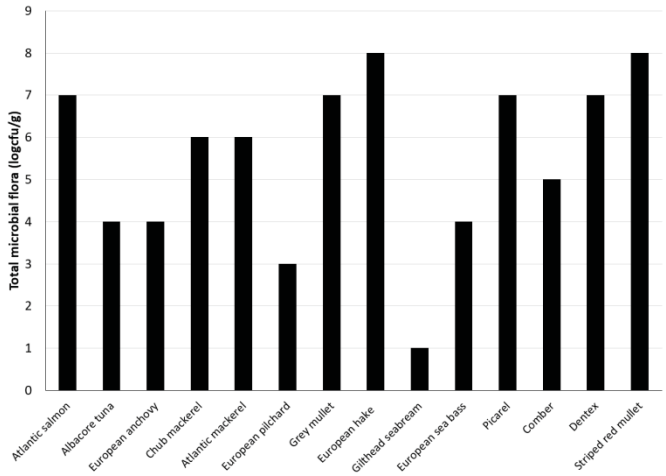

Figure 3. Mean value (log cfu/g) of total microbial flora in fish inoculated with *Morganella morganii* stored isothermally at 0°C, for 0-8 days enumerated using culture techniques

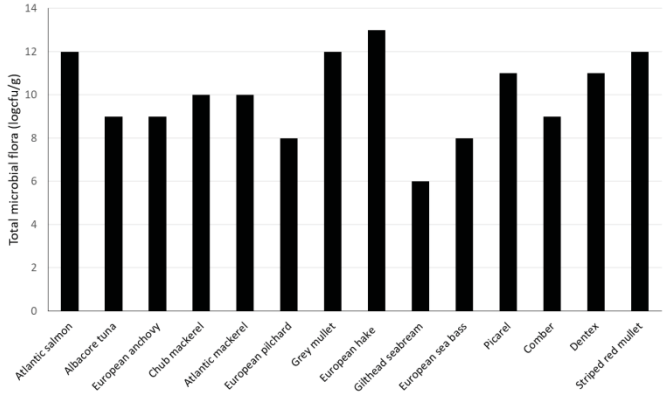

Figure 4. Mean value (log cfu/g) of microbial load in fish inoculated with *Morganella morganii* stored isothermally at 10°C for 0 to 8 days, enumerated using culture techniques

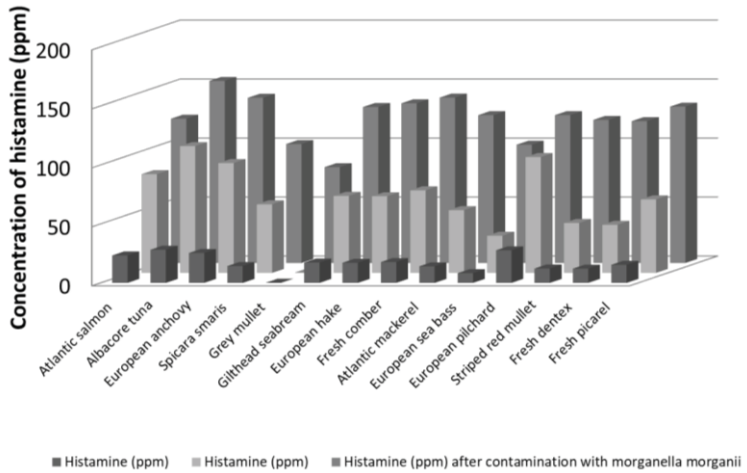

Figure 5. Concentration of histamine (ppm) in fish samples after 4 and 8 days at 10°C

Table 1. Main Identified bacteria with NGS in different fish species correlated with the production of Histamine after 4 and 8 days at 10°C

Main Identified bacteria	**Fish species**	**Histamine (ppm) T=10°C 4 days**	**Histamine (ppm) T=10°C 8 days**
Blastococcus, Kosuria Propionibacterium Brochothrix streptococcus, Janthinobacterium Shewanella Acinetobacter Pseudomonas	Atlantic salmon	23,2	83,7
Chryseobacterium, Flavobacterium Soonwooa, Sphingobacterium Paracoccus, Comamonas Acinetobacter Enhydrobacter Moraxella Psychrobacter Pseudomonas Stenotrophomonas Xanthomonas	Albacore tuna	28,1	107,5
Corynebacterium Microbacterium Propionibacterium Staphylococcus Lactobacillus Streptococcus Aeromonas Shewanella Acinetobacter, Enhydrobacter Psychrobacter Pseudomonas Aliivibrio Photobacterium Vibrio	European anchovy	25,3	93,1
Chryseobacterium Acinetobacter Enhydrobacter Moraxella Pseudomonas Photobacterium Vibrio	Spicara smaris	14,2	58,2
Propionibacterium Flavobacterium Brochothrix Staphylococcus Lactobacillus Streptococcus	Grey mullet	0,1	0,37
Flavobacterium, Brochothrix Lactobacillus Streptococcus Janthinobacterium Shewanella Acinetobacter Psychrobacter Pseudomonas	Gilthead seabream	4,3	25,3
Flavobacterium Brochothrix Streptococcus Shewanella Acinetobacter Psychrobacter Pseudomonas	European hake	17	65
Flavobacterium Janthinobacterium Shewanella Acinetobacter Enhydrobacter Psychrobacter Pseudomonas Photobacterium	Fresh comber	17,6	70
Streptococcus Acinetobacter Psychrobacter Pseudomonas Photobacterium	Atlantic mackerel	14	53,2
Shewanella	European sea bass	6,2	28,5
Corynebacterium Propionibacterium Brochothrix Staphylococcus Lactobacillus Lactococcus Streptococcus Aeromonas Shewanella Acinetobacter Psychrobacter Pseudomonas Vibrio	European pilchard	27,6	98,2
Propionibacterium Flavobacterium Staphylococcus Streptococcus Janthinobacterium Shewanella Acinetobacter Psychrobacter Pseudomonas	Striped red mullet	12,1	42,3
Propionibacterium Streptococcus Acinetobacter Pseudomonas	Fresh dentex	12,0	40,8
Chryseobacterium Acinetobacter Enhydrobacter Moraxella Pseudomonas Photobacterium Vibrio	Fresh picarel	15,3	62,3

Table 2. Main Identified bacteria with NGS in different fish species after the contamination with Morganella Morganii correlated with the production of Histamine after 4 and 8 days at 10°C

Main Identified bacteria	Fish species	Histamine (ppm) T=10°C 4 days
Blastococcus, Kosuria Propionibacterium Brochothrix streptococcus Janthinobacterium Shewanella Acinetobacter Pseudomonas morganella morganii	Atlantic salmon	122,3
Chryseobacterium, Flavobacterium Soonwooa, Sphingobacterium Paracoccus, Comamonas Acinetobacter Enhydrobacter Moraxella Psychrobacter Pseudomonas Stenotrophomonas Xanthomonas morganella morganii	Albacore tuna	154,3
Corynebacterium Microbacterium Propionibacterium Staphylococcus Lactobacillus Streptococcus Aeromonas Shewanella Acinetobacter Enhydrobacter Psychrobacter Pseudomonas Aliivibrio Photobacterium Vibrio morganella morganii	European anchovy	140
Chryseobacterium Acinetobacter Enhydrobacter Moraxella Pseudomonas Photobacterium Vibrio morganella morganii	Spicara smaris	100,8
Propionibacterium Flavobacterium Brochothrix Staphylococcus Lactobacillus Streptococcus morganella morganii	Grey mullet	81,2
Flavobacterium , Brochothrix Lactobacillus Streptococcus Janthinobacterium Shewanella Acinetobacter Psychrobacter Pseudomonas morganella morganii	Gilthead seabream	42,1
Flavobacterium Brochothrix Streptococcus Shewanella Acinetobacter Psychrobacter Pseudomonas morganella morganii	European hake	135,3
Flavobacterium Janthinobacterium Shewanella Acinetobacter Enhydrobacter Psychrobacter Pseudomonas Photobacterium morganella morganii	Fresh comber	140,2
Streptococcus Acinetobacter Psychrobacter Pseudomonas Photobacterium morganella morganii	Atlantic mackerel	125,3
Shewanella morganella morganii	European sea bass	40,2
Corynebacterium Propionibacterium Brochothrix Staphylococcus Lactobacillus Lactococcus Streptococcus Aeromonas Shewanella Acinetobacter Psychrobacter Pseudomonas Vibrio morganella morganii	European pilchard	125,3
Propionibacterium Flavobacterium Staphylococcus Streptococcus Janthinobacterium Shewanella Acinetobacter Psychrobacter Pseudomonas morganella morganii	Striped red mullet	121,3
Propionibacterium Streptococcus Acinetobacter Pseudomonas morganella morganii	Fresh dentex	120,2
Chryseobacterium Acinetobacter Enhydrobacter Moraxella Pseudomonas Photobacterium Vibrio morganella morganii	Fresh picarel	132,5

4. Conclusions

The objective of the study was the assessment of the microbial ecology and safety of fish in Greece using NGS and the correlation of the species of microbial flora with the production of histamine. Fourteen different fish species were obtained from local fish stores (Greece) within 1 day from capture. Using a Next Generation Sequencing approach, a rapid and sensitive method for the identification of bacterial species in polymicrobial samples was demonstrated. The main pathogenic bacterial species identified in the tested fish samples included *Vibrio* spp., *Clostridium* spp., *Staphylococcus*, *Flavobacterium* and *Janthinobacterium*, which represent native freshwater habitats and contaminants arising from sources such as sewage, wild animals, livestock, and feed. The initial spoilage microbiota of fish consisted of several psychrotrophic Gram-negative bacteria, mainly *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Shewanella*, *Psychrobacter*, *Lactobacillus*, *Brochothrix* and *Photobacterium*. The results of the study show the potential of the application and the usefulness of NGS for the determination of microbial flora associated with food-borne diseases and spoilage in fish products. Histamine concentration in Control fish and in samples inoculated with *Morganella morganni* and stored isothermally at 0 or 10°C was evaluated after 4 and 8 days. In general, histamine concentration reached higher values in fish stored at 10°C compared to iced storage. Low concentrations of histamine in some fish species was attributed to either a low free-histidine level in the fish flesh of low microbial counts referring to microorganisms such as *Morganella morganii*, *Raoultella* spp. and *Photobacterium phosphoreum* (for Control fish samples). Histamine formation correlated with the valid reads (concentration and number of bacteria) and slightly with the genus of the identified microorganisms. The activity of *Morganella morganii* in the contaminated samples indicated a synergistic effect with the natural microflora for the formation of histamine during isothermal storage of fish, especially at 10°C.

It is necessary to study the prevalence of bacteria in fish to ensure a better understanding of ecology and distribution of pathogens and spoilage microorganisms in the food chain. The determination of fish microbiota is currently carried out mainly by phenotypic tests (morphological, biochemical) after the isolation of microorganisms using various non-selective and/or selective growth media. The application of novel molecular tools and "omics" techniques in food safety and quality has the potential to answer questions which conventional microbiological methods are not able to address. In the present study, the application of NGS for the microbial assessment of chilled fish is introduced. In general, Good Hygienic Practices are a measure to prevent fish contamination during handling, distribution and storage and to provide safe, high quality fish and fish products. The conventional methods for determination of microbial flora in food products have been proven to detect species when the potential bacteria are already defined or expected in advance as the discriminatory analytical methods for their identification have to be specifically tailored. On the other hand, NGS technologies have improved DNA analysis methods by combining sequencing and quantification of DNA in a single step. By using universal primers to amplify several regions, it is possible to obtain an internal validation of the results derived by the concordance of the assigned reads to a species. Technological developments in the field of microbiology, such as NGS techniques and the omics approach in general, have significantly enhanced our understanding of the behaviour of microorganisms and particularly their physiological state. The perishability of fish and fish products results in their limited shelf life, especially considering the temperature fluctuations that occur in the actual food cold chain which affect significantly the safety and quality level of these products at the time of consumption. The quality and shelf life of fish can be improved if the spoilage mechanism of these products is well defined and clearly understood. The spoilage potential of some members of the fish microbiota is not yet investigated or characterized. The detection of non-cultivable bacteria in fish products remains a challenge, as thee microorganisms may play a significant role in the safety and spoilage process of fish. These innovative approaches could reveal patterns of responses that cannot be detected by classical methods and have the potential to ultimately uncover new and powerful methods to control hazards in food and feed. This may potentially bring more insight than just the usual 'snapshot' in the farm-to-fork contamination process analysis and therefore contribute to the next generation of risk assessment (den Besten et al., 2018). Under this context, the present study might be a baseline for further investigation of the pathogenic and spoilage potential of the identified microorganisms present in Greek (and consequently Mediterranean) fish products. The identified microorganisms should be further studied for their potential corellation with physical, chemical and sensory quality indices changes during storage at simulated (isothermal) conditions and in the real chill chain (variable temperature conditions). A similar approach may be expanded to other food products, such a meat, dairy etc., where the definition of the microbiota is of significant importance.

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Chemical Composition and Particle Size of Grape Seed Flour and Their Effects on the Characteristics of Cookies

Razakou Maman[1] & Jianmei Yu[1]

[1]Department of Family and Consumer Sciences, North Carolina Agricultural and Technical State University, 1601 East Market Street, Greensboro, NC, 27411, USA

Correspondence: Jianmei Yu, Department of Family and Consumer Sciences, North Carolina Agricultural and Technical State University, 1601 East Market Street, Greensboro, USA. E-mail: jyu@ncat.edu

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Abstract

This study investigated the effect of particle size and chemical composition of grape seed flour (GSF) on the physical, chemical and sensory quality of cookies. Results indicate that the chemical composition of GSF varied significantly with particle sizes and smaller particle fraction had higher ash, fat, protein and extractible polyphenol contents but lower dietary fiber. Inclusion of 2.5-10% GSF in the cookie formula enhanced darkening, increased thickness and hardness, increased polyphenol and dietary fiber contents in dose-dependent manner, but reduced diameter and consumer acceptability of cookies to various degrees. The impacts of GSF on the cookie quality and sensory properties were strongly associated with the inclusion level and particle size of GSF. Higher GSF (5%) inclusion and smaller GSF particle size (104 μm) resulted in lower consumer acceptability. To minimize the undesirable effect of GSF on cookie quality, up to 5% of GSF with average particle size 209 μm is recommended.

Keywords: grape seed flour, particle size, cookies, physical properties, chemical properties, consumer acceptability

1. Introduction

Cookie is one of the nonperishable but unhealthy baked foods because it is high in fat and sugar but low in bioactive compounds and dietary fiber. The addition of fruit pomace to cookies, especially to those made of white flour, may significantly enrich their composition with dietary fiber and phenolic compounds and improve cookie's nutritional value (Tańska et al., 2016). Some studies have been done to improve the antioxidant activity of cookies using grape pomace (Acun & Gul, 2014; Zhu et al., 2015; Kuchtová et al., 2016). Our previous study with bread model shows that the bread with 5% grape pomace had similar loaf volume but darker color compared to the control, but increasing grape pomace content to 10% in the bread formula resulted in reduced preference in both taste and texture of the bread (Smith & Yu, 2015). In addition, grainy mouth feel was detected which suggests the need for further particle size reduction.

Grape seeds comprise about 5% of the fruit weight (Choi & Lee, 2009) and 37-44% of the dry grape pomace (Yu, 2014). Grape seeds are rich in dietary polyphenols, particularly, catechins and B-type procyanidins or condensed tannins although the polyphenol composition varies with grape variety and grown location (Shi et al., 2003; Mandic et al., 2008). It is well known that these polyphenols contribute to the color and astringent taste of grapes and wine. The health benefits of grape seed polyphenols have been extensively studied worldwide. Muscadine grapes are native to the United States. Muscadine Carolos and Noble are two varieties widely used for wine making and they have thicker skins and fewer seeds compared to the *Vitis vinifera* wine grape varieties (Yu, 2014). The chemical composition other than polyphenols of Muscadine grape seeds were rarely reported. Therefore, it is important to have a complete understand of their chemical composition which is very important to the nutritional value, chemical and sensory properties of the target product.

Grinding is a traditional method for particle size reduction in food industry for better acceptance of final products. Particle size reduction also affects polyphenol extractability, digestibility and functional and physicochemical properties of polyphenol and dietary fiber rich food ingredients. The reduction in the particle size from 1127 to 550 micrometer resulted in increased hydration properties of coconut fiber (Raghavendra et al., 2006). Superfine grinding of red grape pomace improved the solubility, polyphenol extractability and antioxidant

activity of the pomace (Zhao, et al., 2015). Similar results were found for ultrafine grinding of wheat bran (Zhu et al., 2010). However, it is unknown what effects the superfine grinding grape pomace/seeds have on food product development.

The hypothesis of this study was that chemical compositions of grape seed flour (GSF) fractions of different particle sizes were different, which had significant impact on the product quality and consumer acceptance of cookies. Therefore, the chemical composition including proximate composition, total dietary fiber and polyphenol composition of Muscadine Carolos and Muscadine Noble GSF of different particle sizes were determined and their effects on the quality of short bread cookies were evaluated in this study.

2. Materials and Methods

2.1 Preparation of Grape Seed Flour

Pomaces of Muscadine Carlos and Noble were kindly provided by a winery located in North Carolina, USA. The pomace was vacuum dried and seeds were separated manually from skins. The seeds were ground into powder which was separated into three fractions of different particle size ranges using a set of mesh sieves (fine: 80-100 mesh; medium: 60-80 mesh; coarse: 40-60 mesh). The medium and coarse fractions were ground and sieved again until their particles could not be further reduced. The particle sizes of different fractions of ground grape seeds were determined by Laser Particle Size Analyzer (Microtrac, Montgomeryville, PA, USA). The average particle sizes of three fractions were 104 μm, 209 μm, and 486 μm, respectively. The GSF fractions of different particle sizes were stored in moisture proof plastic bags at 4^{o}C.

2.2 Cookie Preparation

The GSF fraction was mixed with other ingredients of short bread cookie to 2.5-10.0% using a kitchen aid to form cookie dough, thus percentage of other ingredients (all-purpose flour, baking soda, sugar, butter, egg) except vanilla extract were proportionally reduced. The dough was split into small pieces of equal weight which were rolled in small balls and pressed in a cookie cutter to the same height. The cookie dough was placed on a cookie sheet (24 per sheet) and baked in a preheated oven at 177°C for 20 minutes, then cooled to room temperature on the cooling shelf.

2.3 Measurements of Physical Properties of Cookies

After cooling, cookie weight, diameter, thickness, color and texture were measured. The cookie without GSF was used as control The weight of cookies was recorded using METTER TOLEDO AB 265-S balance after cookies were cooled to room temperature. The measurement was triplicated for each type of cookies. The diameter was measured using a ruler and the thickness was determined by a caliper. Four measurements were taken for each cookie.

Color was measured by CIEL color system using Konica-Minolta MC-3500d Spectrophotometer (Tokyo, Japan). The color of cookies denoted L*, a*, and b* was determine, where L* signifies the lightness from 0 (black) to 100 (white). The color channels, *a** and *b**, represent true neutral gray values at *a** = 0 and *b** = 0, represents green and blue at negative *a** and *b**values, and red and yellow at positive *a** and *b** values, respectively. Measurements were made at 5 spots on the top side of each cookie. The results were expressed as the mean of 15 measurements (3 cookies per treatment).

The hardness of cookie was measured using a TA-XT2 Texture Analyzer (Texture Technologies Corporation, Scarsdale, NY). The program used was Bakery/Biscuit cutting- BIS2_KB.PRJ with the probe HDP/ BSK (Blade Set with Knife) to determine the maximum force needed to cut the cookie. The measurement was conducted in triplicate.

2.4 Proximate Composition Analysis of Different Fractions of GSF and Cookies

Moisture, ash, crude protein, crude fat, total carbohydrate and dietary fiber contents of GSF and cookie samples were determined using AOAC methods, and total carbohydrates were calculated by difference. The moistures were determined by vacuum drying method using an Isotemp Vacuum oven (Fisher Scientific, Georgia, USA). The crude fat contents were determined by modified AOAC Method 945.16 (AOAC, 2005) with petroleum ether as solvent using FOSS Soxtec TM 2050 Extractor (Hoganas, Sweden). The ash was determined using AOAC Official Method 923.03 (AOAC, 2005) with a Barnstead Thermolyne 30400 muffle furnace (Dubuque, Iowa, USA). The protein content was determined by a combustion method (AOAC, 2006) using PerkinElmer Series II CHN 2400 Analyzer (PerkinElmer, Inc., Waltham, MA USA) in which the total nitrogen content of the GP sample was determined. The nitrogen to protein conversion factors used for GSF and cookies were 5.46 and 5.7, respectively (Mariotti et al, 2008; Fujihara et al., 2008). Ash and protein were determined using defatted samples,

then converted back to the non-defatted basis according to the fat contents of GSF and cookies. For each of GSF or cookie sample, the measurement was conducted in triplicate.

2.5 Determination of Dietary Fiber of GSF Fractions and Cookies

Total dietary fiber (TDF) of GSF and cookie was determined using the enzymatic-gravimetric method by AOAC method 991.42 (AOAC, 1996) with TDF assay Kit (Sigma-Aldrich, St. Louis, MO, USA), which uses heat stable α- amylase and amyloglucosidase to hydrolyze and remove starch, and protease to remove protein.

2.6 Determination of Polyphenols of GSF Fractions and Cookies

The polyphenols in GSF and ground cookies samples were extracted using 70% ethanol. The sample size was 1.000g for GSF and 3.000g for cookie. A two-step extraction process was used for both GSF and cookie samples as described previously (Smith & Yu, 2015), and the extraction was conducted in triplicate for each sample. The total polyphenol (TP) concentration of the extract was determined by Folin-Ciocalteu method (Singleton et al., 1999) with small modification, and expressed as mg gallic acid equivalent (GAE)/g GP. The total anthocyanin (TA) was determined by AOAC method 2005.02 and calculated as cyanidin-3-glucoside equivalents for the samples (Lee, 2005). The total flavonoid (TF) was determined by the aluminum chloride ($AlCl_3$) colorimetric method and expressed as mg catechin/g sample (Hosu et al., 2014). The condensed tannin (CT) concentration was determined by Vanillin-HCl Assay and were expressed as mg catechin equivalent (mg CE)/g sample (Hagerman, 2002).

2.7 Sensory Evaluation of Cookies

A quantitative affective method was used to evaluate the liking of sensory attributes including color, aroma, texture, flavor and overall liking of cookies. The sensory evaluation of cookie was conducted the day after baking using 55 untrained panelists composed of students, staff and faculty members ages 18 to 55. Each attribute was scored using a 9-point hedonic scale with 1 being dislike extremely, 5 being neither like nor dislike and 9 being like extremely. Samples were assigned 3-digit codes and were randomized served in the plate. Each participant received a total of 5 cookies consisting of 0% GSF (control), 2.5% GSF (104 μm), 2.5% GSF (209 μm), 5% GSF (104 μm), and 5% GSF (209 μm). Water was provided for rinsing mouth between samples.

2.8 Data Analysis

Data of proximate composition, physical properties, and sensory evaluation were analyzed by ANOVA and Duncan Multiple Range Comparison tests using SAS 9.2 software (Cary, NC) to determine whether significant difference exist among samples containing different particle size/amounts of GSF at 5% significance level. The relationship between cookie polyphenol contents and GSF inclusion level was analyzed by numerical regression method.

3. Results

3.1 Proximate Compositions of GSF Fractions of Different Particle Sizes

The proximate compositions of fractions of different particle size differ from each other (Table 1). Overall, finer GSF fractions had high fat, mineral and protein contents but lower carbohydrate and TDF contents regardless grape cultivar (Table 1). When the particle size was reduced from 486 um (coarse) to 209 um (medium), the fat content of increased from 8.7% to 15.45% for Noble seed and from 9.07% to 17.95% for Carolos seed flour, but further reduction of particle size did not change the fat content too much. The coarse fraction (486 um) showed highest carbohydrate, lowest fat, mineral and protein concentrations. Extensive reduction of particle size due to grinding liberate more reactive components due to cell breakage, which explains the increased fat and protein contents detected. Carlos GSF had higher TDF than Noble GSF at same particle size, but TDF decreased significantly for both Carlos and Noble GSF as particle size decreasing. The lowest TDF content (32.32%) was observed in the fine fraction (104 μm) of Noble GSF and the highest TDF content was in the coarse fraction (486 μm) of Carlos GSF. It was reported that GSF contains 43% of TDF (Aghamirzaei et al., 2015). At particle size 104 and 209 um, Carlos GSF showed higher TDF than total carbohydrate because the interaction between condensed tannin with protein and other components which could greatly reduce the digestibility of GSF (Yu et al., 2016).

Table 1. Proximate Compositions and TDF of Grape Seed Flour Fractions of Different Particle Sizes

GSF Type	Particle Size (μm)	Moisture (%)	Crude Fat (%)	Mineral (%)	Crude Protein (%)	Carbohydrate (%)	TDF (%)
Muscadine Noble	104	6.33±0.40 [a]	16.70±0.05 [a]	5.74±0.03 [a]	14.35±0.08 [a]	56.88	32.21±1.79 [a]
	209	6.24±0.20 [a]	15.45±0.16 [b]	4.85±0.07 [b]	14.77±0.19 [b]	58.68	45.09±2.72 [b]
	486	6.68±0.08 [a]	8.70±0.06 [c]	2.80±0.04 [c]	10.53±0.19 [c]	71.29	59.26±0.28[c]
Muscadine Carlos	104	5.03±0.36 [b]	17.59±0.56 [d]	6.33±0.02 [d]	15.74±0.18 [d]	55.32	48.17±0.43[d]
	209	3.89±0.45 [c]	17.95±0.71 [d]	6.08±0.06 [e]	17.53±0.48 [e]	54.56	59.19±0.62 [e]
	486	4.56±0.21 [d]	9.07±0.62 [ce]	3.31±0.07 [f]	11.89±0.91 [f]	71.16	67.22±0.28 [f]

In the same column, data with different superscript are significantly different at $P<0.05$.

3.2 Polyphenol Compositions of GSF Fractions of Different Particle Sizes

Reducing particle size of GSF significantly increased extractability of total polyphenol TP (Fig.1A), total flavonoid TF (Fig.1B), total anthocyanin TA (Fig. 1C) and total condensed tannin (Fig.1D). The only exception is that the TA content in the fine fraction of Muscadine Carlos GSF was significantly lower than that in the medium and coarse fraction. The extractable TP, TF, CT were only 5 mg/g, 2.5-3.0 mg/g and 1.2-3 mg/g in the coarse GSF fractions, but they increased to 12-14 mg/g, 5-6 mg/g and 5-9 mg/g in medium fractions, and 18-20, 6-7, 7-13 mg/g in the fine fractions. Our results are in good agreement with what reported by Brewer et al. (2014) who found that fine fraction of wheat bran had higher phenolic acid, flavonoid, anthocyanin, and carotenoid contents than coarse fraction. It was reported that crushing increased extractable TP and TF for Cabernet Sauvignon and Pinoir Sirah late seeds, but had little effect on anthocyanin extractability of Cabernet Sauvignon and reduced anthocyanin availability of Pinoir Sirah late seeds (Myeyer et al., 1997). Similar results were found in the studies of polyphenol extraction from tea and ginger, in which more TP and TF were extracted when particle size decreased from 1.18 mm to 0.71 mm, but further reduction of particle size to 0.425mm resulted in decreased TP and TA extractability from tea, but increase for ginger (Makanjuola, 2017). Combining our results and the findings of other researcher, we conclude that polyphenol compositions of GSF fractions of different particle sizes vary greatly which may affect the characteristics of food product if GSF is used as an ingredient in food product development.

3.3 Effects of Particle Size and GSF Level on the Physical Properties of Cookies

The physical properties including diameter, thickness, weight, hardness, and color of cookies were measured and the results were summarized in Tables 2. Compared with the control, cookie diameter decreased but thickness increased ($P<0.05$) in the presence of GSF although cookie weight only showed slight changes. Increasing GSF level from 2.5 to 5% reduced the diameter but increased the thickness of cookies, whereas, reducing particle size of GSF from 209 μm to 104 μm resulted in increased cookie diameter but did not change cookie thickness regardless the type of GSF. The hardness of cookies increased significantly in the presence of GSF and high GSF level resulted in harder cookies ($P<0.05$), especially Muscadine Noble GSF. The findings are different from the that of Acun & Gül (2016) who found that addition of 5-15% of whole grape pomace or 5-10% of grape seed powder did not cause significant changes in cookie diameter and thickness. Our previous study and Hoye's study about the fortification of while bread using grape pomace and GSF also found that an increase in grape pomace or GSF replacement above 5% of wheat flour caused significant decrease in loaf volume (Smith & Yu, 2015; Hoye, 2009).

The mechanical texture of cookies was evaluated by measuring hardness and expressed as maximal force required to cut the cookie (Table 2). GSF cookies, especially those containing Noble GSF, were found to be harder than the control cookie ($P<0.05$). The increase of hardness with GSF concentration is marginally significant ($P\leq0.05$) for Carlos seed cookies and smaller particle GSF fractions of GSF (104 um) had larger impact on the hardness of cookie in general with exception of Noble GSF at 5% inclusion level. The findings in this research are in good agreement with the findings of Samohvalova et al. (2016) who found that addition of grape seed powder strengthened wheat flour gluten in the dough and increased the hardness of butter biscuit. Similar results were also obtained in the studies of bread fortification using grape pomace and GSF (Smith & Yu, 2015; Hoye, 2009). The hardness of cookies with GSF may also be attributed to the loss of moisture (Table 4) and the interaction between condensed tannin and protein (gluten) which result in the formation of tannin-protein complex.

Table 2. The physical measurements of cookies (thickness, diameter, weight, and hardness) with GSF of Muscadine Noble

Type of GSF	GSF (%)	Particle Size (μm)	Weight (g)	Diameter (cm)	Thickness (cm)	Hardness (kg)
	0 (Control)		30.95 ±0.11^{a}	7.62 ±0.05^{a}	1.66 ±0.06^{a}	6.54 ±1.12^{a}
Muscadine Carlos	2.5	104	30.94±0.07^{a}	7.06±0.06^{b}	1.63±0.06^{a}	7.18±1.04^{ab}
		209	31.21±0.10^{b}	7.03±0.06^{b}	1.57±0.05^{b}	6.71±0.78^{a}
	5.0	104	31.35±0.04^{bc}	6.77±0.12^{c}	1.73±0.15^{c}	7.77±1.30^{b}
		209	31.41±0.08^{c}	6.87±0.10^{c}	1.70±0.07^{c}	7.25±1.14^{b}
Muscadine Noble	2.5	104	31.02 ±0.03^{a}	6.73 ±0.06^{d}	2.0 ±0.01^{d}	8.64 ±1.08^{c}
		209	30.97 ±0.04^{a}	7.03 ±0.15^{b}	1.93 ±0.15^{de}	8.36 ±1.27^{c}
	5.0	104	31.37 ±0.01^{bc}	6.33 ±0.12^{e}	2.13 ±0.06^{f}	8.55 ±0.89^{c}
		209	31.02 ±0.01^{a}	6.73 ±0.12^{c}	2.17 ±0.06^{f}	9.23 ±1.23^{d}

In the same column, data with different superscripts are significantly different (P<0.05).

The color parameters (CIEL L*, a*, and b* values) of top surfaces of cookies were presented in Table 3. All GSF containing cookies had smaller L* values than the control, indicating reduced brightness. At same GSF level and particle size, cookies with Carlos GSF had higher L* values, but lower a* and b* values than cookies with Noble GSF. Carlos GSF had less impact on L*, more impacts on a* and b* of cookies than Noble GSF, which might be caused by higher anthocyanin content of Noble GSF as shown in Fig.1C. At same particle size, the higher the GSF level, the smaller the L*, a* and b* values were. At same GSF concentration, smaller particle size resulted in reduced L* but increased a* and b* values. Overall, the addition of GSF in the cookie formula resulted in cookie darkening and reducing particle size of GSF enhanced the darkening. This is in agreement with the findings of Hoye (2009) in a cookie study and the findings of Smith & Yu (2015) in a bread study. Co-pigmentation of anthocyanins with other polyphenols can produce black pigments (Heras-Roger, et al., 2016). Oxidation of polyphenols during baking may also contribute to the darkening of GSF containing cookies.

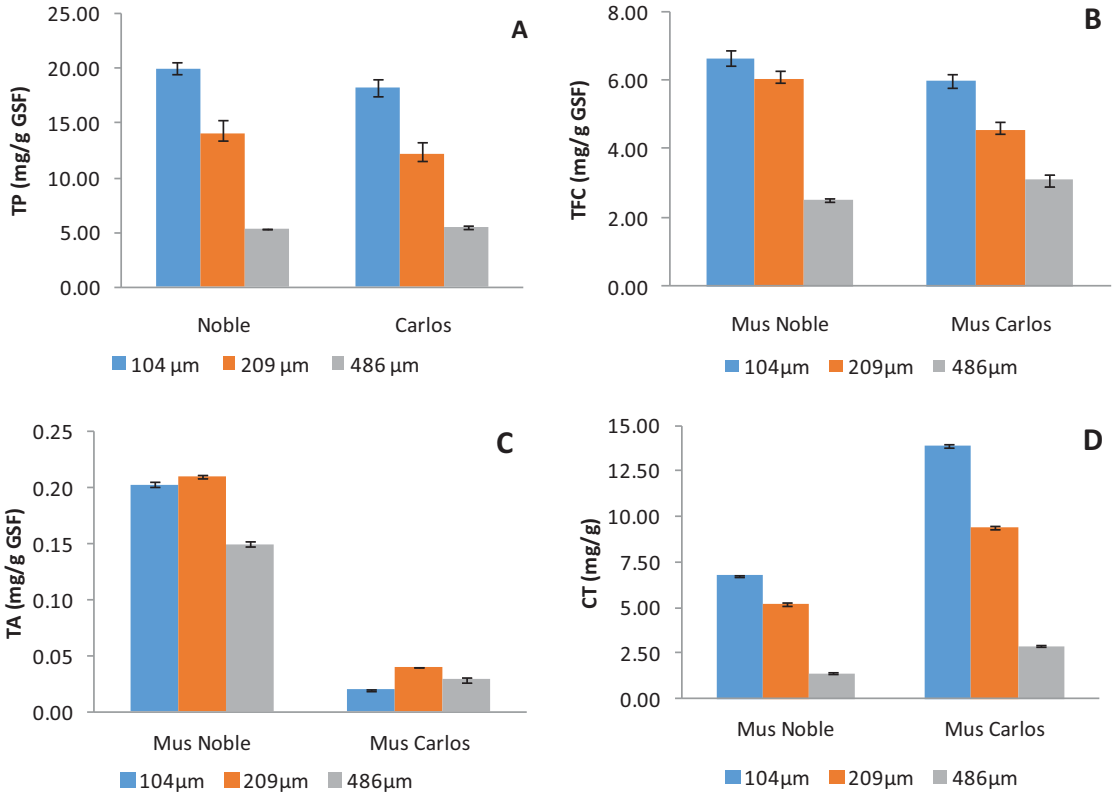

Figure 1. Effects of particle size of grape seed flour on polyphenol extractability and composition

A-total polyphenol, B-Total Flavonoids, C-Total Anthocyanin, D-Condensed Tannin, Mus-Muscadine

Table 3. Color parameters of cookies containing GSF at different concentrations and particle sizes

GSF Added (%)	Particle Size (μm)	Muscadine Noble			Muscadine Carlos		
		L*	a*	b*	L*	a*	b*
0	Control	54.87±0.82^{a}	7.92 ±0.11^{a}	16.11±0.33^{a}	54.87±0.82^{a}	7.92 ±0.11^{a}	16.11±0.33^{a}
2.5	104	41.21 ±0.8^{b}	9.0 ±0.33^{b}	20.99 ±0.45^{b}	42.17±0.43^{b}	5.88±0.21^{b}	7.074±0.15^{b}
	209	45.04 ±2.49^{c}	7.74 ±0.65^{a}	21.29 ±0.81^{b}	45.17±1.79^{c}	6.79±0.09^{c}	8.96 ± 0.25^{b}
5.0	104	36.99 ±1.17^{d}	8.59 ±0.20^{c}	17.52 ±0.53^{c}	44.67±0.52^{d}	6.99 ± 0.19^{c}	9.19±0.30^{bc}
	209	46.32 ±0.09^{c}	4.57 ±0.15^{d}	7.28 ±0.75^{e}	46.58±0.43^{ce}	7.13±0.20^{d}	10.61±0.27^{d}

In the same column, data with different superscripts are significantly different (P<0.05). (L* representing lightness (L* = 0: black, L* = 100 complete white); a* = 0 and b* = 0 representing true neutral gray values, negative a* and b* values representing green and blue respectively, positive a* and b* values representing red and positive yellow respectively.

3.4 Proximate Composition of Cookies

Table 4 shows that the inclusion of GSF significantly affected the proximate composition of cookies, but the degree of effect varied with the type, quantity and particle size of GSF. In the presence of GSF, the moisture of cookies decreased from 3.25% (control) to 0.9-1.34% depending on the quantity and particle size of GSF. Larger particle size resulted in lower water retention except cookies with 5% Carlos GSF. The lower moisture of cookies in the presence of GSF could be caused by the high lignin content of grape seeds which has low water holding capacity (Yu, & Ahmedna, 2013). The cookie ash contents increased with increasing GSF level and decreasing GSF particle size, which can be explained by the high ash content of GSF and the higher ash content of finer GSF fraction (Table 1). GSF inclusion slightly but significantly increased fat contents of cookie (P<0.05), particularly for Carlos GSF, but the effect of GSF particle size on cookie fat content was very limited. The fat content Carlos and Noble GSF fractions used for cookie making were about 18% and 16%, respectively (Table 1), while the wheat flour and sugar were low- or non-fat ingredients. Therefore, partial replacement of wheat flour, sugar and butter by GSF increased fat content of cookie. Cookies containing GSF has slightly higher protein content than control cookies (7.07%). Significantly higher protein contents were noticed at 5% and particle size 209 μm of GSF. The main protein source of the original cookie recipe is wheat flour, the proportional replacement of wheat flour, sugar, and fat by GSF, which contains 10.5-17.5% of protein (Table 1), should increase the protein content of cookies. Table 4 indicates that total carbohydrate in cookies was not significantly affected by the inclusion of 2.5-5% GSF of different particle sizes.

Table 4. Proximate composition and TDF contents of cookies containing GSF of different particle sizes

Type of GSF	GSF (%)	Particle Size (μm)	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Protein (%)	Carbohydrate (%)	TDF (%)
Control	0		3.25±0.09^{a}	0.10±0.00^{a}	23.07±0.20^{a}	7.068±0.36^{a}	66.56	2.51±0.10^{a}
	2.5	104	0.99±0.15^{d}	0.13±0.03^{b}	24.21±1.20^{b}	7.39±0.03^{ab}	67.28	6.61±0.07^{b}
Muscadine Carlos		209	0.91±0.04^{c}	0.16±0.06^{bc}	24.94±0.31^{b}	7.52±0.06^{c}	66.47	5.77±0.23^{c}
	5.0	104	1.22±0.01^{d}	0.23±0.04^{d}	24.62±2.72^{b}	7.68±0.09^{cd}	66.25	8.05±0.26^{d}
		209	1.58±0.07^{e}	0.20±0.06^{d}	25.08±0.26^{c}	7.87±0.00^{e}	65.27	7.39±0.22^{e}
	2.5	104	1.14±0.16^{d}	0.27±0.01^{e}	23.67±0.84^{d}	7.20±0.09^{a}	67.72	4.57±0.07^{f}
Muscadine Noble		209	0.90±0.06^{c}	0.20±0.00^{d}	23.60±0.52^{d}	7.47±0.03^{b}	67.83	4.06±0.52^{g}
	5.0	104	1.34±0.05^{f}	0.31±0.05^{f}	23.88±0.43^{d}	7.60±0.17^{c}	66.87	6.76±0.30^{b}
		209	1.08±0.05^{gd}	0.26±0.03^{e}	23.39±0.42^{ab}	7.62±0.13^{c}	67.65	6.89±0.34^{b}

In the same column, data with different superscripts are significantly different (P<0.05).

3.5 Polyphenol Contents of Cookies

The total phenolic (TP), total flavonoid (TF) and condensed tannin (CT) in cookies were assessed, but total anthocyanin (TA) was not because TA content in GSF was too low (Fig. 1C) and anthocyanin was very unstable under alkaline condition (Oancea & Drăghici, 2013). The TP and TF contents of cookies increased linearly with increasing GSF level and decreased with the increasing of particle sizes of GSF at each GSF particle size (Fig.2A, 2B, 2C and 2D). The CT increased with GSF inclusion level in dose-dependent manner, but not linearly (Fig. 3E, 3F). Overall, cookies with smaller GSF particles yielded higher TP, TF and CT than those with larger GSF particle size, which is correspondent to the higher TP, TF and CT contents of smaller particle fractions (Fig.1). Cookies containing Muscadine Carlos GSF showed lower TP but higher TF and CT than cookies

containing Muscadine Noble GSF at same inclusion level and particle size. These findings suggest that GSF can be good source of flavonoid and condensed tannin of cookies, and particle size of GSF plays important role in polyphenol accessibility in food products. The smaller particle size results in better accessibility, thus better bioavailability of polyphenols. Condensed tannins are known to interact with proteins and contribute to astringent taste of cookies (Hoye, 2009; Davidov-Pardo et al., 2012). Therefore, the inclusion level and particle size of GSF need to be well controlled to ensure consumer acceptability of the product.

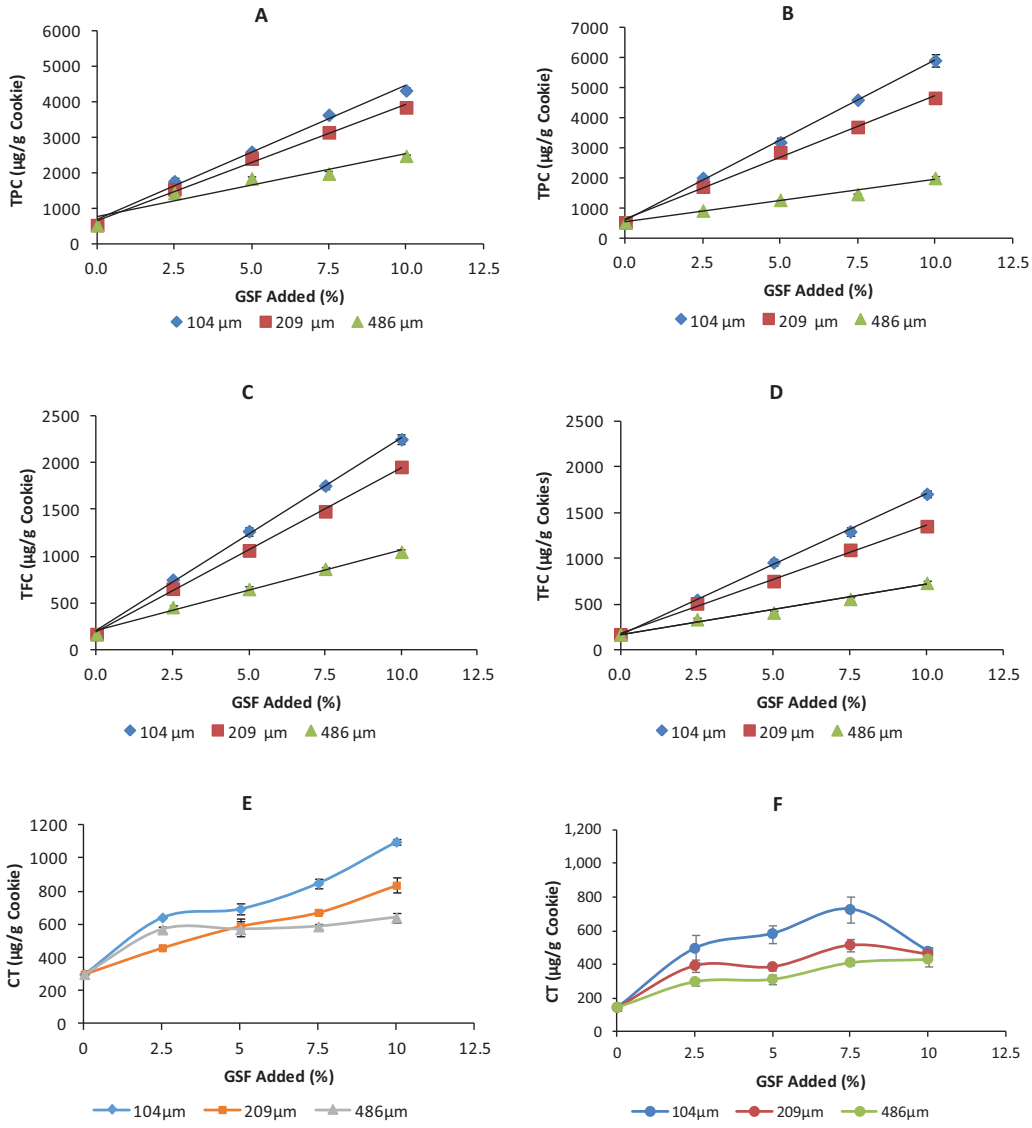

Figure 2. Effect of GSF inclusion level and GSF particle size on the total polyphenol content (TPC), total flavonoid content (TFC) and condensed tannin (CT) content of cookies

A, C, and E-cookies containing Muscadine Carlos GSF, B, D, and F-cookies containing Muscadine Noble GSF

3.6 Total Dietary Fiber Content of Cookies

Regression analysis of cookie TDF versus GSF level shows the TDF of cookies increased with GSF content linearly (R^2= 0.9604 – 0.9927) (Fig. 3). This is expected because GSF fractions contain about 32-67% TDF (Table 1). Overall, the cookie with coarse GSF fraction showed higher TDF. At particle size 104 and 209, every 1% addition of Noble GSF increased cookie TDF by 0.935 and 0.911%, respectively; and every 1% addition of Carlos GSF increased cookie TDF 0.938 and 1.02, respectively; which were higher than the TDF of GSF. This might be attributed to the high polyphenol, particularly, condensed tannin content of GSF because polyphenols tend to interact with macronutrients to form indigestible complex and to inhibit the activity of digestive enzymes

(Yu et al., 2016; Velickovic & Stanic-Vucinic, 2018). Therefore, GSF can be good for TDF fortification in cookie product, but the level of inclusion must be controlled.

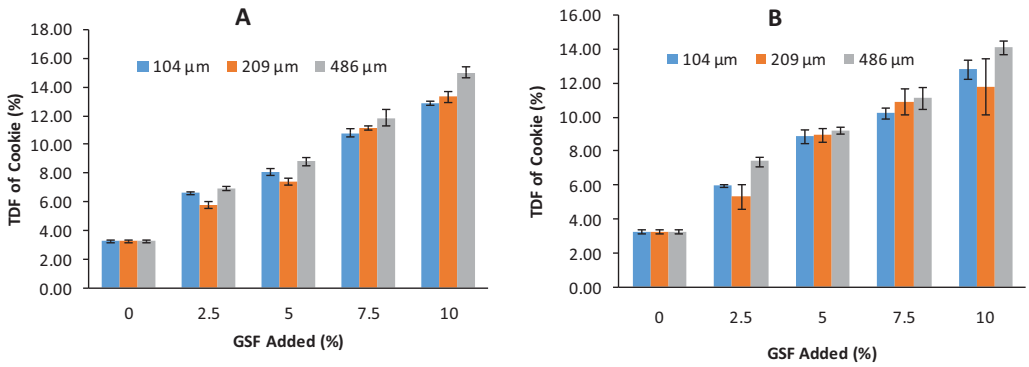

Figure 3. Effect of GSF content and particle size on the TDF of cookies

A-cookie containing Muscadine Carlos flour, B-cookies containing Muscadine Noble flour

3.7 Consumer Acceptance of Cookies

Cookies made with 2.5% and 5% Muscadine Noble GSF of fine (104 µm) and medium (209 µm) particle sizes were evaluated by consumer test because cookies containing higher GSF levels were obviously different from control in color and flavor as tested by a focus group of 4 trained panelists. Results are summarized in Table 5. The control cookie had the highest scores in all the sensory attributes and overall liking, followed by the cookies with 2.5% of 209 µm GSF. The cookies with 5% GSF of 104 µm received lowest scores. Statistically, both concentration and particle size of GSF had significant impacts on the color, taste and overall liking of cookies ($P<0.05$), but not on the aroma and texture. The particle size reduction of GSF did not obviously influence the consumer acceptance of cookies at 2.5% level, but negatively affected consumer's perception of all sensory attributes at 5% GSF level ($P<0.05$). Overall, the level of GSF had more impact on the sensory quality and consumer acceptance of cookies than the particle size for the GSF at the concentrations and particle sizes used in this study. It was reported that the cookies containing 5% GSF (red) was most appreciated in terms of sensorial properties and purchasing intent but the general acceptability significantly decreased as the level of GSF exceeded 10% (Acun & Gul, 2014). Although another study found that adding up to 15.0 % (on the weight of flour) of GSF did not reduce organoleptic properties of butter biscuit comparing with reference (Samohvalova et al., 2016), it is unknown whether the GSF used in that study was from red or white grapes. Therefore, 5% GSF might be the highest inclusion level and the fraction with average 209 µm might be the best for cookie making if the GSF is from red grapes.

Table 5. Sensory Evaluation results of cookies containing Muscadine Noble GSF of different particle sizes (n=55)

Particle Size	GSF (%)	Color	Aroma	Taste	Texture	Overall
Control	0.0	7.19±1.70^{a}	6.81±1.74^{a}	6.93 ±1.37^{a}	6.52 ±1.55^{a}	7.00 ±1.26^{a}
104 µm	2.5	6.22±1.66^{b}	6.67 ± 1.77^{a}	6.11±.49^{b}	6.18±1.73^{ab}	6.27±1.62^{b}
	5	5.92 ±1.81^{b}	5.92 ±1.53^{b}	5.43 ±1.69^{c}	5.92 ±1.81^{b}	5.92 ±1.53^{c}
209 µm	2.5	6.36±1.89^{b}	6.62 ± 1.58^{a}	6.44±1.69^{ab}	6.27±1.55^{ab}	6.25±1.28^{b}
	5	6.11±1.99^{b}	6.29 ± 1.81^{ab}	5.96±1.70^{bc}	6.15±1.75^{ab}	6.22±1.56^{b}

In the same column, data with different superscripts are significantly different ($P<0.05$).

4. Conclusion

This study indicates that the chemical composition of GSF varied significantly with particle sizes and finer fraction had higher ash, fat, protein and extractible polyphenol contents, but lower TDF content. Inclusion of GSF in the cookie formula had significant influences on cookie's physical, chemical and sensory properties. The impacts of GSF inclusion on the cookie quality and sensory properties were strongly associated with the quantity of inclusion and the particle size of GSF. Higher inclusion level (5%) and smaller particle size (104 µm) resulted

in lower consumer acceptability. Therefore, the quantity of inclusion and particle size of GSF have to be well controlled/balanced to ensure the product quality. According to the results obtained from this study, the GSF fraction with average particle size 209 μm (the fraction passes 60 mesh sieve) can be added up to 5%. These findings provide important insights on the value added utilization of grape seed by food manufacturers or general consumers.

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Conflict of Interest

The authors declare no conflict of interest.

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Effect of Whole Purple Potato Flour on Dough Properties and Quality of Steamed Bread

Tianyu Zhang[1, 2], Fengjie Zhang[1, 2], Yanfei Cao[1, 2], Haijing Zhang[1], Zhe Yang[1] & Hongjun Li[1, 2]

[1]Laoling Xisen Potato Industry Group Co., LTD, Dezhou, China

[2]School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo, China

Correspondence: Hongjun Li, Laoling Xisen Potato Industry Group Co., LTD., Dezhou, China. E-mail: lhj6812@163.com

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Abstract

WPPF was added into the wheat flour (WF) flour with different addition amount (0%~50%) to study the influence of whole purple potato flour (WPPF) on dough properties and quality of steamed bread. Result revealed that the WPPF addition significantly influenced the dough properties and quality of steamed bread. The water absorption, the maximum height of the gas release, total volume of CO_2 release and the hardness of steamed bread significantly increased with the increase of WPPF addition amount, while decreased the dough stability, the maximum height of dough, the gas holding capacity and the specific volume. Moreover, peak viscosity, final viscosity and setback value had a remarkable decrease when 10% WPPF added, but increased following the addition of WPPF. Considering the sensory evaluation, the steamed bread with 20% WPPF is acceptable. Appropriate addition amount of WPPF improves the nutrition value and variety of steamed bread and did not effect on the quality of the quality of dough and steamed bread.

Keywords: whole purple potato flour, pasting properties, fermentation properties, texture properties, sensory evaluation

1. Introduction

Purple potato is a cross-breed potato variety native to South America and introduced to China in recent years(Gan, Xin, & Yun, 2017).Purple potato has a high nutritional value due to the large amount of starch, dietary fiber, amino acids, minerals and vitamins. Each 100 grams of fresh purple potato contains about 11.0 mg of calcium, 1.2 mg of iron, 343.0 mg of potassium, 22.9 g of magnesium, 16.0 mg of vitamin C and 40 mganthocyanins(Qiu, Wang, Song, Deng, & Zhao, 2018), which can effectively compensate for the deficiencies of traditional staple foods(Gan et al., 2017).

Compared with other major crops potato has stronger adaptability to barren drought, severe climate and has higher production per units, it's part for human consumption up to 85% (Lutaladio, Castaldi, & Lutaladio, 2009).In 2015, the Ministry of Agriculture of China proposed a strategy of potato staple food, and promoted the potato into a staple food such as steamed bread, noodles and synthetic rice. The potato will become another staple food other than rice, wheat and corn. Comparing with the consumption model of international China's potato is mainly used for fresh food, starch raw materials, and feed raw materials and so on. Increasing the consumption of potato in China can improve the fulfillment of the nutritional needs of the residents and promote the sustainable development of agriculture(Wang, Liu, & Zhao, 2016).

Lack of gluten in the respective products leads to weak dough structure and deterioration of crumb quality, therefore, potato food products are made from a mixture of potato flour and wheat flour, both to solve the problem of whole potato powder poor processing performance, and can remedy the nutritional limitations of potato flour.

The addition of whole purple potato flour changed the proportions of starches, protein and other components. Their presence influences water absorption, pasting properties and farinograph properties of the dough as well as texture and staling of the steamed bread, which allows the manufacture of products with strictly designed and controllable properties(Zhu, 2014).

In this research, the quality characteristics of steamed bread with high proportion of purple potato powder were

investigated to provide a basis for further development of purple potato steamed bread.

2. Materials and Methods

2.1 Materials

Wheat flour (WF) was purchased from Yunhai Flour Factory, Zibo, CHN. Whole purple potato flour (WPPF) was obtained from LaolingXisen Potato Industry Group Co. LTD, Dezhou, CHN. Yeast was purchased from Angel Yeast Co., Ltd, Chifeng, CHN.

The flaky whole purple potato flour was crushed with an ultramicro grinder and sieved with 80 mesh sieve. WF and sieved whole potato flour were mixed at the ratio of 10:0, 9:1, 8:2, 7:3, 6:4 and 5:5, respectively, to get six kinds of blends.

2.2 Chemical Composition Analysis

The WPPF and WF were analyzed for moisture, ash, crude protein and crude starch according tothe AOAC methods (AOAC, 2007). The wet gluten content was determined according to GB/T 14608. All the valueswere measured in triplicates.

2.3 Farinograph Properties

Thefarinograph properties were investigated by Yucebas farinograph (Yucebasmakine, Izmir, TUR) according to the IS0 5530-I method.

2.4 Pasting Properties

The pasting properties were investigated by starch viscoanalyzer (DFY-1, Fangrui Instrument Co., Ltd, Shanghai, CHN). Sample (2 g) and distilled water (20 g) were mixed and then added into an aluminum canister. Then sample was heated to 95 °C at a rate of 2 °C/min, held at 90 °C for 20 min and cooled to 50 °C at 2.25 °C/min and then held for 10 min. The measurements werecarried out duplicate for each sample.

2.5 Rheofermentometer Rheological Measurements

Rheofermentometer rheological properties were investigated by RheofermentometerF4 (Chopin Technologies, Paris, FRA). Dough (315g, 1% yeast, water addition according table 2) was prepared and placed intothe instrumentat constant temperature of 30°C for 3 h and 2 000 g was used as a restrain.

2.6 Steamed Bread Making Process

Dry yeast was dissolved with 36°C distilled water and poured into 200 blended flour, surplus water (total water addition was determined by farinograph water absorption rate, table 2) was added to form dough.The dough fermented in a fermentation room (Brandone Equipment, Guangzhou, CHN) for 60 min at 36 °C and 80% relative humidity. Then, the dough was divided into 100 g dough piece, and each dough pieces were kneaded and shaped by hand. The second fermentation performed for 20 min at the same conditions as first fermentation.After fermentation, dough was steamed for 20 min and cooled for 60 min at room temperature.

2.7 Steamed Bread Quality Evaluation

2.7.1 Textural Profile Analysis (TPA) of Steamed Bread

The steamed bread was sliced into 20 mm pieces. TPA was measured using a TA.XT.plus Texture Analyser (Stable Micro Systems, Survey, UK).The measurement probe was used P/36R. The parameter settings below: compression degree was 60%; trigger force was auto-5 g; before testing speed was 2mm/s; testing speeding and post testing speed were 1mm/s; the interval time between two compression was 5s.

2.7.2 Determination of the Specific Volume of Steamed Bread

The specific volume of steamed bread (volume/mass) was determined using millet displacement for volume and electronic balance for mass.

2.7.3 Color

Color parameters were measured using a CM-3600A desktop color measurement instrument (Konica Minolta Investment Ltd., Shanghai, CHN). The equipment was standardized each time with white and black standards. Samples were scanned to determine lightness (L*), red-green (a*) and yellow-blue (b*) color components.

2.7.4 Sensory Evaluation

The sensory evaluation standard was according to the Gao et al.(2017). The sensory quality testing was performed by eight trained panelists in triplicate. All samples with randomized numbers were tested by panelists at the same time. The values of specific volume, appearance, color and luster, odor, viscosity, chewiness,

elasticity and internal structure were scored by the panels. The best scores were20, 15, 10, 10, 10, 10, 10 and 15, respectively, and the total score was 100.

2.8 Statistical Analysis

Data were analyzedby averages, Duncan's t-test, ANOVA and least significant difference (LSD) test using SPSS Statistics 22 (IBM Corp., NY, USA). The least significant difference at the 5%probability level ($P < 0.05$) was calculated for eachparameter.

3 Results and Discussion

3.1 Proximate Composition from Two Flours

Table 1. Moisture, ash, crude starch, crude protein, crude ash and wet gluten contents of WPPF and WF(%)

	Moisture	Ash	Crude starch	Crude protein	Crude fat	Wet gluten
WF	12.59± 0.23	0.53± 0.01	70.56± 0.21	12.86± 1.25	0.87± 0.01	34.14± 0.16
WPPF	6.59± 0.16	2.17± 0.09	69.16± 0.12	11.98± 0.56	1.55± 0.01	0

The basic composition of wheat flour and whole purple potato flour are presented in table1.The WF ash content was 0.53%,which indicated that the wheat flour used in this experiment had superior quality and high processing degree. The WPPF ash content was 2.17%, it is about four times as much as WF.This result showed that the whole purple potato powder is rich in minerals, such as iron, phosphorus, potassium, calcium, zinc and so on.

The protein content of the whole purple potato flour is slightly lower than that of wheat flour.But proteins are quite different in composition between WF and WPPF. WF protein mainly includes albumin (3%~5%), globulin (6%~10%), gliadin (40%~50%), glutenin (30%~40%) and other proteins (Osborne, 1907). WPPF protein mainly includes patain (about 40%), protease inhibitors (about 50%) and other macromolecular proteins (about 10%). Gliadin and glutenin are the main ingredient of gluten, lack of gliadin and glutenin resulted in gluten content of WPPF was no detectable and resulted in poor dough properties.

Studies have shown that the potato protein contains 8 kinds of essential amino acids (Bártová et al., 2015). In addition, the protein in wheat flour lacks lysine, can cause nutrition to unbalance. Therefore, from the nutritional value of protein, WPPT outbalance WF. Compared with WF, the mixed flour with WPPF had higher nutritional value.

3.2 Farinograph Properties

Table 2. Effect of WPPF on *farinograph* properties of wheat dough

Addition level (%)	Water absorption rate (%)	Development time (min)	Stability time / (min)	Degree of softening(FU)	Flour Quality Coef.
0	58.7	2.4	3.1	115	52
10	67.8	2.5	2.1	266	38
20	72.2	2.5	2.5	279	36
30	87.5	4.2	1.2	240	34
40	93.6	4.2	1.2	255	24
50	103.2	5.8	1.1	257	39

Table 2 showed the influence of WPPF on farinograph properties of WF. With WPPF addition, an increase in the water absorption was observed (from 58.7% to 103.2%), which might be due to the low moisture content and high damage starch content of WPPF. There also been a significant increase in development time (from 2.4 to 5.8 min), and the reason could be the high water absorption of WPPF results in the uneven water distribution in dough. Decrease in stability time (from 3.1 to 1.1 min) and degree of softening (from 115 to 257 FU) indicated the gluten strength of blends was weakened. The gluten protein was diluted with the increase of WPPF proportion. The interchain disulfide bond between the gluten polypeptide and the secondary bond is more likely to be broken. The ability to recombine to form the gluten network and the strength of the gluten skeleton were weakened, resulting in shortened dough stability time, increased degree of softening of the blends, and decreased four quality coefficients (Verwimp, Courtin, Delcour, & Hui, 2006).

3.3 Pasting Properties

Pasting behavior of different ratios blends determined using starch viscoanalyzer is presented in figure 1 and appendix A.The stir and heat resulted in the swelling of the starch and increases the viscosity at the initial stage

(S1), and then the blends system was homogenized and decreased the viscosity(Zhang, Mu, & Sun, 2018).

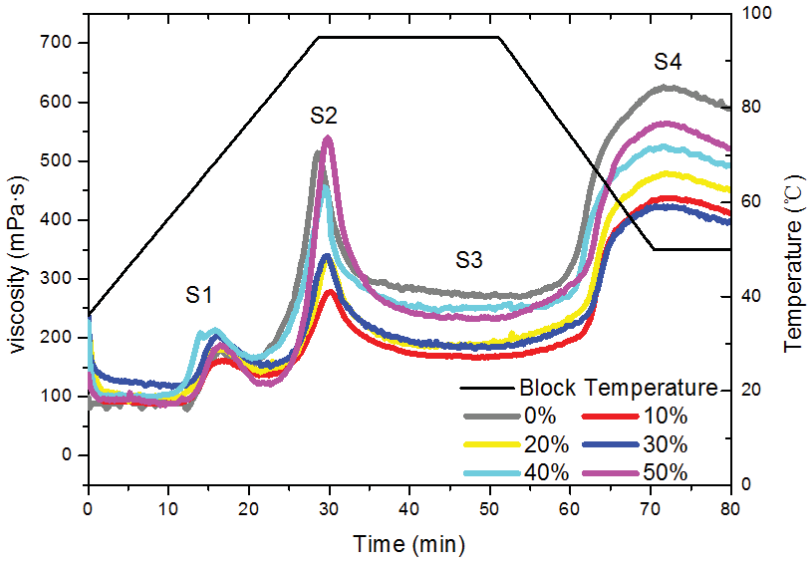

Figure 1. Pasting properties of the WPPF-WF blends systems

Note. WPPF, whole purple potato flour; WF, wheat flour. S1, the maximum dough consistency at the initial mixing stage; S2, the peak viscosity during the heating stage; S3, the minimum viscosity during the heating period; S5, the maximum viscosity obtained after cooling at 50 °C

S2 is the peak viscosity (PV) of the WPPF-WF blends system during the heating stage. The peak PV of 0% was 527 mPa.s, whereas the 10%, 20%, 30%, 40% and 50% addition levels showed a PV of 278 mPa.s, 333.2 mPa.s, 339 mPa.s, 473.4 mPa.s, and 590.2 mPa.s, respectively. There was a rapid decrease of PV when 10% WPPF was added, and then heightened gradually with the ratio of WPPF increase. This might be due to the fact that the WPPF did not contain gluten protein, and the WPPF was added to the flour to play the role of diluting gluten. Moreover, since the starch in the WPPF had good adhesion property, the more stringy and cohesive textural properties of the potato starch made the blends more stable against stir (Swinkels, 1985), thereby increased the PV. The interaction of protein and starch made the WPPF addition affected the PV.

The PV of 0% came early (28.5 min) compared with WPPF addition blends (from 29.3 to 30.2min). The PV of the above blends system migrated to the high temperature direction because the WPPF starch competed with the wheat starch for water absorption, so that the gelatinization temperature of the mixed powder was increased. This is consistent with previous water absorption results in table 2.

The difference between S3 to S4 representation the setback value of WPPF-WF blends system. The setback value decreased with the increase of the WPPF addition, indicating that the addition of WPPF increased the anti-retrogradation performance of the blends. This may be mainly due to the strong water absorption of the whole potato powder, which limited the moisture available to the flour and hindered the expansion of wheat starch, which is not sufficient for the gelatinization of the system, resulting in the final viscosity, regenerative value (Zaidul, Yamauchi, Kim, Hashimoto, & Noda, 2007).

The mechanism of starch gelatinization was that the water molecules in the system move to the inside of the starch molecule and compete for hydrogen bonds(Lei, Tina, Sun, & Chun, 2008). When the energy of the water molecules was greater than the hydrogen bond energy between the starch molecules, the hydrogen bonds between the molecules and the molecules will break. So that the structure of the starch molecule was destroyed, fully stretched, and loose(Yang, 2009).

3.4 Fermentation Properties

Table 4. Effect of WPPF on fermentation rheological properties of wheat dough

Addition level / %	H_m/ mm	h/ mm	(Hm-h)/Hm	H'_m/ mm	V_T/ mm	V_R/ mm	V_C/ mm	V_r/V_t
0	25.5± 0.13d	24.7± 0.19e	3.1± 0.01b	68.1± 1.23a	1473± 5.28a	1145± 3.99a	328± 10.25a	77.7± 0.12e
10	19.1± 0.15c	19.1± 0.20d	0a	90.8± 2.15b	1963± 11.96b	1347± 10.02b	616± 11.52b	68.6± 0.01d
20	15.9± 0.12bc	15.9± 0.08b	0a	109.3± 0.58d	2463± 11.27e	1498± 24.95e	965± 6.28e	60.8± 0.50b
30	16.6± 0.13b	16.6± 0.06c	0a	113.9± 1.23f	2572± 15.68f	1530± 4.52f	1042± 14.76f	59.5± 0.01a
40	10.5± 0.05a	10.5± 0.15a	0a	106.3± 1.58c	2409± 25.52d	1471± 9.68d	939± 9.58d	61.0 ± 0.24c
50	9.6± 0.02a	9.6± 0.05a	0a	109.9± 1.41e	2293± 9.61c	1439± 23.44c	855± 8.46c	62.7± 0.58c

Note. H_m, dough height at maximum development time; h, height of dough at the end of the test; H'_m, the maximum height of the gas release; V_T, total volume of CO_2 release; V_R, CO_2 volume still retained in the dough at the end of test; V_C, CO_2 volume that the dough has lost during test.Different letters in the same column are significantly different ($P < 0.05$).

The maximum dough height (H_m) and final dough height (h) were significantly lowered by the WPPF addition (table 3), which indicated dough development was significantly decreased. The reason might be speculated that WPPF restrained the extension of wheat dough during fermentation process (Penella, Collar, & Haros, 2008). There were a negative of dough development and stability with WPPF addition, which was also confirmed by the farinograph properties.

As the proportion of WPPF increased the maximum height of the gas release (H'_m), the total volume of CO_2 release (V_T), the CO_2 volume still retained in the dough (V_R) and the CO_2 volume that the dough lost (V_C) gradually increased, especially the addition of 30% WPPF was the most obvious. The reason might be that potato starch was made by high temperature, the structure of potato starch was changed, active sites of starch were emerged, the enzyme could easily contact the starch active sites, and then catalyze it into sugars which can be utilized by yeast. But as the WPPF increased further, the content of alpha-amylase decreased and affected the total volume of gas(Liu, Taihua, Sun, Zhang, & Chen, 2016). Despite the volume of gas produced in the WPPF dough was increased remarkably, the V_r/V_t was decreased which indicated gas holding capacity was weakened.

3.4 Quality of Steamed Bread

3.4.1 Texture Properties

Table 3. Effect of WPPF on texture properties of steamed bread

Addition level / %	hardness	adhesiveness	resilience	cohesion	springiness	chewiness
0	2973.01±349.18a	-15.24±1.35b	0.32±0.01cd	0.76±0.01ef	0.94±0.01abc	2119.66±223.54a
10	3447.87±411.97ab	-17.32±1.25b	0.41±0.01f	0.76±0.09f	0.94±0.01abc	2466.32±257.02ab
20	4166.75±451.65b	-18.42±1.47b	0.41±0.01f	0.75±0.015def	0.94±0.02abc	2933.94±271.93b
30	6360.66±495.23d	-34.45±1.38b	0.38±0.01cd	0.72±0.010c	0.95±0.04c	4401.68±319.66c
40	13881.32±575.19f	-28.00±1.37b	0.39±0.01cd	0.72±0.013bc	0.92±0.01ab	9197.94±481.88e
50	12125.78±521.12e	-73.42±5.54a	0.3614±0.01b	0.70±0.01b	0.92±0.01a	7825.14±488.88d

Different letters in the same column are significantly different ($P < 0.05$).

The effect of the amount of purple potato powder added on the texture of steamed bread was shown in Table 3. It can be seen from Table 3 that the total trend of the hardness of the steamed bread is increased with the increase of the total amount of purple potato powder. When the added amount was less than 30%, the hardness increased slowly; when the added amount reached 30%, the hardness increased greatly; while the added amount ranges from 30% to 50%, the hardness of the steamed bread did not change much. The resilience of the steamed bread was gradually increased and the structure of the steamed bread was firmed. The compression required a large force, and the force released during the recovery process was also large; the springiness and cohesiveness were gradually reduced, and the chewiness was directly related to the hardness, and the increase was obvious. Since the whole purple potato powder did not contain gluten protein, the whole purple potato powder was added to the flour to dilute the gluten protein, resulting in poor formation of the gluten network or partial tearing, the dough collapses, and the structure becomes compact. This also led to a decline in the cohesiveness of the steamed bread, which may be due to insufficient coverage of the starch molecules by the gluten network(Marston, Khouryieh, & Aramouni, 2016).

3.4.2 Specific Volume

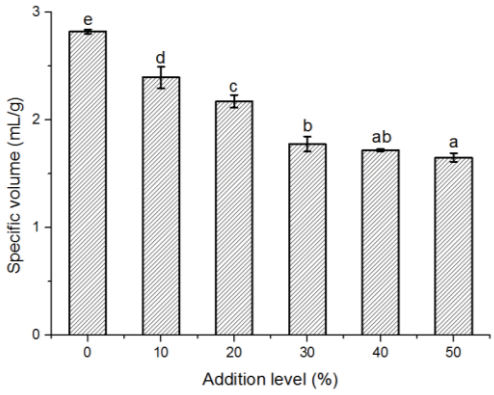

Figure 2. Effect of potato granule addition on specific volume of steamed bread

It can be seen intuitively from figure 2 that the specific volume of the steamed bread gradually decreased with the increased of the WPPF addition level. When the addition level was less than 20%, the larger specific volume indicated that the addition of a small amount of WPPF did not have much influence on the specific volume of the steamed bread. When the total amount of purple potato powder was higher than 20%, the specific volume is reduced. For one thing, it can be seen at 3.4, the gas production capacity increased with WPPF addition, but more volume of CO_2 released, which made dough a worse gas holding capacity(Arendt, Ryan, & Bello, 2007). For another, it diluted and damaged the structureof gluten network, that's why WPPF samples werewith slight collapse and smaller specific volume.

3.4.3 Color

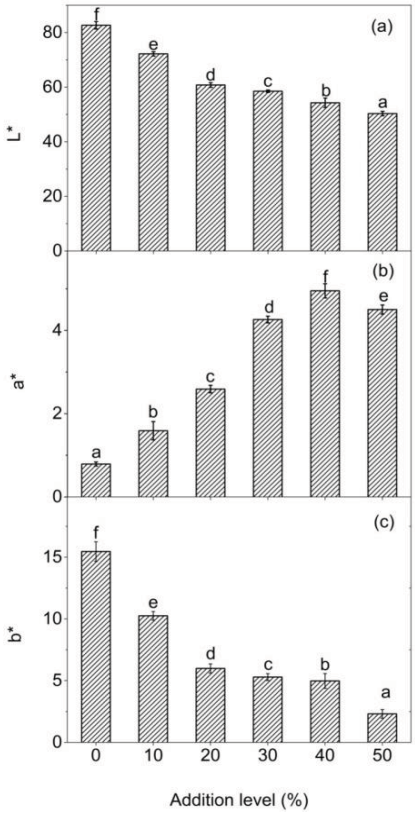

Figure 3. Color of the six kind of WPPF addition level steamed bread

Note. The L* value indicates brightness, 0 for black, 100 for white; (b) for a* value for red-green bias, positive for red, negative for green; (c) for b* for yellow-blue bias, positive for positive yellow, negative for blue.

Figure 3 showed the results of L*, a*, and b* evaluated of the steamed bread samples. Through the determination of desktop color measurement instrument, different ratio blends L*, a*and b* value, asshown in figure 3. Figure 3 (a) shows that the brightness of WF is above 80, with the increased of the WPPF, the L* value of became smaller and smaller, that is, the color becomes darker and darker. Figure 3 (b) shows that the a* value of WF is close to 0.5, hardly showing red color, while the a * value of 40% addition is about 4.5, showing obvious red color. As can be seen from figure 3 (c), there is a negative influence in the b* value of the WPPF. WF tends to be yellow, WPPF tends to be blue. Such changes could be largely attributed to the anthocyanins in WPPF (Zhu & Sun, 2019).

3.4.4 Sensory Evaluation

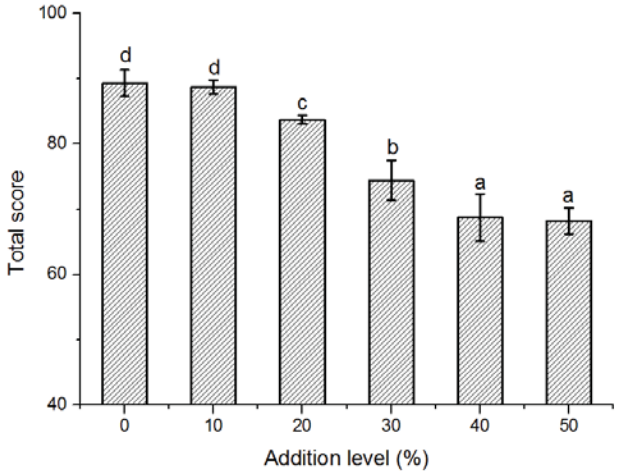

Figure 4. Effect of WPPF on sensory evaluationscore of steamed bread

Sensory evaluation of steamed bread is shown in figure 4 and appendix B. Total score was decreased with the WPPF added. The sensory evaluation dropped significantly when the WPPF addition exceeded 20%. Steamed bread increased the hardness with the WPPF added, thereby had hard texture and worse chewiness. In addition, the viscosity had a conspicuous decrease that had a strong impact on sensory evaluation. It probably due to the high damage starch content, the damaged starch was easy to absorb water and expand, and the volume was also increased. The space structure of the gluten was squeezed in space, destroying the gluten network structure. It was also had a negative effect on specific volume of steamed bread.

4. Conclusion

The WPPF addition increased the water absorption and development time, but decreased the dough stability. Peak viscosity, breakdown viscosity, final viscosity and setback value of 10% WPPF addition were found to be lower than thatof 0% addition. However, the values gradually increased by the WPPF addition from 10% to 50%. As the WPPF addition increased, there was a stronger gas production capacity, but the gas cannot beretained, and the gas holding capacity decreases.When the WPPF addition to more than 20%, specific volume, viscosity, chewiness, elasticity and internal structure decreased greatly, resulted in lower sensory value. The sensory value of steamed bread with 20% WPPF is acceptable.

Acknowledgments

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Appendix

Appendix A

Effect of WPPF on fermentation rheological properties of wheat dough

Addition level (%)	Peak viscosity (mPa·s)	Trough (mPa·s)	Break down (mPa·s)	Final viscosity (mPa·s)	Setback value (mPa·s)	Peak time (min)
0	514.1	269.0	245.1	624.8	355.8	25.9
10	278.5	167.0	111.5	437.8	270.8	30.0
20	334.2	187.2	147	479.5	292.3	29.8
30	338.9	181.0	157.9	423.6	242.6	30.0
40	455.6	247.0	208.6	525.7	278.7	29.4
50	540.0	234.6	305.4	563.01	328.41	29.8

Appendix B

Effect of WPPF on sensory evaluationscore of steamed bread

Addition level (%)	Specific volume	Appearance	Color and luster	Odor	Viscosity	Chewiness	Elasticity	Internal structure	Total score
0	20	14	8.7	8.2	8.9	8.4	8.5	12.9	89.3
10	20	12.5	9	8.2	8.8	8.5	8.9	12.9	88.7
20	19	11.8	8.2	8.7	8.1	8.2	8.1	11.5	83.8
30	15	11.3	8.3	7.9	6.6	7.7	7.3	10.3	74.4
40	14	11.2	7.6	7.4	6.1	7.0	6.8	8.8	68.7
50	13	11.4	8.9	7.3	5.8	7.0	6.1	8.7	68.2

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Association between Fruit Characteristics and Postharvest Stability of Different Pumpkin (*Cucurbita*) Species

Nulu Nansikombi[1], John Herbert Muyonga[1] & Yusuf Byenkya Byaruhanga[1]

[1]School of Food Technology, Nutrition and Bio-engineering, Makerere University, Uganda

Correspondence: Yusuf B. Byaruhanga, School of Food Technology, Nutrition and Bio-engineering, Makerere University P. O Box 7062 Kampala, Uganda. Tel: 256-772-445-113. E-mail: ybbyaru@gmail.com

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Abstract

This study aimed at understanding peel and flesh characteristics of *Cucurbita maxima Duchesne* subsp *maxima*, *Cucurbita pepo* L var. *fastigata* and *Cucurbita moschata Decne* pumpkin varieties and how the fruit characteristics relate to its postharvest stability. Mature fruits were stored at ambient conditions (28^{o}C and 78% Relative humidity) for eight months. Cell microscopic structure, intercellular space size, sugars, starch, cellulose, hemicellulose, lignin, degree of esterification of pectin, polygalacturonase activity, and cumulative moisture lost were determined monthly. Sucrose, starch, cellulose and hemicellulose content and degree of esterification of pectin of different varieties decreased with storage. Glucose and fructose content increased to a maximum and then reduced. Polygalacturonase activity and lignin generally increased with storage time. Size of intercellular spaces, and cumulative moisture lost from both flesh and peel increased with storage. Sucrose breakdown was key during 0 to 3 months, while moisture loss was important at 4 months of storage. Polygalacturonase activity became paramount from 7th month of storage. Postharvest stability of pumpkins is determined by an interplay of factors. The deterministic factor changes with time. Pumpkin varieties with more lignified peel had lower rates of sucrose breakdown and moisture loss and tended to be more stable postharvest. Varieties with low level of hemicellulose had their cell walls degrade early, while deterioration of pumpkins with highly esterified pectin and closely packed cells was delayed.

Keywords: pumpkin varieties, fruit, stability determinants, factor analysis

1. Introduction

Pumpkin (*Cucurbita spp*) is reported to be agronomically a less demanding and weather tolerant crop. Besides, FAO (1994) reported existence of diverse pumpkins species. Although there are five domesticated species, three species namely *Cucurbita maxima*, *Cucurbita pepo* and *Cucurbita moschata* are considered of economic importance. Yet still, the three species of economic importance have many varieties under them.

FAO, (1994) classified pumpkin as a neglected crop and describes neglected crops as those either; associated with low income generation, strongly linked to their places of origin, locally produced or wild species or with their facts poorly documented for example their distribution, biology, cultivation, uses among others. Total crop land for Africa was estimated at 1.4billion ha in 2016. As of 2017, pumpkins, squash and gourds production covered only 344,755ha, corresponding to 0.02% (FAO, 2019).

Just like any other agricultural product, pumpkins are subject to changes in quality postharvest. For example Balandrán-Quintana, Mendoza-Wilson, Vargas-Arispuro, & Martínez-Telléz (2007) reported an increase in the activity of polygalacturonase enzyme leading to depolymerization of pectin during senescence of Zucchini (*Cucurbita pepo L*) stored at 2.5^{o}C and 12^{o}C for 16days. Jariene, Danilčenko, Vaitkevičienė, & Nataliia (2015) reported a decrease in dry matter of great pumpkins (*Cucurbita maxima* D) during storage at 15^{o}C and 70% relative humidity for four months. However, commercially pumpkins are stored in the open at ambient temperatures. Rahman, Miaruddin, Khan, Masud, & Begum (2013) reported an initial increase in total soluble solids until 45days of storage followed by a decrease in titratable acidity, weight, β–carotene, and ascorbic acid of BARI pumpkin (*Cucurbita moschata* Poir) during storage at ambient conditions (27-31^{o}C and 75-90% relative humidity) for a period of 120days.

The postharvest stability of the different pumpkin varieties varies depending on the composition of the peel and

the flesh. According to Kader and Barret (2004), fruits generally differ in chemical constituent such as metabolizable carbohydrates, and pectin and in the expression of metabolic enzymes. This is likely to impact differently on their stability postharvest. The peel of fruits is reported to regulate the rate of transpiration and respiration, the key deteriorative processes postharvest (Kader & Barret, 2004). Hence, differences in peel structural components such as cellulose, hemicellulose and lignin and their degradation are likely to affect the postharvest stability of different fruits.

The pumpkin fruit peel and flesh is the key distinguishing feature between species and varieties. However, there is dearth of knowledge about the fruit peel and flesh characteristics of the different varieties and how these relate to the postharvest stability of the different pumpkin varieties. To address these knowledge gaps, the changes in composition of the peel, flesh and the structure changes of the flesh of mature fruits of *Cucurbita maxima Duchesne* subsp *maxima*, *Cucurbita pepo* L var. *fastigata* and *Cucurbita moschata Decne* stored at ambient conditions were studied over a storage period of 8months. The results of this study will increase our understanding of the variability in *Cucurbita spp* but also form a basis for tapping into the commercial potential of the different pumpkin varieties.

2. Materials and Methods

2.1 Raw Material Selection and Experimental Design

Three pumpkin varieties namely *C. maxima Duchesne* subsp *maxima*, *C. pepo* L var. *fastigata* and *C. moschata Decne* varieties were grown on randomized plots on a farm in Luwero district, Central Uganda. The experiment was conducted in triplicate. Fruits were harvested at maturity (drying of the tendrils next to the fruit was used as the maturity index). Mature and defect free fruits weighing about 3.0 to 3.6kg (*C. maxima Duchesne* subsp *maxima*), 2.6 to 3.1kg (*C. pepo* L var. *fastigata*) and 3.2 to 3.3kg (*C. moschata Decne*) were stored at ambient conditions (28^{o}C average temperature and 78% Relative humidity) for 8months. At monthly intervals, three fruits of each variety were randomly picked and monitored for change in cellulose, hemicellulose, lignin, starch, sugars, pectin esterification, polygalacturonase activity, moisture, and cell microscopy.

2.2 Test Methods

2.2.1 Cellulose, Hemicellulose and Lignin Content of the Peel

Cellulose, hemicellulose and lignin were determined as described by Van Soest and Robertson (1990). Pumpkin peel dried at 60^{o}C was finely ground and 0.5g weighed into a crucible and 100ml of neutral detergent solution, 0.5g of sodium sulfite and three drops of n-octanol added. The mixture was heated to boiling, refluxed for 60min, filtered and the residue washed thrice with boiling water, then twice with cold acetone. Residue was dried for 8hr at 105°C, cooled in a desiccator and weighed. The neutral detergent fibre, composed of cellulose, hemicellulose, lignin and fibre bound compounds was calculated using equation 1.

$$\text{NDF}\ (\%) = \frac{\text{Weight of residue after treatment with neutral detergent solution}}{\text{Weight of sample}} \times 100 \quad (1)$$

To determine the acid detergent fibre (ADF), 0.5g of ground sample was weighed into a crucible, 100ml of acid detergent solution, three drops of n-octanol were added, heated to boiling and refluxed for 60min. The mixture was filtered, the residue washed thrice with boiling water, then twice with cold acetone. The Acid detergent fibre (residue, composed of cellulose, lignin and minerals), was dried at 105°C for 8hr, cooled and weighed and calculated using equation 2.

$$\text{ADF}\ (\%) = \frac{\text{Weight of residue after treatment with acid detergent solution}}{\text{Weight of sample}} \times 100 \quad (2)$$

Hemicellulose and cellulose were determined as shown in equations 3 and 4 respectively. Lignin was determined after treating the Acid detergent fibre with 72% sulphuric acid for 3hr with occasional stirring. The remaining residue, composed of lignin and minerals was oven-dried at 105^{o}C for 8hr, incinerated in a muffle furnace at 550^{o}C for 3hr and ash content calculated as shown in equation 5.

$$\text{Hemicellulose}\ (\%) = \text{NDF}\ (\%) - \text{ADF}\ (\%) \quad (3)$$

$$\text{Cellulose}(\%) = \text{ADF}(\%) - \text{Residue}(\%)\ \text{of ADF treated with 72\% sulphiric acid} \quad (4)$$

$$\text{Lignin}(\%) = \text{Residue}(\%)\ \text{of ADF treated with 72\% sulphiric acid} - \text{Ash}(\%) \quad (5)$$

2.2.2 Starch in Pumpkin Flesh

Starch was quantified using the amyloglucosidase/α-amylase method as per Megazyme total starch assay kit

(Megazyme, 2016). To finely grated pumpkin (0.1g) in a test tube, 5ml of 80% (v/v) ethanol was added, incubated at 80°C for 5min, vortexed, 5ml of 80% (v/v) ethanol added, centrifuged (Fischer scientific 225 centrifuge, Fisher Scientific Co. St. Louis, MO) for 10min at 1141x*g* and supernatant discarded to get rid of any D-glucose and maltodextrins. Then 3ml of 1:30 mixture of thermostable amylase and 100mM sodium acetate buffer pH 5 was added, incubated in a boiling water bath for 6min to hydrolyse starch to maltodextrins, cooled to 50°C, 0.1ml amyloglucosidase added and held at 50°C in a water bath for 30min to hydrolyse maltodextrins to D-glucose. Tube contents were reconstituted to 100ml using distilled water and an aliquot centrifuged at 1141x*g* for 10min. To each 0.1ml of filtrate, glucose standard and distilled water (blank), 3ml of glucose oxidase peroxidase (GOPOD) was added to oxidize glucose to D-gluconate and hydrogen peroxide. Hydrogen peroxide produces a quinoneimine dye whose absorbance was read at 510nm (Genesys 10S UV Visible spectrophotometer, Thermo Electron Corporation USA) and used in determining the starch content as shown in equation 6.

$$\text{Starch}\ (\%) = \Delta A \times \frac{0.1}{(A_{gc} - A_b)} \times \frac{FV}{0.1} \times \frac{100}{W} \times \frac{162}{180} \quad (6)$$

Where; ΔA = absorbance sample-absorbance blank: W = weight of sample (mg): FV = final volume (100 ml): A_{gc} = absorbance glucose control: A_b = absorbance blank**:**

$$\frac{100}{W} = \text{factor to express starch as a percentage of sample}$$

$$\frac{162}{180} = \text{adjustment from free D} - \text{glucose to anhydro D} - \text{glucose of starch}$$

2.2.3 D-glucose, D-fructose and Sucrose

D-glucose, D-fructose and sucrose were determined spectrophotometrically at 340nm (Genesys 10S UV Visible spectrophotometer, Thermo Electron Corporation USA) as described in the Megazyme D-glucose, D-fructose and sucrose assay kit (Megazyme, 2014). Finely grated pumpkin flesh (5g) was slurred to one litre using distilled water, filtered using Whatman filter paper, 0.1ml filtrate pipetted into a test tube, 2.1ml distilled water was added, then 0.1ml buffer and 0.1ml of nicotinamide adenine dinucleotide phosphate and adenosine 5 triphosphate ($NADP^+$/ATP). Absorbance A_1 of mixture was read after 3min. To the above mixture, 20µl of a mixture of hexokinase and glucose 6 phosphate dehydrogenase was added, mixed and absorbance A_2 read after 5min. Glucose was quantified as shown in equations 7 and 8.

In presence of hexokinase and adenosine 5 triphosphate, fructose in the above sample is phosphorylated to fructose 6 phosphate. Phosphoglucose isomerase (20µl) was added to isomerize fructose 6 phosphate to glucose 6 phosphate which reacts with nicotinamide adenine dinucleotide phosphate forming gluconate 6 phosphate and reduced nicotinamide adenine dinucleotide phosphate leading to a rise in absorbance that was read after 10min ($A_{3)}$ and used to quantify fructose as shown in equation 9.

$$\text{Glucose}\ (g/L) = \frac{2.42 \times 180.16}{6300 \times 1 \times 0.1} \times (A_2 - A_1)_{\text{sample}} - (A_2 - A_1)_{\text{blank}} \times F \quad (7)$$

$$\text{Glucose}\ (g/100g) = \frac{\text{Concetration glucose}_{(g/l)}}{\text{sample weight}_{(g/l)}} \times 100 \quad (8)$$

Where; 2.42 = final volume of the mixture (ml): 180.16 = molecular weight of glucose (g/mol): 1 = light path (1cm): 0.1 = sample volume (ml): F = dilution factor (200): and 6300 = extinction coefficient of NADPH at 340 nm (1 x mol^{-1} x cm^{-1}).

$$\text{Fructose}\ (g/L) = \frac{2.44 \times 180.16}{6300 \times 1 \times 0.1} \times (A_3 - A_2)_{\text{sample}} - (A_3 - A_2)_{\text{blank}} \times F \quad (9)$$

Where; 2.44 = final volume of the mixture in the cuvette (ml): 180.16 = molecular weight of fructose (g/mol)

Sucrose was determined from the difference in absorbance for D glucose concentration before and after hydrolysis of sucrose to glucose. To the test tubes except for blank, 0.1ml slurred filtered sample was added. Beta fructosidase (0.2ml) was added, mixed and held at 30°C for 5min followed by 2ml distilled water, 0.1ml buffer and 0.1ml of $NADP^+$/ATP. Absorbance of the mixture (A_1) was read after 3min, a mixture of hexokinase and glucose 6 phosphate dehydrogenase (0.02ml) added, mixed and absorbance A_2 read after 5min. Sucrose was

quantified as shown in equation 10.

$$\text{Sucrose (g/L)} = \frac{2.42\times342.3}{6300\times1\times0.1} \times \Delta A_{\text{sucrose}} \times F \quad (10)$$

$$\Delta A_{\text{sucrose}} = A_{\underset{(sucrose\ sample)}{\text{total D-glucose}}} - \Delta A_{\underset{(glucose\ sample)}{\text{D-glucose}}}$$

$$\Delta A_{\underset{(sucrose\ sample)}{\text{total D-glucose}}} = (A_2 - A_1)_{\text{sample}} - (A_2 - A_1)_{\text{blank}}$$

Where; 342.3 = molecular weight of sucrose (g/mol): $\Delta A_{Sucrose}$ = change in absorbance for sucrose sample - change in absorbance for glucose sample.

2.2.4 Pectin's degree of Esterification

Pectin was extracted as described by Fertonani et al., (2006). To 5g of pumpkin flesh dried overnight at 70°C and finely ground, 10ml of 70% ethanol was added, mixed, 100ml distilled water added followed by 100ml of 210mM hot nitric acid solution, stirred for 10min, and cooled to 4°C. Pectin was precipitated by slowly pouring the mixture into a beaker containing ethanol to a final proportion of 1:3 (v/v) of pectin solution: ethanol. Mixture was filtered using a cheese cloth, pectin put in 70% ethanol overnight to remove the remaining acid, filtered using filter paper and oven dried at 30°C for 8hr and then overnight at 60°C.

Free carboxy groups (K_f%) and esterified carboxy groups (K_e%) were determined using potentiometric titration method (Bochek, Zabivalova, & Petropavlovskii, 2001). Dried pectin (0.2g) was wetted with ethanol, 20ml distilled water at 40°C added, stirred for 2hr, titrated with 0.1N NaOH in the presence of phenolphthalein to a pale rose color and titre used to determine free carboxy groups (K_f%). Then 10ml of 0.1N NaOH solution was added, stirred for 2hr to saponify the esterified carboxy groups, 10ml of 0.1N HCl added, the excess HCl titrated with 0.1N NaOH and titre used to calculate esterified carboxy groups (K_e %) as shown in equation 11. The degree of esterification was calculated using equation 12.

$$K_f \text{ or } K_e(\%) = \frac{N_{NaOH}\times V_{NaOH}\times 0.045}{a} \times 100 \quad (11)$$

$$DE\ (\%) = \frac{K_e}{K_t} \times 100 \quad (12)$$

Where K_t (total carboxyl groups (%) = K_f + K_e and DE is the degree of esterification:

a is the sample weight of pectin

N_{NaOH} and V_{NaOH} are the normality and volume (ml) of sodium hydroxide, respectively.

2.2.5 Polygalacturonase Activity

Crude extracts with polygalacturonase activity were obtained by washing 10g grated pumpkin in 30ml cold 1% sodium bisulphite, thrice. The residue mixed with 30ml of 1M sodium chloride, pH adjusted to 6.0 using 0.1N NaOH, held at 2.5°C for 2hr, stirring occasionally and centrifuged (Fischer scientific 225 centrifuge, Fisher Scientific Co. St. Louis, MO) at 10,062x*g* for 10min. The supernatant (200µl) was reacted with of 0.025% polygalacturonic acid (LM-106 AS-YA GENU pectin, CPKelco, Lille Skensved-Denmark) at 30°C for 1hr, cold borate buffer pH10 (1ml) added then 200µl of 1% cyanoacetamide, held in a boiling water bath for 10min, cooled to room temperature and the absorbance read at 276nm (Genesys 10S UV Visible spectrophotometer, Thermo Electron Corporation USA). A blank, and standard curve for D-galacturonic acid were also prepared. The enzyme activity was reported as µmol of D-galacturonic acid produced in 1hr (Gross, 1982).

2.2.6 Moisture Loss

A sample (2g) of peel and flesh was weighed into a moisture dish, dried in a hot air oven at 105°C for 16hr and moisture content (%) determined using the following equation (Bradley, 2003). Monthly average moisture loss was computed and expressed as a percentage of the initial using equation 13.

$$\%\ \text{Moisture}\ \left(\frac{wt}{wt}\right) = \frac{\text{weight of wet sample} - \text{weight dry sample}}{\text{weight of wet sample}} \times 100 \quad (13)$$

2.2.7 Microscopy of Cells and Intercellular Spaces

A piece of the pumpkin was cut transversely from three fruits of the different pumpkin varieties, trimmed to

completely remove traces of peel and soft regions of the ovary wall where seeds attach, leaving a piece of about $1cm^3$. The $1cm^3$ piece was fixed in cold formalin: acetic acid: ethanol solution (2:1:10 v/v) for 7.8hr. Thin (5mm) sections were cut, subjected to 7.8hr tissue processing in a tissue processor (SLEE mtp, SLEE MAINZ, Germany) and then embedded in paraffin wax (Bio-plast, Bioptica, Italy) before sectioning (3μm) using a rotary microtome (cut 4062, SLEE MAINZ Germany). Sections were deparaffinized and stained using Toluidine Blue as described by Li et al., (2017). Stained sections were viewed using a Nikkon Eclipse E800 light microscope with a camera (Isaza, Barcelona, Spain) at a magnification of x40 and micrographs taken. Area occupied by intercellular spaces was determined using ImageJ/Fiji 1.46 software.

2.3 Statistics

All data analysis was done using Minitab 18 statistical software. One way ANOVA analysis was done to compare the differences between varieties at different storage times and mean separation was done using the Tukey Pairwise Comparisons method ($p \leq 0.05$) in order know which varieties differed significantly. In addition, One way ANOVA was used to compare different times of storage within the same variety and Mean separation was done in order know after how long a significant difference existed in a given parameter of a particular variety. Factor analysis using principal components was carried out on the mean values of the 12 quality parameters to obtain the major determinants of postharvest stability.

3. Results

3.1 Cellulose, Hemicellulose and Lignin Content of the Peel

Initial cellulose content varied significantly *($p<0.05$)* with *C. maxima Duchesne* subsp *maxima* having the highest and *C. pepo* L var. *fastigata* the least. In the 8months, cellulose decreased by 41.7% in *C. maxima Duchesne* subsp *maxima*, 50.4% in *C. pepo* L var. *fastigata* and 67.9% in *C. moschata Decne* (Figure 1A). A significant decrease in each variety was observed after 4months, 3months and 2months respectively.

Initial hemicellulose content varied significantly *($p<0.05$)* with *C. maxima Duchesne* subsp *maxima* having the highest and *C. moschata Decne* the least. Hemicellulose reduced with storage (Figure 1B). *C. maxima Duchesne* subsp *maxima* lost 75.3% of its hemicellulose, *C. pepo* L var. *fastigata* lost 96.1% and *C. moschata Decne* lost 97.7% with significant reductions after 4months, 3months and 2months, respectively.

Initial varietal lignin content did not differ (Figure 1C), and increased significantly after 4months of storage. Lignin increased by $15.9gkg^{-1}$FWB in *C. maxima Duchesne* subsp *maxima*, $6.0gkg^{-1}$FWB in *C. pepo* L var. *fastigata* and $8.8gkg^{-1}$FWB in *C. moschata Decne*.

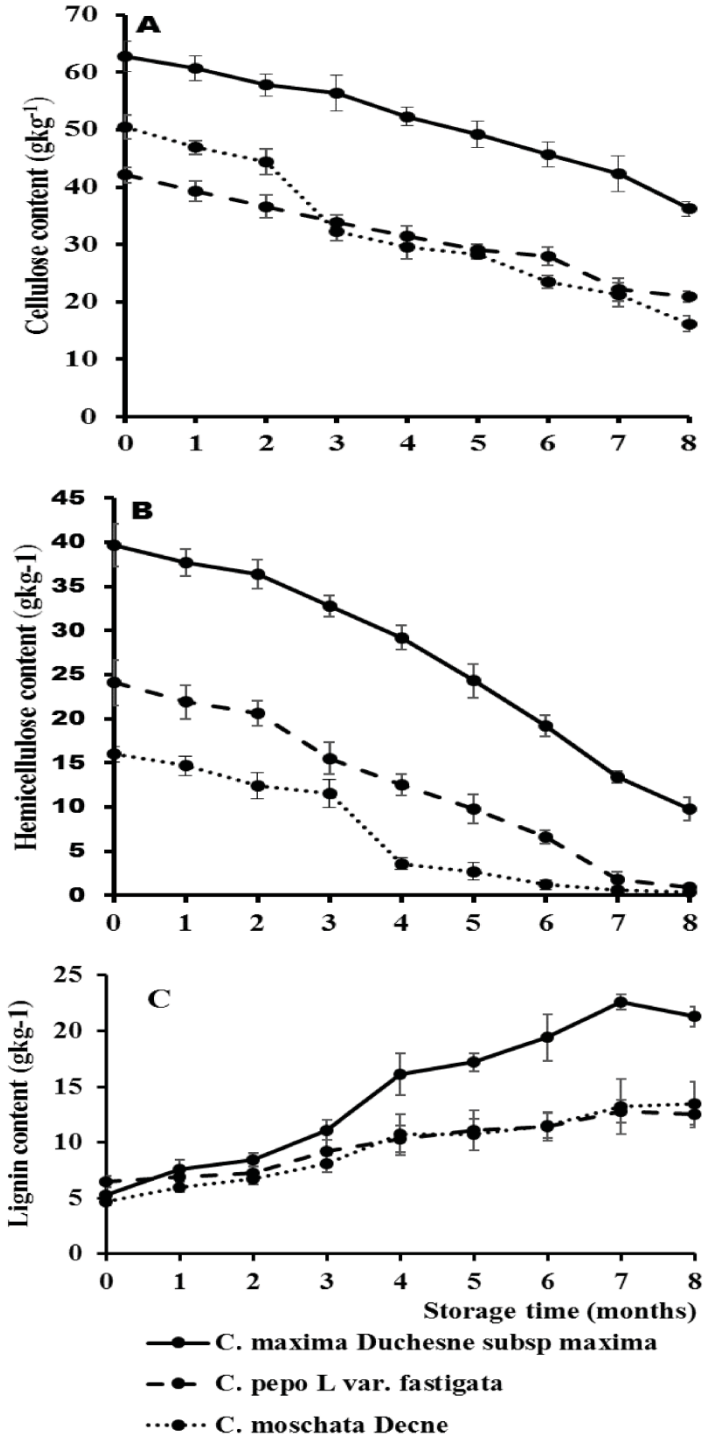

Figure 1. Change in cellulose (A), hemicellulose (B) and lignin (C) content of the pumpkins peel during storage

3.2 Starch, D-glucose, D-fructose and Sucrose Content of the Flesh

Initial starch content varied significantly among varieties and decreased during storage. *C. maxima Duchesne* subsp *maxima* had highest *($p<0.05$)* starch content and *C. moschata Decne* the least throughout the storage period (Figure 2A). In all the three varieties, starch breakdown was fastest from 0-3months of storage.

The initial glucose level of *C. maxima Duchesne* subsp *maxima* and *C. moschata Decne* was not statistically different but *C. pepo* L var. *fastigata* had significantly higher glucose. Glucose level in *C. maxima Duchesne* subsp *maxima* and *C. moschata Decne* increased to a peak at 4 to 5months and then decreased. However, the

glucose content in *C. pepo* L var. *fastigata* remained fairly constant throughout (Figure 2B).

Varietal sucrose content initially varied significantly, with *C. maxima Duchesne* subsp *maxima* having the highest. Sucrose content decreased during storage (Figure 2C) for all varieties. In both *C. maxima Duchesne* subsp *maxima* and *C. moschata Decne,* the rate of sucrose breakdown was highest from 0-5months of storage with respective slopes of 5.1 and 3.8 $gkg^{-1}month^{-1}$. Sucrose breakdown then changed to 4.6 and 3.4 $gkg^{-1}month^{-1}$ for *C. maxima Duchesne* subsp *maxima* and *C. moschata Decne,* respectively. For *C. pepo* L var. *fastigata* the rate of sucrose breakdown was 2.2$gkg^{-1}month^{-1}$ from 0-5months and 2.6$gkg^{-1}month^{-1}$ from 5-8months of storage.

The initial fructose content of different varieties was the same. Fructose content generally increased to a peak at 4-6 months and then decreased among the varieties (Figure 2D).

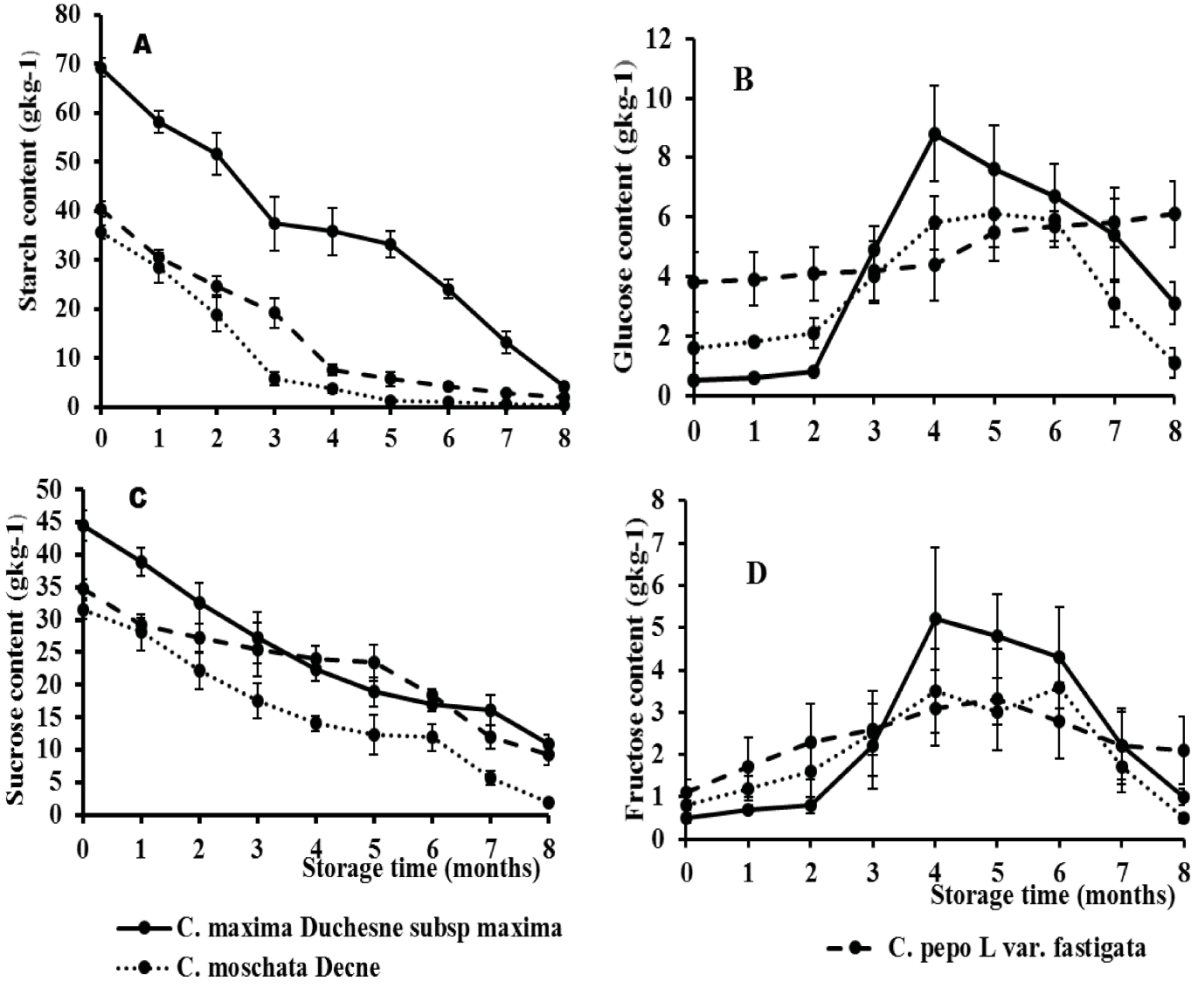

Figure 2. Change in carbohydrates content of the flesh of the pumpkin varieties during storage

3.3 Degree of Esterification of Pectin

The esterification of pectin of different varieties varied significantly *($p<0.05$). C. pepo* L var. *fastigata* had the most esterified pectin and *C. moschata Decne* the least. Generally, the degree of esterification reduced during storage (Figure 3A). The esterification of pectin reduced by 64.9, 57.4, and 48.6% in *C. moschata Decne*, *C. maxima Duchesne* subsp *maxima* and *C. pepo* L var. *fastigata* respectively.

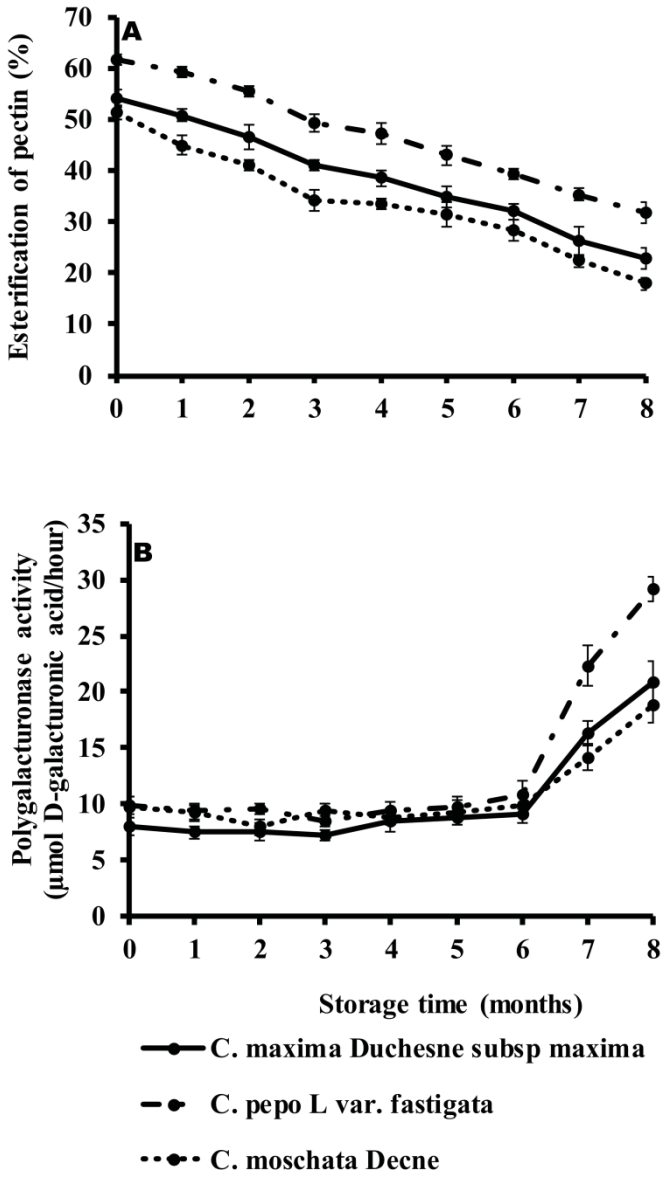

Figure 3. Change in degree of esterification of pectin (A) and polygalacturonase activity (B) of the flesh of different pumpkin varieties during storage

3.4 Polygalacturonase Activity

Polygalacturonase activity was the same, remained constant until the 7thmonth when it increased. *C. pepo* L var. *fastigata* registered higher enzyme activity than *C. moschata Decne* and *C. maxima Duchesne* subsp *maxima*, which were statistically not different between 7 and 8 months of storage (Figure 3B).

3.5 Moisture Loss

The initial moisture content of the flesh of the three pumpkin varieties was statistically the same and ranged from 900 to 924gkg^{-1}. Also the moisture content of the peel did not differ ($p<0.05$), ranging from 890 to 910gkg^{-1}. Cumulative moisture lost from flesh and peel increased with storage. Statistical difference in cumulative moisture loss of the peel was detectable starting from 5thmonth but that from the flesh was not statistically different throughout storage (Figure 4).

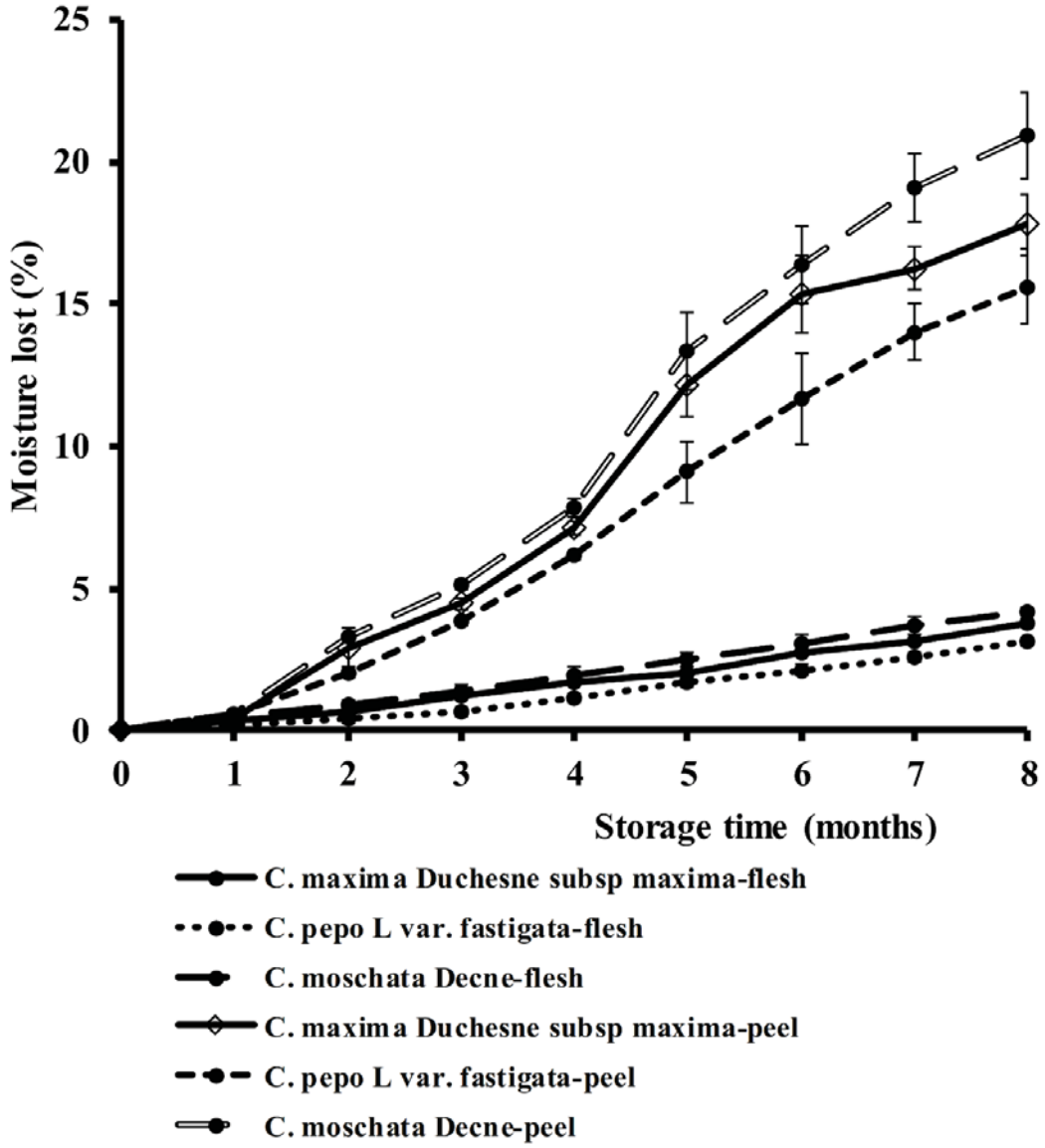

Figure 4. Cumulative moisture (%) lost from the peel and flesh of different pumpkin varieties during storage

3.6 Microscopy of Cells and Intercellular Spaces

The area occupied by intercellular spaces (%) in all the three varieties was not statistically different at month zero. The size of intercellular spaces increased with storage (Table 1). The degree of cell separation was most pronounced in *C. moschata Decne* and by the sixth month, cells had started shrinking and the cell walls shriveled (Figure 5).

Table 1. Area of intercellular spaces (%) in three pumpkin varieties stored for 8 months

Month	*C. maxima Duchesne* subsp *maxima*	*C. pepo* L var. *fastigata*	*C. moschata Decne*
0	$1.0^{a} \pm 0.1$	$0.9^{a} \pm 0.1$	$0.9^{a} \pm 0.1$
1	$1.1^{ab} \pm 0.2$	$1.0^{b} \pm 0.1$	$1.4^{a} \pm 0.1$
2	$1.6^{ab} \pm 0.1$	$1.4^{b} \pm 0.1$	$1.9^{a} \pm 0.2$
3	$2.1^{ab} \pm 0.1$	$1.9^{b} \pm 0.1$	$2.6^{a} \pm 0.2$
4	$2.6^{a} \pm 0.1$	$2.2^{b} \pm 0.1$	$2.9^{a} \pm 0.2$
5	$2.8^{a} \pm 0.1$	$2.4^{b} \pm 0.1$	$3.5^{c} \pm 0.1$
6	$3.3^{a} \pm 0.4$	$2.9^{a} \pm 0.1$	$5.0^{b} \pm 0.2$
7	$3.9^{ab} \pm 0.2$	$3.5^{b} \pm 0.3$	$4.3^{a} \pm 0.3$
8	$3.0^{a} \pm 0.2$	$4.0^{b} \pm 0.2$	$3.5^{ab} \pm 0.3$

Data are means of triplicate parameter values ± standard deviation. Means in the same row with the same letter are significantly different ($P \leq 0.05$)

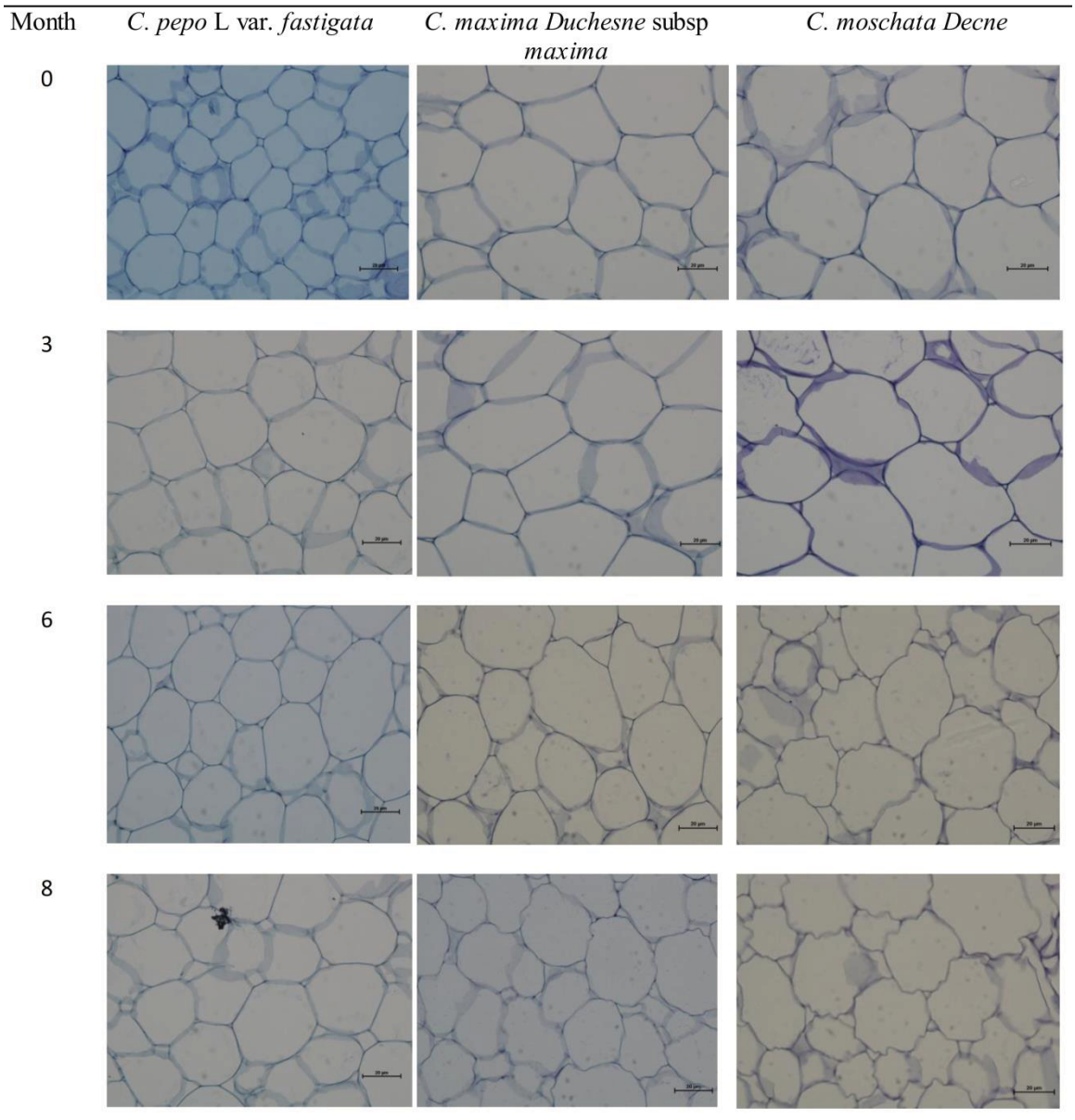

Figure 5. Change in appearance of cells and size of intercellular spaces of the flesh of three pumpkin varieties during the eight months of storage

3.7 The Major Determinants of Postharvest Stability as Determined by Factor Analysis

Factor analysis revealed that factors 1 and 2 accounted for 64.9% and 16.1% of the total variation, respectively. Reduction in sucrose, esterification of pectin, starch, hemicellulose and cellulose had strong negative association with Factor 1 (Figure 6A), with factor loadings of -0.97, -0.88, -0.87, -0.82 and -0.79 respectively. These were the major changes from 0 to 3months (Figure 6B). Moisture loss from the flesh and peel, change in size of intercellular spaces, breakdown of glucose and fructose positively associated with Factor 1 from 4 to 7months, with factor loadings of 0.95, 0.94, 0.93, 0.51, and 0.37 respectively. Yet polygalacturonase activity solely positively associated with Factor 1 (0.64) from 7 to 8months.

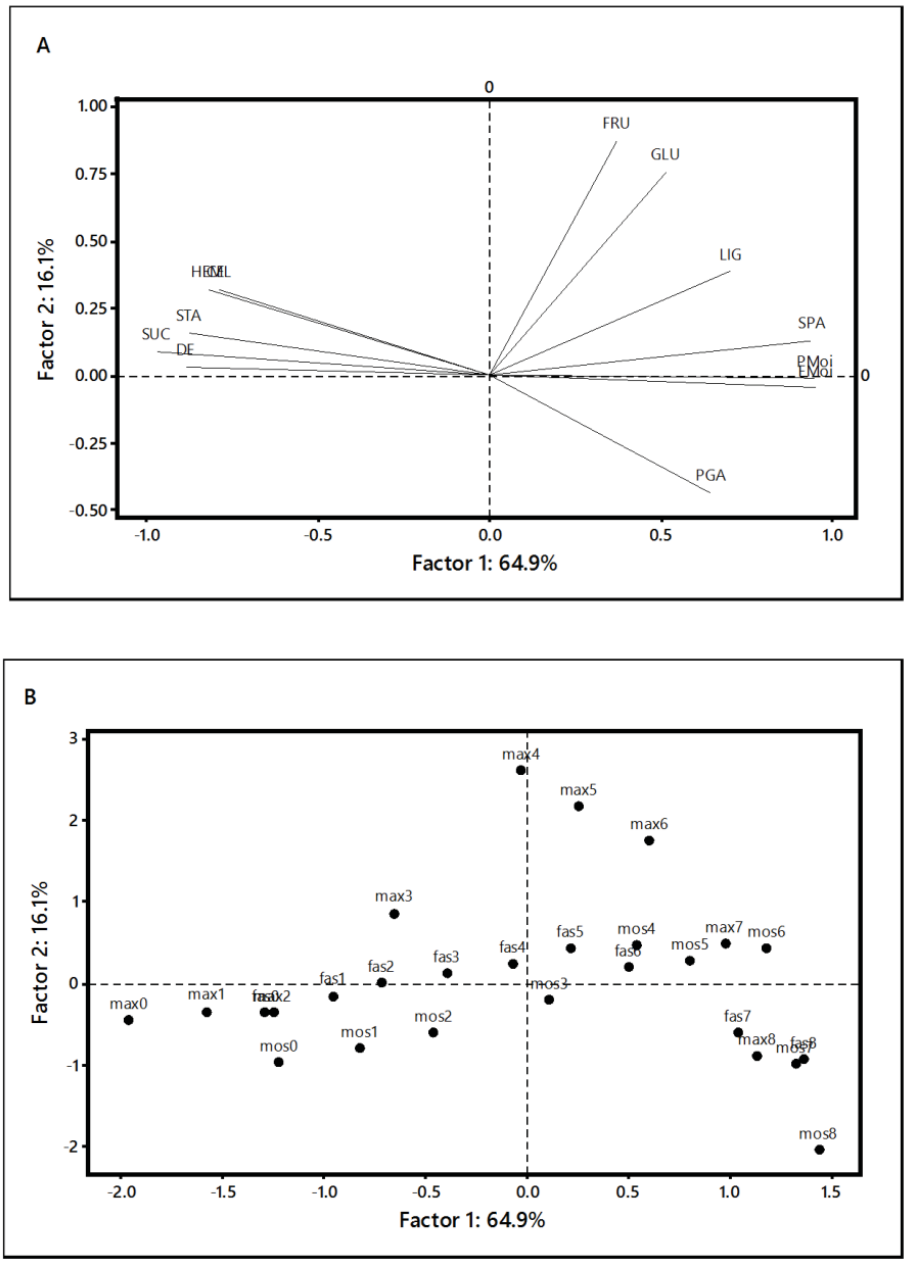

Figure 6. Loading plot (A) contribution of the different parameters to postharvest stability and score plot (B) position of the three varieties at different storage times in the quadrants of the multivariate space of the first 2 factors

FRU: fructose; GLU: glucose; LIG: lignin; SPA: size of intercellular spaces; PMoi: peel moisture; FMoi: flesh moisture; PGA: polygalacturonase activity; DE: pectin's degree of esterification; SUC: sucrose; STA: starch HEM: hemicellulose and CEL: cellulose. Max 0 to 8; Fas 0 to 8; and Mos 0 to 8 refer to *C. maxima Duchesne* subsp *maxima, C. pepo L. var fastigata* and *C. moschata Decne* at 0 to 8th month of storage respectively.

4. Discussion

4.1 Cellulose, Hemicellulose and Lignin Content of the Peel

Cellulose and hemicellulose results (Figure 1) agree with those of Jariene et al. (2015) who observed a significant decrease in crude fibre of stored pumpkins. Plant cell walls reportedly comprise of 90% polysaccharides namely cellulose, hemicellulose and pectin. Hemicellulose especially xyloglucan tethers cellulose microfibrils through hydrogen bonds thus reinforcing the cell wall. Hydrolysis of cellulose is slow in orderly, well bound cellulose microfibrils but hemicellulases reportedly alter ligation of hemicelluloses to cellulose in addition to cleaving the backbone of hemicelluloses (Goulao & Oliveira, 2008). This possibly explains why *C. moschata Decne* that had the least hemicellulose content also had the highest decrease in cellulose and hence deteriorated fastest. By the 4thmonth, *C. maxima Duchesne* subsp *maxima*, *C. pepo* L var. *fastigata* and *C. moschata Decne* had lost 16.6%, 25.2% and 41.5% of their cellulose, respectively. Besides, it is the degradation of cellulose microfibrils that leads to breakdown of cell walls and their eventual disintegration (NCBE, 2000) and cellulase enzyme activity was reported to increase towards ripening of pumpkin fruit (Sharma & Rao, 2013).

The observed increase in lignin could be attributed to continued lignification of cells postharvest (Cai et al., 2006) coupled with moisture loss from the peel that concentrates the matrix. An increment in liginification was also observed in postharvest storage of 'Luoyangqing' loquat fruits (Cai et al., 2006). Besides degradative changes in the plant cell wall are reported to occur in cellulose, hemicellulose and pectin not lignin (Goulao, Almeida, & Oliveira, 2010).

4.2 Starch, D-glucose and Sucrose Content of the Flesh

Starch and sucrose pattern observed is in agreement with Jariene et al., (2015) who reported a decrease in dry matter during storage of different pumpkin cultivars. Wills, Lim, -& Greenfield (1984) also observed a decrease in starch of Cavendish bananas during storage but in about 7days, nearly all the starch had been converted to sugars unlike in the studied pumpkins. This is explained by the fact that pumpkins are non-climacteric whereas bananas are climacteric fruits hence bananas characterized by an increase in respiration postharvest (Kader & Barret, 2004).

The pattern observed in glucose and fructose in this study is in agreement with that of Rahman et al., (2013), where total soluble solids of *C. moschata* Poir increased until 1.5months and then decreased. Initial increase in glucose and fructose despite their utilization in respiration was attributable to breakdown of starch and sucrose. Probably at that stage, the rate of generation of glucose from starch and sucrose as well as fructose from sucrose was greater than the rate of glucose and fructose breakdown. Respiration is an oxidative process and conventionally, glucose is the compound that is preferentially involved in the initial oxidative step. Starch is degraded first to glucose while sucrose is hydrolysed to glucose and fructose that are then oxidised to other respiratory substrates (Kader & Barret, 2004). All pumpkin varieties were principally starchy at harvest, with low levels of readily metabolizable sugars. The high starch and sucrose breakdown from 2 to 3months and from 2 to 4months, respectively was concomitant with the peak in glucose and fructose from 4 to 5months.

The rate of respiration indicates metabolic activity of the tissue (Stitt & Sonnewald, 1995) and thus a useful guide to storage life. Hence *C. moschata Decne* that had least starch and sucrose contents is likely to have a shorter postharvest life. Besides at 4months, *C. maxima Duchesne* subsp *maxima, C. pepo* L var. *fastigata and C. moschata Decne* had lost 48.3%, 81.1% and 89.7% of their starch and 49.6%, 30.7% and 55.5% of their sucrose respectively.

4.3 Degree of Esterification of Pectin

Esterification of pectin refers to the proportion of methyl groups present in the pectin (Bochek et al., 2001). The observed reduction in esterification of pectin during storage of pumpkins concurs with Gross & Wallner (1979) who observed a reduction in methylation of pectin from 90% in green tomato to 35% in ripe fruit. Gross & Wallner (1979) further reported the decrease in methylation of the pectin as one of the earliest detectable changes during ripening of fruits. The structural and compositional changes that occur to most fruit cell wall polymers during ripening are reported to have common features (Balandrán-Quintana et al., 2007) although progression is fastest in climacteric fruits than non-climacteric fruits (Kader & Barret, 2004). The higher decrease in esterification in *C. moschata Decne* implies that *C. moschata Decne* either has a higher quantity of pectin methyl esterase enzyme or its pectin is more sensitive to the enzyme than other varieties. Pectin methylesterase is responsible for splitting methoxy groups off the pectin, forming less esterified pectin (NCBE, 2000).

4.4 Polygalacturonase Activity

Polygalacturonase activity remaining fairly constant for the first 6months in all pumpkin varieties justifies a

non-climacteric nature. This is explained by the fact that the transcription of polygalacturonase enzyme gets activated only during ripening and the progression of ripening is reportedly slow in non-climacteric fruits (Nicholass, Smith, Schuch, Bird, & Grierson, 1995). Shooting-up in the 7thmonth is in agreement with Balandrán-Quintana et al., (2007) who reported polygalacturonase enzyme to be majorly responsible for pectin depolymerization during senescence of non-climacteric fruits like Zucchini (*Cucurbita pepo L*). Despite showing highest degree of polygalacturonase activity, *C. pepo L var. fastigata* had least change in size of intercellular spaces, probably due to the high esterification making its pectin less sensitive to polygalacturonase enzyme (NCBE, 2000). Besides endo and exo-polygalacturonases only break bonds between the non-esterified galacturonic acid molecules (NCBE, 2000) yet the ease with which plant cells separate from one another depends on how easy pectin of the middle lamella can be solubilized (Goulao et al., 2010). This postulation also agrees with the study in which Scifresh and Royal Gala apple varieties were studied for cell wall structures leading to cultivar differences in softening rates. The tricellular junctions of Scifresh apple, a slow softening variety were found to be rich in highly esterified pectin thus stronger cell adhesion and increased resistance to development of large air spaces (Ng et al., 2013).

Noteworthy, texture of fruits is derived from turgor pressure and from the middle lamella that holds cells together (Barrett, Beaulieu, & Shewfelt, 2010). Turgor pressure is attributed to the moisture and starch contents of the cells. Loss of turgor pressure (Goulao et al., 2010) and degradation of pectin in the middle lamella (Mollendorf, Villiers, Jacobs, & Westraad, 1993) are reported to result into a mealy texture. This implies that *C. moschata Decne* that exhibited the largest increase in size of air spaces and starch breakdown is likely to get mealy easily and also have a shorter postharvest life.

4.5 Moisture Loss

The highest moisture lost from the peel of *C. moschata Decne* was partly attributable to its low initial hemicellulose content and high hemicellulose and cellulose breakdown. Fruits cells majorly contain cellulose, hemicellulose and pectin. However some tissues also contain lignin (NCBE, 2000), for example the pumpkin peel but initial lignin did not vary among studied pumpkin varieties.

4.6 The Major Determinants of Postharvest Stability as Determined by Factor Analysis

Factor loadings show the relationship of each variable to the underlying latent factor, in this case the postharvest stability of pumpkins. Variables with strong association to postharvest stability of pumpkins are in the order of sucrose breakdown (-0.97), moisture loss from flesh (0.95) and peel (0.94), change in intercellular spaces (0.93), de-esterification of pectin (-0.88), breakdown of starch (-0.87), hemicellulose (-0.82) and cellulose (-0.79).

The position of the three varieties at different storage times in the quadrants of the multivariate space revealed *C. maxima Duchesne* subsp *maxima* of 0 to 3months, *C. pepo* L var. *fastigata* of 0 to 4months and *C. moschata Decne* of 0 to 2months to be strongly linked to reduction in sucrose, starch, cellulose, Hemicellulose, and esterification of pectin. This implies that these changes took place early in *C. moschata Decne* (2months) compared to other varieties hence *C. moschata Decne* likely to have a shorter postharvest life. In addition a strong association exited between *C. maxima Duchesne* subsp *maxima* of 5 to 7months, *C. pepo* L var. *fastigata* of 5 to 6months and *C. moschata Decne* of 3 to 6months to moisture loss from the peel and change in size of intercellular spaces. This again shows that these changes took place early in *C. moschata Decne* compared to other varieties.

5. Conclusion

C. moschata Decne deteriorates fastest of all studied varieties. The postharvest stability of pumpkins is determined by an interplay of factors. The major cause of variation in stability of different pumpkin species were difference in rates of reduction in sucrose, starch, cellulose, hemicellulose, and esterification of pectin. These changes took place earliest in *C. moschata Decne* (2months) compared to other varieties. In addition *C. moschata Decne* of 3 to 6months was strongly associated with moisture loss from the peel. Generally pumpkin varieties with high hemicellulose content, which is meant to tether cellulose microfibrils will have slower rate of cellulose reduction hence delayed breakdown of the cell walls. This will in turn reduce the rate of moisture loss from the peel, and the rate of breakdown of sucrose and starch.

Conflict of interest

None

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