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Physicochemical and Nutritional Characteristics of Solar and Sun-dried Tomato Powder

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Abstract

Tomato (*Solanum lycopersicum*) is one of the most important vegetable plants in the world used in cooking foods. In Ghana, at peak seasons of harvesting tomato, high postharvest loses are incurred because of the absence of industrial tomato processing facilities for value addition into other convenient forms to extend shelf-life. Solar drying is a more efficient and low cost method of enhancing the quality and adding value to tomato. This study was carried out to design a natural mixed mode solar dryer suitable for drying tomato and to investigate the quality characteristics of the dried products. Fresh tomatoes were pre-treated with: a) 1% potassium metabisulfite solution; b) 1 % ascorbic acid solution; and c) used untreated as control. Pre-treated tomatoes were dried for three days on the average till there was no significant change in the final moisture content. Open-sun drying was used as a control for comparison purposes. The final moisture content for pre-treated solar dried and sun-dried tomato was 14 - 15 % and 19 - 22 %, respectively. The ash content was higher in the sun-dried samples compared to the solar dried samples, an indication of contamination with extraneous materials from the environment. Sulfur dioxide content of 740.8 mg/Kg d.w., for solar dried tomato pre-treated with potassium metabisulfite was much lower than the maximum legal limit of 2000 mg/Kg d.w., of sulfur dioxide recommended in fruits. Solar and sun-dried tomato pre-treated with potassium metabisulfite had significantly higher carotenoids, lycopene and beta carotene contents than pre-treated samples, and the controls. The quality of pre-treated solar-dried tomato was better enhanced by the use of the solar dryer compared to sun-drying.

Keywords: lycopene, post-harvest loses, pre-treatment, quality, solar drying tomato

1. Introduction

Ghana consumes about 25,000 tonnes of tomatoes per annum worth $25 million and is currently ranked the world's second highest country in canned tomato importation (Aryeetey, 2006). These massive imports have negatively affected the livelihoods of tomato farmers. Improving domestic tomato processing would help to minimize the over dependence on canned tomato imports, offer employment opportunities to the youth (Owureku-Asare *et al.,* 2013) and reduce the high post-harvest losses during the peak season as well as strengthen the tomato value chain. Processing technologies, such as drying, have the potential to help farmers preserve agricultural produce and distribute them to markets, even in other countries (Jon & King, 2008). Drying is one of the oldest methods of food preservation. It is a relatively inexpensive technology and it preserves foods by removing enough moisture to prevent decay and spoilage by microorganisms. Sun drying is an effective method of preserving and maintaining the quality of fresh produce. It lowers the water activity of the produce and consequently reduces microbial activity. Sun drying however, is associated with safety and quality problems such as contamination with dust and other extraneous materials, color degradation and poor rehydration characteristics. It is also associated with high microbial loads in the products due to slow drying rates of

agricultural materials (Doymaz, 2004, 2005b). It has been established that the use of solar and hot-air dryers can significantly improve the safety and quality issues associated with sun-dried food products (Adom *et al.,* 1997; Gogus & Maskan, 1999; Doymaz & Pala, 2002).

During the drying process, heated air initiates continuous physical and biochemical changes. The physical changes may result in changes in size, shape, color and texture whereas biochemical reactions cause oxidation of carotenoid and chlorophyll pigments, changes in the flavor, color and degradation of heat labile and UV sensitive nutrients in the produce (Lenart, 1996; Lin et al., 1998). Handling and preparation procedures prior to processing also cause substantial variation in nutrient loss (Gallali et al., 2000) and other physical properties of the dehydrated product. Some fruits and vegetables are pre-treated with different agents including calcium chloride, sulfur dioxide, ascorbic acid, vinegar, etc before drying to preserve certain vitamins, maintain color, reduce microbial contamination and prevent storage changes (Prabhakar, 2014).

Gaseous sulfur dioxide, sodium or potassium bisulfite and metabisulfite are commonly used for pre-treating some fruits and vegetables during processing. This process known as sulfiting, protects the product against non-enzymatic browning during drying and storage (Ngoddy & Ihekoronye, 1985). lycopene content of dried tomato was enhanced when tomato slices were pre-treatment with sodium metabisulfite solution before convection drying (Owureku-Asare *et al.,* 2014). Potassium metabisulfite was used pre-treatment of tomato slices prior to drying significantly affected rehydration ratio, yeast count and color because sulfite plasmolysed the cells which facilitated the drying process (Gould & Russel, 1991). However, due to health concerns over the use of sodium, potassium metabisulfite is a better substitute for pre-treating tomato.

Central to the drying operations is the need to maintain the quality and functionality of dehydrated food materials (Prabhakar, 2014). Assessment of dried tomato involves evaluation of sensory, microbial load, physical characteristics, nutritional parameters, as well as microstructural and rehydration characteristics (Ranganna, 1986; Gallali, Abujnah & Bannani, 1999). Consequently, the quality of dried products is often used to assess the performance and efficiency of the dryer system (Chen et al*.,* 2005). During the drying process, agricultural fresh produce undergoes continuous physical and biochemical changes. Typical physical changes of dried food product may result in changes in size, shape, color and texture whereas the biochemical reactions cause changes in the flavor, color and nutrients of perishable produce like tomato (Lin et al., 1998; Lenart, 1996).

The heated air used in the drying process initiates changes in the nutritional and chemical properties of tomato by causing oxidation of carotenoid and chlorophyll pigments. Stability of phytochemicals (such as lycopene and carotene) should be investigated because there is increasing awareness and interest in the health benefits it's stability during food processing should be investigated (Tonucci et al., 1995).

The aim of this study was to assess the physicochemical, nutritional quality characteristics of pre-treated solar dried tomato powder, processed using a mixed mode solar dryer.

2. Materials and Methods

2.1 Sample Preparation

Fresh tomato (Roma variety) was purchased from a farmer and transported in wooden crates at ambient temperature to the laboratory, where it was stored in an air-condition room at 16 ^{o}C. Ripe but firm tomato was selected, washed under running tap water and then with 1% sodium metabisulfite solution. The tomato was cut into slices of 5 mm, using tomato slicer (Jaccard stainless steel mandolin, USA). This size was selected based on results from preliminary studies (unpublished data). The initial moisture content of tomato was determined using the air-oven method (AOAC, 2000).

2.3 Pre-treatments Prior to Dehydration Process

The tomato slices were divided into three equal portions of 4 kg each and randomly assigned to three pre-process treatments as follows: dipping in (a) a solution of 1 % Potassium metabisulfite (KMS) for 10 min, (b) 1 % ascorbic acid solution for 10 min and (c) untreated as control. The choice of these selected pre-treatment was based on preliminary studies carried out prior to this study.

2.4 Dehydration Processes

Drying experiments were performed in a mixed-mode natural convection solar dryer (MNCSD) (Figure 1a & b) designed for tomatoes. The MNCSD was constructed by the Engineering Unit, Food Technology Research Division of the CSIR- Food Research Institute, Accra. The dimensions of the dryer chamber were length (1.0 m), width (0.6 m) and height (1 m) with a collector tilt angle of 15.6^{o} placed in a South North position for optimum solar radiation in Accra, Ghana which is located at 49 m above sea above sea level at 5.6301N 0.1801W

(accuracy: 3 m radius, device info: Garmin eTrex 30). Pre-treated tomato slices (4 kg) were uniformly spread on rectangular mesh trays (87 cm x 53 cm) and placed in the drying chamber of the solar dryer over three-day period till moisture content of 13-14 %. In order to compare the performance of the cabinet dryer with that of open sun drying, 4 kg of sliced tomatoes were placed on drying trays and sun-dried (Figure 2). Triplicate sun drying experiments were carried out alongside the solar dryer.

After drying, both sundried and solar dried tomato samples were milled separately into powder using Kenwood dry mill blender (BL335, United Kingdom) at 450W speed for 3 min.

a. **b.**

Figure 1a & 1b. Solar cabinet dryer

Figure 2. Sliced tomatoes on drying racks in open sun drying.

2.4 Experimental Design

A 3 x 2 factorial for pretreatment (1 % ascorbic acid solution, 1% metabisulfite solution and no pretreatment) and drying methods (solar and sun-drying) was used in the study. Tomato slices were subjected to three different pre-treatments (dipping in 1 % potassium metabisulfite for 10 min, dipping in 1 % ascorbic acid for 10 min, or using it as control without any pre-treatment). Each of the three samples was then dried under two drying conditions; solar and sun drying over a period of three days. Moisture content of samples was assessed at 2 h interval throughout the drying period. Experiments were carried out in triplicates between 9:30 and 16:30 h on sunny days (using British Broadcasting Corporation (BBC) weather forecast) during December 2015 to March 2016.

2.5 Physicochemical Quality Analysis of Fresh and Dried Tomato

Fresh tomato was blended in a waring blender to obtain tomato juice. Ten percent (10 %) tomato solution was also prepared from tomato powder by mixing 10 g of tomato powder with 100 ml of deionized water. Tomato solution and tomato juice (from fresh tomato) were used for the following analysis.

2.5.1 Moisture and Total Solids

The average initial moisture content for both the fresh and dried tomato samples were determined by drying 3 g of samples in a convection oven at 105 ^{o}C for 10 h (AOAC, 2000). Total solids were estimated by subtracting moisture content from 100 %. Moisture content and total solids of fresh and dried tomato samples were determined in triplicates.

2.5.2 Water activity (a_w)

Water activity was determined using a water activity meter (Paw kit, Model Series 3 TE, Decagon Devices, Inc., Pullman, WA, USA) for fresh and dry tomato samples, respectively.

2.5.3 Color

Tristimulus color of the fresh tomato and dried tomato (tomato powder) samples were measured using the chroma meter (LABSCAN XE Hunterlab, VA, USA) and reported in CIELAB color scales. The L* value being the degree of lightness to darkness, a* value the degree of redness to greenness, and b* value, is degree of yellowness to blueness. The chromameter was calibrated against a white tile (L* = 100). The total color difference ΔE was calculated as:

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

2.5.4 Total Soluble Solids (TSS)

Total Soluble Solids of tomato juice was measured in triplicate using a digital Refractometer (AR 200, Reichert Analytical instrument, NY, USA). TSS of tomato powder was determined by reconstituting tomato powder into 10 % solution before placing a drop of the juice on the lens of the refractometer after which subsequent reading were taken.

2.5.5 pH

The pH of the tomato juice was determined using pH meter (Symphony SB70P VWR, Radnor, PA, USA). The pH of dried tomato was measured from 10 % tomato solution made from tomato powder and determined with a pH meter. The pH readings were taken in triplicates for both fresh and dried tomato.

2.5.6 Total Titratable Acidity

Titratable acidity of fresh tomato and 10 % was calculated as percentage citric acid according to method by Elfalleh 2011. Ten grams of tomato powder was mixed in 100ml of distilled water and filtered. Ten milliliters of the filtrate was titrated with a solution of NaOH (0.1N) till pH end point of pH 8.1. The TTA was expressed as citric acid equivalent.

$$\%\ \text{TTA}\ \frac{Titre\ value\ x\ Normality\ x\ M.eq.wt\ of\ acid x 100}{volume\ of\ sample} x\ 100 \quad (2)$$

Milli-equivalent weight of citric acid=0.06404

2.5.7 Ash

The AACC Method 08-01(American Association of Cereal Chemists, 2000) was used to determine the ash content of tomato powder. Approximately, 4 grams of sample was weighed accurately into a silica ashing crucible which had previously been ignited, cooled in a desiccator and weighed. The samples were incinerated in a muffle furnace until a light grey ash was obtained, cooled in a desiccator and weighed. Ash was calculated as follows:

$$\%\ \text{Ash}=\frac{weight\ of\ crucible+ash)-weight\ of\ empty\ crucible\ x\ 100}{weight\ of\ sample} \quad (3)$$

2.5.8 Sulfur Dioxide

Total sulfur dioxide content (ppm dry weight basis) was determined using the modified Reith Williams Method (FAO, 1986). Sulfur dioxide was determined by dispersing 25 g of tomato powder was dispersed in 20 mL of

water and diluted with 25 mL of dilute sodium hydroxide. It was allowed to stand for 5 min and diluted with 10 ml sulfuric and allowed to stand for another 5 min, and 1 ml of starch indicator added. It was titrated with standard iodine solution to a permanent purple color.

2.6 Phytochemical Analysis

2.6.1 Extraction of Carotenoids

Using 0.5 g of the hydrated powered tomatoes, the samples were crushed in a crucible mortar with 1.0 g sodium bicarbonate and celite to neutralize organic acid release. Twenty (20) mL of extraction solution (acetone: petroleum ether (0.1 % BHT) 1:1) was added to each sample through a vacuum filter into a flask. The filtrate was quantitatively collected and the process repeated three times until the residue was devoid of color. Forty (40) ml of 40 % potassium hydroxide in methanol (w/w) was added and placed on magnetic stirrer for 10 minutes at room temperature. Combined extract is transferred into a 250 mL separating funnel and washed with distilled water. Ten (10) ml of saturated NaCl was added to break emulsions. Aqueous layer was drained, and the petroleum ether layer was quantitatively collected into a 25 mL volumetric flask with a funnel plug made of glass cotton wool. Aliquot of extract was dried under nitrogen atmosphere and dissolved in 1 mL of methanol: Ethyl Acetate (1:1) for HPLC analysis.

2.6.2 HPLC Conditions

A water liquid chromatographic instrument consisting of a Model 626 Pump, 717 plus auto sampler and column heater) and a Model 2996 photodiode array detector was used. Operation and data processing were performed by Empower software. Separation of carotenoids was performed using a YMC C_{30}, 250 x 4.6 mm, 5µm pore size with gradient elution of (A) Ethyl Acetate, (B) Methyl tertiary butyl ether and (C) methanol, in which elution started with 20 % A and 80 % C, which changed to 40 % A and 60 % B in 2 min then to 100 % A in 12 min and then 20 % A and 80 % C in 13 min and stayed isocratic for 7 min. The flow rate was 1.0 ml/min for 20 min. PAD spectrum of carotenoids was displayed between 200 and 700 nm. Peak identification was based on comparison of retention time and spectral characteristics of carotenoids standards.

2.6.3 Microstructural Evaluation

Dried tomato slices were fixed in 2.5 % glutaraldehyde for one hour and rinsed in buffer for 5 min. It was immersed in 2 % aqueous osmium tetroxide for 90 min. Osmium was rinsed out with deionized water for 5 min. This was repeated three times. Samples were then dehydrated in ethanol (ETOH) series of 50 %, 70 % and 95 % for 10 min each repeated three times. Specimen samples were placed in metal baskets, coded and processed in a critical point dryer and then mounted on carbon tape for sputter coating with platinum for 120 sec. The samples were imaged with an FEI Quanta 3D FEG scanning electron microscope (FEI Company, Hillsboro, OR) using the Everhart-Thornley (ET) detector at high vacuum. Parameters for imaging, 5kV, spot 5, 50µm aperture and working distance of ~10 mm.

2.6.4 Particle Size Analysis of Tomato Powder

Tomato particle size and shape distribution was determined using a Morphologi G3-ID (Malvern Instrument, Malvern, United Kingdom). A total of 10,000 particles were analyzed to obtain the number distribution of circle equivalent (CE) diameter, convexity, and high sensitivity (HS) circularity. CE-diameter is the measurement of particle size, expressed as the diameter of a circle with the same area as the particle image. Convexity is the measurement of the surface roughness of a particle and it is calculated by dividing the convex hull perimeter by the actual particle perimeter. The value of convexity ranges from 0 to 1. HS-circularity quantified how close the shape is to a perfect circle by the ratio of the particle area to the square of the perimeter of the object.

2.7 Data Analysis

All data were analyzed using Minitab version 7. Means and standard deviations of the data were presented. ANOVA and Duncan's multiple range test carried out to determine differences among treatments at the significant level of $p \leq .05$.

3. Results and Discussion

3.1 Physicochemical Assessment of Tomato Powder

The moisture content of fresh tomato was high at 94.73 % ± 0.43 (Table 1) making tomato highly susceptible for spoilage. According to USDA database, lycopene content of fresh tomato ranges from 0.88–4.2 mg/100 g fresh weight, and b-carotene content lies slightly (0.1–0.7 mg/100g fresh weight) (http://www.nal.usda.gov/fnic/foodcom). The lycopene content of fresh roma tomato used in this study fell within this range (Table 1).

Table 1. Physicochemical quality of fresh Roma tomato

Analysis	Fresh tomato
Moisture (%)	94.73 ±0.43
Dry matter (%)	5.27± 0.43
Ash dwb (g/100g)	8.50±0.78
Acidity (g/100g)	0.43±0.02
pH	4.13±0.01
L*	44.65±0.82
a*	22.71±1.34
b*	14.50±2.38
Lycopene	1.49mg/100g
Beta carotene	0.55mg/g
°Bx	4±0.0
a_w	0.99±0.01

3.2 Effect of Pre-treatment and Drying Method on Physicochemical Parameters of Dried Tomato

The moisture content of solar dried tomato powder ranged from 13.94 ± 0.75 % to 14.57 ± 0.21 % was observed whereas 19.38 ± 0.36 % to 21.63 ± 2.36 % for sundried tomato. The moisture content of solar dried tomato was significantly ($p \leq 0.05$) lower than sundried tomato (Table 2). The relative humidity of ambient air was consistently higher than that of the solar dryer during drying and as such moisture was readily absorbed by tomato at a faster rate from the surrounding ambient air than that of a more controlled environment created within the solar dryer. Dried tomato contains sugars and cellulose which has hygroscopic properties and high affinity to moisture, enables it to absorbs and hold unto moisture from its surrounding environment.

Table 2. Physicochemical Quality characteristics of solar and sundried pre-treated tomato powder

Drying Method	Pre treatment	Moisture (%)	Dry matter (%)	a_w	TTA (g/100g)	pH	°Bx	Ash (g/100g) dw	L*	a*	b*	ΔE
Solar	**Control**	13.99^{xA}	86.01^{yB}	0.37^{xA}	1.70^{xA}	4.16^{xA}	7.0^{yB}	9.68^{xA}	48.63^{xyB}	20.41^{yB}	30.26^{xB}	-
		(0.40)	(0.40)	(0.01)	(0.56)	(0.6)		(0.99)	(2.82)	(2.16)	(1.37)	
	KMS	13.94^{xA}	86.06^{yB}	0.38^{xA}	2.87^{yB}	4.09^{xA}	6.0^{xA}	9.40^{xA}	50.35^{yB}	20.26^{yB}	31.76^{xB}	3.52^{xB}
		(0.75)	(0.75)	(0.05)	(0.03)	(0.08)	(0.0)	(0.91)	(2.9)	(1.39)	(1.88)	(0.32)
	AA	14.57^{yA}	85.43^{xB}	0.35^{xA}	1.71^{xA}	4.07^{xA}	7.0^{yB}	9.55^{xA}	46.44^{xB}	20.38^{xAB}	28.87^{xB}	3.00^{yB}
		(0.21)	(0.21)	(0.01)	(0.56)	(0.02)	(0.0)	(0.9)	(1.2)	(1.53)	(2.33)	(2.82)
Sun	**Control**	19.38^{xB}	$80.63y^{A}$	0.53^{xB}	1.75^{xA}	4.16^{xA}	6.0^{xA}	9.30^{xA}	37.81^{xA}	20.91^{yB}	23.37^{xA}	-
		(0.36)	(0.36)	(0.02)	(0.76)	(0.01)	(0.0)	(0.15)	(0.89)	(0.72)	(0.96)	
	KMS	21.36^{xyB}	78.64^{xyA}	0.56^{xB}	3.10^{yB}	4.08^{xA}	6.0^{xA}	10.03^{yB}	40.31^{yA}	17.13^{xA}	22.76^{xA}	2.93^{xA}
		(2.07)	(2.07)	(0.04)	(0.02)	(0.09)	(0.0)	(2.02)	(1.67)	(2.00)	(1.65)	(0.14)
	AA	21.63^{yB}	78.3^{xA}	0.57^{xB}	1.79^{xA}	4.08^{xA}	6.0^{xA}	10.18^{yAB}	41.48^{yA}	18.06^{xAB}	23.25^{xA}	2.55^{xA}
		(2.26)	(2.26)	(0.03)	(0.07)	(0.07)	(0.0)	(0.44)	(2.56)	(3.12)	(1.52)	(0.27)

Values are means of triplicate readings with a standard deviation in brackets.
Mean values in a column for pre-treatments with the same superscript (x, y, z) are not significantly different ($p<0.05$) from each other.
Mean values in a column for drying method with the same superscript (A, B,) are not significantly different ($p<0.05$) from each other.
Dw-On a dry weight basis.
TTA-Total titratable acidity

The moisture content of sundried samples fluctuated in response to the relative humidity of the ambient air. It was challenging reaching desirable low moisture content (10 - 12 %) for sun-dried samples compared to solar dryer which had elevated temperatures and lower final moisture content (13.95 ± 0.75 %). Sun drying requires 7 to 12 days, and results in a product with typically 12 % to 24 % moisture and robust taste. (ECOM, 1997).

Water activity (a_w) is a measure of how much of the water in a product is free and not chemically or physically bound, but which is available for food enzyme activity and microbial growth (Prabhakar et al., 2014). Water activity (aw) for solar dried tomato powder were significantly lower (0.35 ± 0.01 to 0.38± 0.05) than sundried tomato powder (0.53 ± 0.002 to 0.57 ± 0.03) (Table 2). In a similar study by Rajkumar et al., (2007), significant differences were observed for a_w of tomato slices dried in vacuum assisted solar dryers and open sun drying with corresponding moisture content of 11.5 ± 0.01% (w.b). Pre-treatment of solar and sundried tomato powder did not have significant ($p > 0.05$) effect on water activity (Table 2). In general, foods have a_w levels in the range of 0.2 for very dry foods to 0.99 for moist fresh foods. Microorganisms can keep their viability regardless of the water activity, but growth bacteria require $a_w > 0.8$ whiles yeasts and molds grow in $a_w > 0.6$ (ECOM, 1997).

Since dry fruits have a_w of 0.4; thus the a_w of solar dried tomato will enhance a stable storage shelf- life for the product. Water activity lower than 0.6 achieved was considered as microbiologically safe for storage (Wang & Brennen, 1991).

Brix (°Bx) of reconstituted solar dried tomato were significantly ($p \leq .05$) lower (6.0) for all sundried samples (7.0), with the exception of KMS pre-treated solar dried tomato powder which was also 7.0 (Table 2). Generally, sun-dried tomato had lower brix than solar dried tomato. This could be due to the breakdown/fermentation of sugars observed from growth of yeast cells and characterized by off smell observed in sundried samples.

The pH ranged from 4.08 ± 0.07 to 4.16± 0.01 for sun dried and solar-dried tomato. There were no significant ($p \leq 0.05$) differences in the pH for solar and sundried tomato powder. Total titratable acidity (citric acid) was significantly higher for solar (2.87 ± 0.03) and sun-dried (3.10 ± 0.02) tomato pre-treated with KMS than sample with other pre-treatments. KMS solution; an acidic solution releases sulfur dioxide gas during drying. The higher acidity recorded for sundried samples could be an indication of fermentation which occurred during sun drying.

The ash content was slightly higher in the sundried samples (9.30 - 10.18 %) compared to the solar dried tomato samples (9.4 - 9.68 %) (Table 2). Higher ash content is indicative of contamination by dust and other extraneous materials. In a study by Gallali et al., (2000) on dried grapes; significant differences in the ash content were observed for solar dried (2.95 %) and sun dried grapes (12.1 %) indicating higher fermentation associated with sun drying.

The L* value for sundried tomato were significantly affected by the different pre-treatments. L*color dried tomato pre-treated with ascorbic acid and KMS was higher than the control samples without any pre-treatment. The use of KMS and ascorbic acid which are both acidic in nature had a bleaching effect on the red color of tomato prior to drying. Significantly ($p < 0.05$) lower L* (37.81 ± 0.89 - 40.31 ± 1.67) observed in sundried dried tomato indicated that these samples were darker than solar dried samples higher L* values (50.35 ± 2.9 - 46.44 ± 1.2). Significant changes in L* value for pre-treated solar dried tomato. In a similar study by Falade & Shogaolu, (2010), higher L* value was observed for sulfited pumpkin due to the bleaching effect of the sulfite treatment prior to air oven drying.

The presence of pigments susceptible to degradation by non-enzymatic and enzymatic reactions affects the color of fruits, and vegetables. A low a_w in dry products leads to an increase in the half-life of the pigments (Pizzocaro *et al.,* 1993). It was also observed that the color change in sun drying was mainly due to the non-enzymatic browning /Maillard reaction and it was in the solar dried samples compared to sun drying (Pizzocaro et al.,1993).

The color change of solar dried tomato pre-treated with KMS (3.52 ± 0.32) and ascorbic acid (3.00 ± 2.82) significantly differed from the control and was higher than pre-treated sundried tomato (2.93 ± 0.14 - 2.55 ± 0.27). There was higher degradation of the red tomato color characterized by darker red color (brownish) in sundried tomato than solar dried. In a similar study by Latapi & Barrett (2006), significant ($p \leq 0.05$) differences were observed in the color of sundried tomato pre-treated with different concentrations of sodium metabisulfite. In that study color values (Hue°) decreased with increasing concentration of sodium metabisulfite and a more desirable redder color was observed with increasing sodium metabisulfite concentration (6 - 8 %). Also Sun-dried tomatoes that were dipped in an 8 % sodium metabisulfite solution had the highest sulfur dioxide content, and thus the best red color (32.2 Hue°) compared with control sun-dried tomatoes with the highest hue angles before (36.9 Hue°) and after storage (42.0 Hue) with darker brown color. During drying, pre-treatment with sulfur dioxide preserves color and reduces color degradation.

3.3 Nutritional Assessment of Tomato Powder

3.3.1 Effect of Pre-treatment and Drying Method on Beta Carotene, Lycopene and Total Carotenoid Quality of Tomato Powder

Table 4 shows the antioxidant composition of tomato powder. Tomato contains different kinds of micronutrients such as carotenoids, folate, vitamins (C and E), and phenolic compounds (Beecher, 1998; Periago & Garcia-Alonso, 2009). Tomato is a rich source of lycopene, an important carotenoid which has enormous health benefits. Fresh tomato fruit contains about 7.2 to 200 mg of lycopene per kg of fresh weight, which accounts for about 30 % of the total carotenoids in plasma (Stahl & Sies, 1996). In this study, fresh tomato contained 14.9 mg/of lycopene per kg of fresh weight and the exocarp of fresh tomato contains about five times more lycopene than the pulp (Papaioannou & Karabelas, 2012).

Solar-dried tomato pre-treated with KMS had significantly ($p \leq 0.05$) high carotenoids (43.13 ± 1.43 mg/100g), lycopene (50.35 ± 2 .01 mg/100g) and beta carotene (29.16 ± 0.78) compared with the other solar dried samples. Sun-dried tomato pre-treated with KMS also recorded significantly high β-carotene (10.46 ± 1.78 mg/100g),

lycopene (23.01 ± 2.04 mg/100g) and total carotenoid concentrations (33.21 ± 0.76 mg/100g) compared with ascorbic acid pre-treated and control sun-dried samples (Table 4). Drying method significantly ($p \leq 0.05$) affected the lycopene, β -carotene and total carotenoid levels in dried tomato samples. There was significant ($p \leq 0.05$) interaction of pre-treatment and drying method on the lycopene, β-carotene concentration which led to high values recorded for KMS pre-treated tomato. USDA database records lycopene of 45.90 mg/100g and β-carotene of 0.524 mg/100g on dry weight basis for sundried tomato (<http://www.nal.usda.gov/fnic/foodcomp>).

Processing such as drying can affect isomerization, bio-accessibility and concentration of lycopene (Knockaert et al., 2012). In a study carried out by Georgé et al., (2010), the thermal processing of tomato juice to puree, (at 92^{o} C for 10min) significantly ($p \leq 0.05$) reduced total polyphenolic and vitamin C content in both yellow and red varieties, but did not significantly ($p \leq .05$) lower the carotenoid content of red tomato. Freeze drying of tomato juice also reduced the carotenoid content but did not affect total polyphenol content of tomato. In a related study by Owureku-Asare *et al.,* (2014), the lycopene content of conventional oven dried tomato pre-treated with sodium metabisulfite was 93.0 ± 168 mg/100g showing a lowering in degradation compared to samples pre-treated with ascorbic acid. This could be as a result of the protective effect of the metabisulfite. Sun drying, causes considerable carotenoid destruction whiles drying in a solar dryer reduces the exposure to direct sunlight. The destruction of carotenoids in tomato pre-treatment with antioxidant and sulphating agents reduces carotenoid degradation and inhibit the enzyme polyphenol oxidase, demobilizing it (Pizzocaro et al., 1999). In a study by Davoodi et al., 2007, the retarding effect of $CaCl_2$ for browning and protective effect of KMS on lycopene was observed in tomato powders. All the carotenoids assessed were highest in KMS treated sun and solar dried tomato samples.

Table 4. Beta carotene, Lycopene and total carotenoid content of tomato powder

Drying Method	**Pre-treatment**	**β -carotene mg/100g (dw)**	**Lycopene / mg/100g (dw)**	**Total carotenoid /mg/100g (dw)**
Solar	Control	28.34^{zB} (0.23)	15.32^{yB} (1.53)	31.80^{zB} (2.01)
	KMS	29.16^{zB} (0.78)	50.35^{zB} (2.01)	43.13^{zB} (1.43)
	AA	8.753^{xyA} (0.94)	2.69^{xA} (1.82)	9.02^{xA} (0.92)
Sun	Control	9.86^{yA} (1.09)	13.86^{yA} (0.24)	28.54^{yzA} (1.72)
	KMS	10.46^{yA} (1.78)	23.01^{yA} (2.04)	33.21^{zB} (0.76)
	AA	6.39^{xA} (1.39)	11.25^{xA} (0.34)	15.21^{xA} (0.78)

Values are means of triplicate readings with a standard deviation in brackets. Mean values in a column for pre-treatments with the same superscript (x, y, z) are not significantly different ($p \leq .05$) from each other. Mean values in a column for drying method with the same superscript (A, B) are not significantly different ($p \leq .05$) from each other. dw-dry weight.

3.3.2 Particle Size and Shape Distribution of Tomato Powder

3.3.2.1 Size Diameter of Tomato Powders

The physical properties including moisture content and milling methods affect particle size distribution of flours and powders (Gaines, 1985; Dexter *et al.,* 1994). Sun-dried and solar dried tomato samples pre-treated with ascorbic acid and sundried control recorded higher percentage of particles with circle equivalent diameter (CE) of 2 -10 μm (Figure 1). The KMS pre-treated dried tomato powder had highest proportions of small particles between 2-5 μm, there were also high volumes of particles ranging from 2 - 31 μm making the particle larger than the rest of the powders. Sun dried tomato powder pre-treated with potassium metabisulfite and solar dried controlled samples also had relatively higher particle size between 2 - 14 μm.

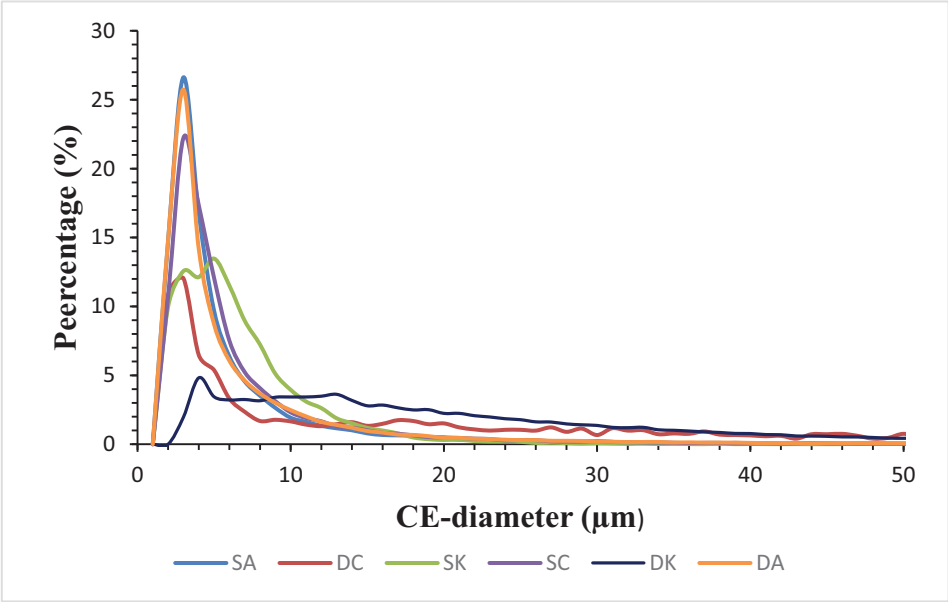


Figure 1. Distribution of tomato powder particle circle-equivalent (CE) diameter

SA- sundried tomato powder pre-treated with ascorbic acid; SK- sundried tomato powder pre-treated with potassium metabisulfite; SC-sundried tomato powder with no pre-treatment; DA- solar dried tomato powder pre-treated with Ascorbic acid; DK- solar dried tomato powder pre-treated with potassium metabisulfite; DC-solar dried tomato powder with no pre-treatment

In a study by Sign et al., 2005, the particles of blanched tomato powder were finer compared with un-blanched tomato powder. Blanched tomato hard a narrow range of particle sizes depicting more stable particles which is desirable in processing into other tomato products. Solar dried tomato powder pre-treated wit KMS (DK) had CE-diameter which was widely distributed compared to all other samples. SC, DA, SA, SK and DC had higher proportions (peak) of particles with size of up to 3 µ while DK was 4 µ. Tomato powder mainly composed of sugars, mineral composition, organic acids, lycopene and total phenols (Verma et al., 2016). Compared to flours/powders from starchy foods, the dried skin/exocarp of the tomato gave the powder a chaffy and coarse texture with, the mesocarp of tomato producing a finer matrix to the powder. The narrow peaks for almost all the samples shows a larger proportion of smaller particles (5 – 10µm), an indication that the milling intensity increased damage of the sugars, resulting in sugars of smaller particle sizes (Verma et al., 2016). In a study of sorghum flour, the particles had diameters that ranged between 5 and 30 µm (Choi et al., 2008), which suggests starch damage enhanced by the grinding process.

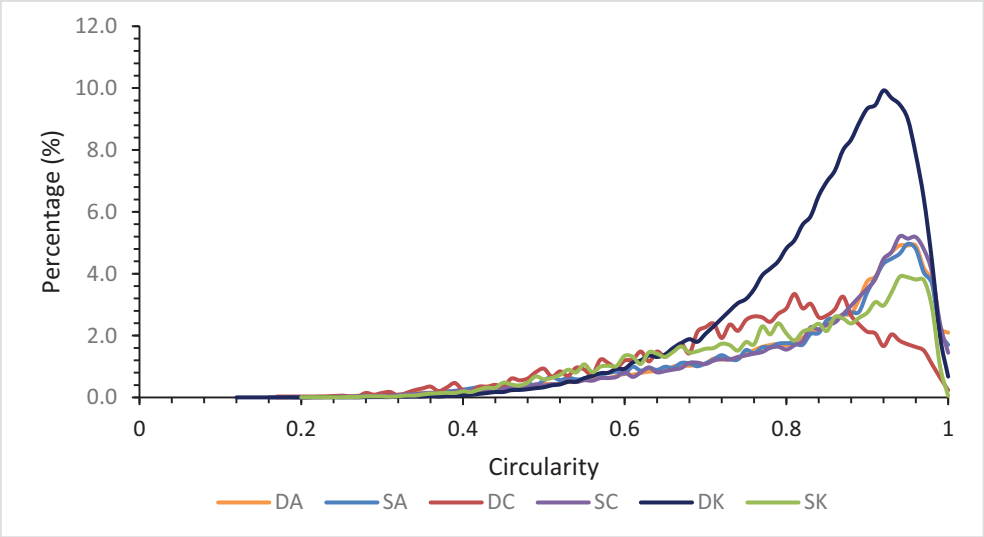


Figure 2. Distribution of tomato powder particle high-sensitivity (HS) circularity

SA- sundried tomato powder pre-treated with Ascorbic acid; SK- sundried tomato powder pre-treated with potassium metabisulfite; SC-sundried tomato powder with no pre-treatment; DA- solar dried tomato powder pre-treated with Ascorbic acid; DK- solar dried tomato powder pre-treated with potassium metabisulfite; DC-solar dried tomato powder with no pre-treatment

Even with the same milling process, the HS-circularity (Figure 2) and convexity (Figure 3) of tomato powder were highly different. The circularity of 10 % of the particles of solar dried tomato powder pre-treated with KMS were 0.92 % making up the highest proportion of the shape of the samples. The convexity of tomato powder granules was higher (0.98 - 1.0) because most of the powder particles were separate and less shape-distorted. On the other hand, exocarp of tomato fractions had less uniform shapes, with low convexity and circularity similar to the bran of cereal flours (Saad et al., 2011). The KMS pre-treated solar dried tomato powder resulted in powder particles with more convex and circular shape compared to the other samples. The treatments with KMS could have weakened the bond between exocarp and mesocarp of tomato as well as between sugars, acids and protein, resulting in a better separation of powder particles. Figure 6 shows some particles of tomato powder. Different particle sizes had varied convexity and circularity and particles fractions that were mostly irregular, elongated and fibrous in shape seem to come from the exocarp of the tomato whiles others were mostly rounded were produced from the mesocarp matrix. From the results, it can be deduced that solar dried tomato pre-treated with KMS would give a smoother texture compared to the other samples when reconstituted into other tomato products.

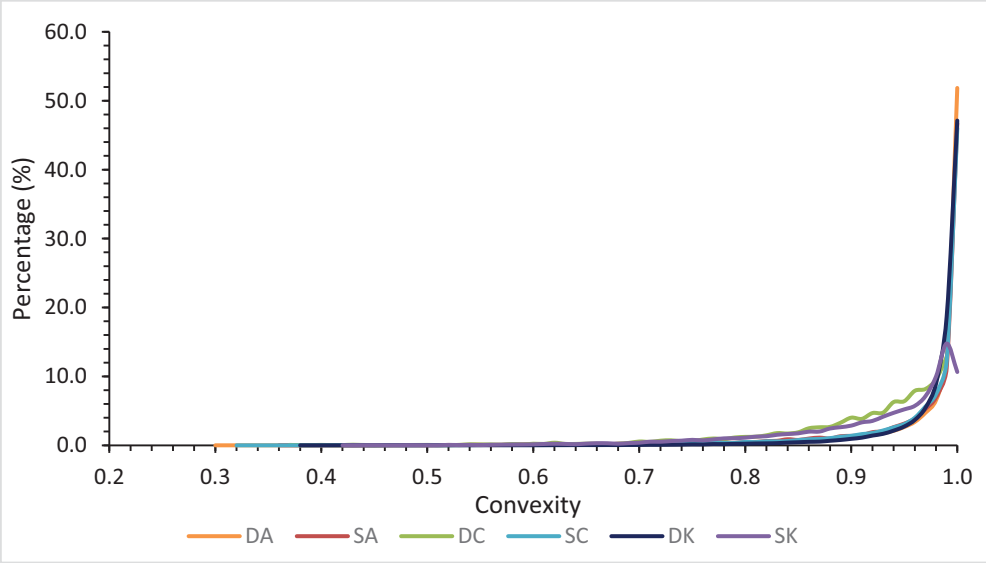


Figure 3. Distribution of convexity of tomato powder particles

SA- sundried tomato powder pre-treated with Ascorbic acid; SK- sundried tomato powder pre-treated with potassium metabisulfite; SC-sundried tomato powder with no pre-treatment; DA- solar dried tomato powder pre-treated with Ascorbic acid; DK- solar dried tomato powder pre-treated with potassium metabisulfite; DC-solar dried tomato powder with no pre-treatment

3.4 Microstructural Evaluation of Dried Tomato

Scanning electron microscopy uses microscopic techniques to examine the changes in the size and shape of the intercellular and cellular spaces and structural changes that occur when food is processed (Aguilera & Lillford, 1996; Alzamora et al., 1996). The cellular structure of tomato influences the mode of transfer of nutrients and water during drying and this has an impact on the quality of dried tomato (Gekas, 1992).

The physical characteristics of foods correlates well with their microscopic structure (Tortoe & Orchard, 2006). SEM of fresh tomato has rigid cell wells with firm edges and that of dried tomato looks depressed, distorted and deformed. Differences in the distortion, shape and appearance of cell walls could be observed for sun and solar dried samples (Figures 4 & 5).

The Depressions are a sign of water loss. The depressions observed for the solar dried tomato are more pronounced at the cellular level compared to the sun dried (final moisture content 19 - 20 %); an indication of higher water loss in solar dried tomato (final moisture content 14 - 15 %).

Zogzas *et al.,* (1994) explained the extent of collapse of the cell wall during drying as being proportional to the quantity of water lost during the drying process. This is similar to what was observed in a study by Owureku-Asare *et al.,* (2014), about the structure of oven dried tomato.

From this observation, pre-treatment did not seem to affect the cell structure of solar and sun dried tomato as there were no visual differences observed in the cellular structures for the different pretreated samples. In similar studies by Sargent, 1998; Tortoe & Orchard, 2006, for apple and banana respectively, osmotic dehydration caused the movement of water and deformation of the pectin, hemi cellulose and cellulose of the cell structure plasma membrane and middle lamella resulting in the collapse of the cell and plasmolysed cells.

Dried tomato is considered to be hygroscopic (Hawlader et al., 1991) and reconstitute well when water is added to it. The water moves freely across the gradient and is absorbed by the cellular membranes of tomato.

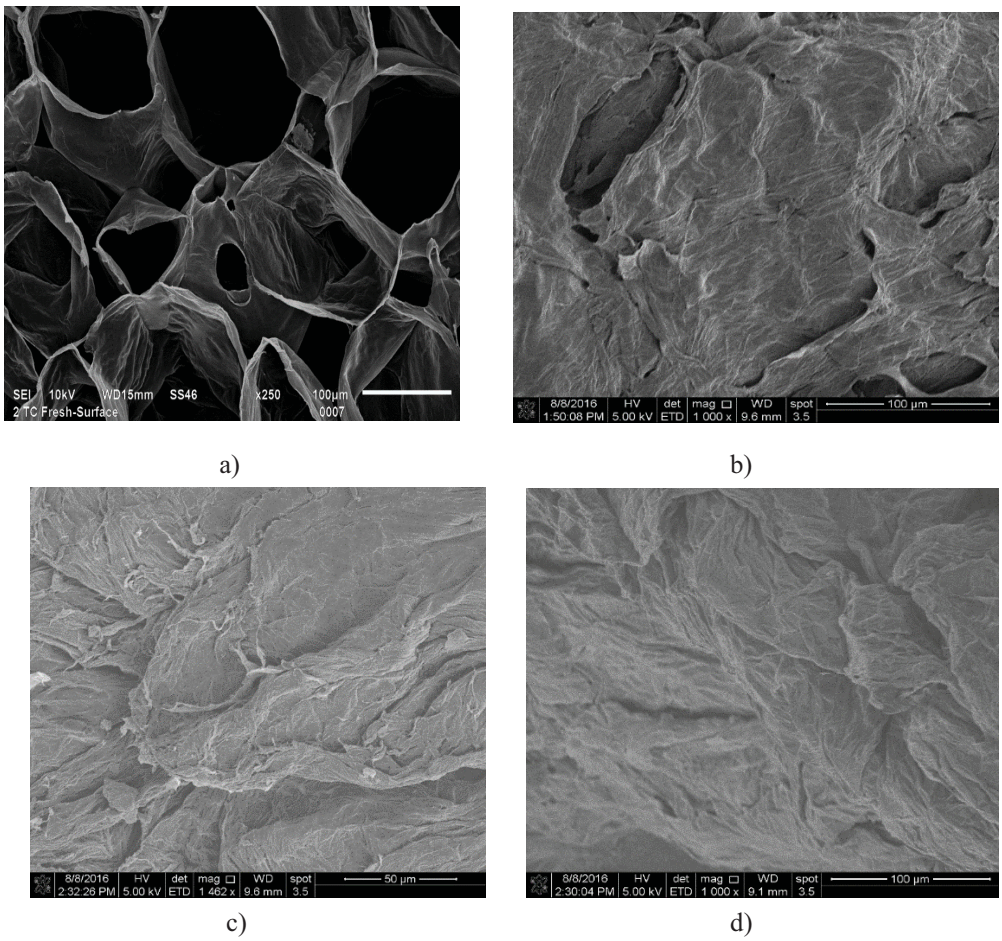
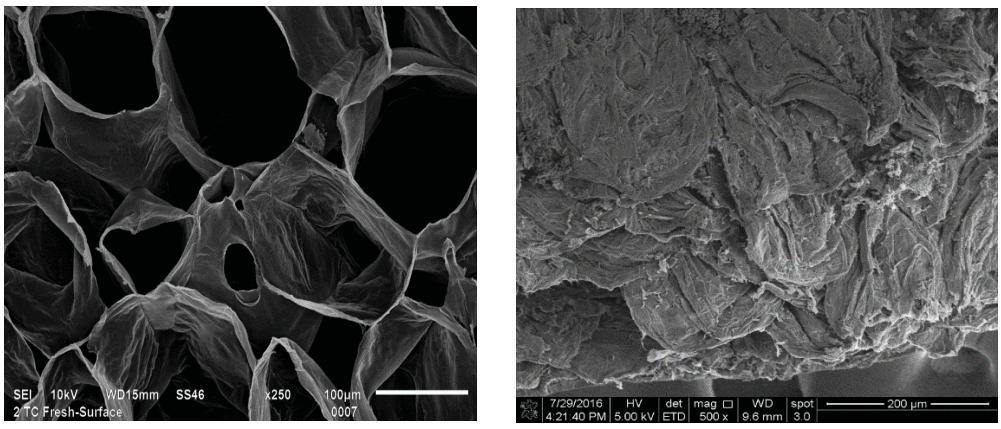


Figure 4. The microstructure of the surface of (a) fresh tomato (b) sun-dried tomato slices with no pre-treatment (c) sun-dried tomato slice pre-treated with ascorbic acid (d) sun-dried tomato pre-treated with KMS at SEM magnification of 250, 100,50 and 100x respectively



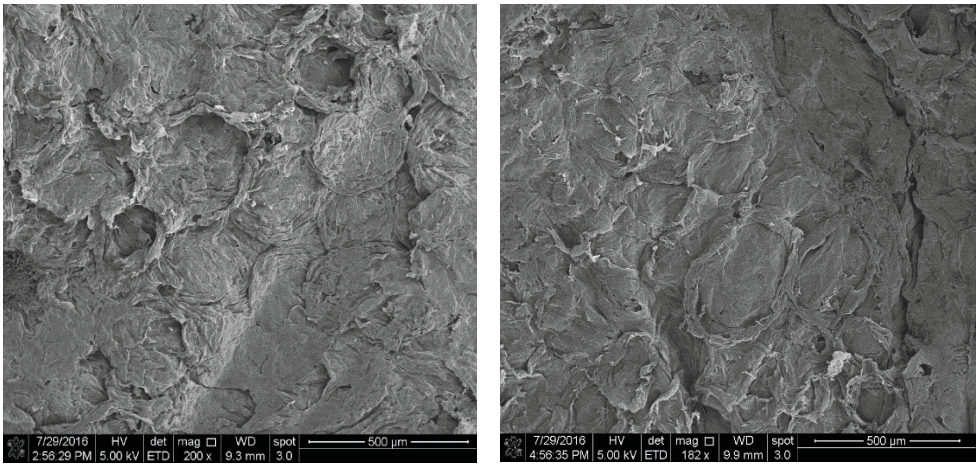


Figure 5. The microstructure of the surface of (a) fresh tomato (b) solar-dried tomato slices with no pretreatment (c) solar-dried tomato slice pretreated with ascorbic acid (d) solar-dried tomato pretreated with KMS at SEM magnification of 100,200,500 and 500x respectively

4. Conclusion

The ash content was higher in the sun-dried samples compared to the solar dried tomato samples, an indication of contamination with extraneous materials from the environment. Sulfur dioxide content of 740 mg/Kg d.w. recorded for solar dried tomato pre-treated with potassium metabisulfite was much lower than the maximum legal limit of 2000 mg/kg d.w. sulfur dioxide recommended in fruits. Solar and sun-dried tomato pre-treated with potassium metabisulfite had significantly high carotenoids, lycopene and beta carotene compared with the other pre-treated samples and controls. Potassium metabisulfite pre-treated solar dried tomato powder particles were more convex and circular in shape compared to the other samples which enhances the reconstitution characteristics of tomato powder; an ideal quality for further processing into other products such as sauce or paste. Processing tomato paste locally by could help reduce huge imports of tomato paste. This dryer which is easy to operate and has the potential of helping to add value to tomato and reduce post-harvest losses incurred along the production and marketing chain.

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References

Adom, K. K., Dzogbefia, V. P., & Ellis, W. O. (1997). Combined effect of drying time slice thickness on solar drying of okra. *Journal of the Science of Food Agriculture*, *73*, 315-320. https://doi.org/10.1002/(sici)1097-0010(199703)73:3%3c315::aid-jsfa718%3e3.0.co;2-n

Aguilera, J. M., & Lillford, P. J. (1996). *Microstructural and imaging analyses are related to food engineering.* In Food Engineering 2000 (Eds. Fito P, Ortega-Rodriguez E, Barbosa-Canovas G.V). Chapman and Hall, N. Y. pp 23-38.

Alzamora, S. M., Gerschenson, L. N., Vidales, S. L., & Nieto, A. (1996). *Structural changes in the minimal processing of fruits*: Some effect of blanching and sugar impregnation. In Food Engineering 2000 (Eds. Fito P, Ortega-Rodriguez E, Barbosa-Canovas G V). Chapman and Hall, N. Y. 117-139.

American Association of Cereal Chemists (2000). Approved Methods of the AACC, 11th ed. The Association: St. Paul, MN.

AOAC, 2000). Association of Official Analytical Chemists, AOAC International. Vol. 1. 18th ed. Arlington: VA, AOAC International.

Aryeetey, E. (2006). Ghana -Second Largest Importer of tin tomato. ISSER-Merchant Bank Development Seminar Series. Ghanaweb. Retrieved from http://ghanaweb.com/GhanaHomePage/NewsArchive/artikel.php?ID=1 Assessed 5th June, 2013

Beecher, G. R. (1998). Nutrient content of tomatoes and tomato products. *Proceedings of the Society for Experimental Biology and Medicine, 218*(2), 98-100. https://doi.org/10.3181%2F00379727-218-44282a

Chen, H., Hernandez, C. F., & Huang, T. (2005). A study of the drying effect on lemon slices using a closed-type solar dryer. *Solar Energy, 78*(1), 97-103. https://doi.org/10.12777/ijse.8.1.69-74

Choi, S. Woo, H., Ko, S., & Moon, T. (2008). Confocal laser scanning microscopy to investigate the effect of cooking and sodium bisulfite on in vitro digestibility of waxy sorghum flour. *Cereal Chemistry, 85*, 65-69. https://doi.org/10.1094/cchem-85-1-0065.

Davoodi, M. G., Vijayan, P., Kulkarnib, S. G., & Ramana, K. V. R. (2007). Effect of different pre- treatments and dehydration methods on quality characteristics and storage stability of tomato powder. *LWT Food Science Technology, 40*, 1832-1840. https://doi.org/10.1016/j.lwt.2006.12.004

Doymaz, I. (2004b). Pre-treatment effect on sun drying of mulberry fruits (Morus alba L.). *Journal of Food Engineering, 65*, 205-209. https://doi.org/10.1016/j.jfoodeng.2004.01.016

Doymaz, I., & Pala, M. (2002). Hot air drying characteristics of red pepper. *Journal of Food Engineering*, *55*, 331-335. https://doi.org/10.21924/cst.2.2.2017.56

Dumoulin, E., & Bimbenet, J. J. (1998a). *Mechanical, Physical and Chemical Phenomena during Food Drying: Consequences on properties of dried products*. In: Proceedings of Eleventh International Drying Symposium, August 19-22, Halkidiki, Greece, 711-718.

ECOM Canada Ingredients. (1997). Retrieved from http://ecomcanada.com/tomato.html

Falade, K. O., & Omojola, B. S. (2010). Effect of Processing Methods on Physical, Chemical, Rheological, and Sensory Properties of Okra (*Abelmoschus esculentus*). *Food Bioprocess Technology, 3,* 387. https://doi.org/10.1007/s11947-008-0126-2.

Falade, K. O., Olufemi, T., & Shogaolu, O. T. (2010). Effect of pre-treatments on air-drying pattern and color of dried pumpkin (*Cucurbita maxima)* slices. *Journal of Food Process Engineering, 33*, 1129-1147. https://doi.org/10.1111/j.1745-4530.2008.00330.x.

Gallali, Y. M., Abujnah, Y. S., & Bannani, F. K. (2000). Preservation of fruits and vegetable using solar dryer: a comparative study of natural and solar drying III; chemical analysis and sensory evaluation data of dried samples (grapes, figs, tomatoes and onions). *Renewable Energy, 12*, 203-212. https://doi.org/10.1080/23311932.2018.1461731.

Gekas, V. C. (1992). *Transport phenomena of foods and biological materials* (Eds. Singh R.P, Heldman D.R). CRC Press, Boca Raton, FL.

Gogus, F., & Maskan, M. (1999). Water adsorption and drying characteristics of okra (*Hibiscus esculentus* L.). *Dry. Technol, 17*, 883-894. https://doi.org/10.1080/07373939908917576.

Hawlader, M. N. A., Uddin, M. S. Ho, J. C., & Teng, A. B. W. (1991). Drying characteristics of tomatoes. *Journal of Food Engineering, 14*, 259-268. http://www.nal.usda.gov/fnic/foodcomp/Data/car98/car98.html>. Assessed on 16th March, 2017.

Jon, C. K., & Kiang, C.S. (2008). *Food dehydration and developing countries Food drying science and technology*. In: Food drying science and technology, microbiological, chemistry applications. DEStech Publications Inc. Lancester Pensylvannia USA. pp 67-82.

Knockaert, G., Sudheer, K. P., Lemmens, L., Van Buggenhout, S., Hendrickx, M., & Van Loey, A. (2012). Carrot ß-carotene degradation and isomerization kinetics during thermal processing in the presence of oil. *Journal of Agricultural and Food Chemistry, 60*(41), 10312-10319. https://doi.org/10.1021/jf3025776.

Krokida, M. K., Karanthanos, V. T., & Maroulis, Z. B. (2000). Effect of osmotic dehydration on color and sorption characteristics of maple and banana. *Drying Technology, 18*, 937-950.

Latapi G., & Barrett, D. M. (2006). Influence of pre-drying treatments on quality and safety of sun-dried tomatoes. Part I: Use of steam blanching, boiling brine blanching and dips in salt or sodium metabisulfite. *Journal of Food Science, 71*, 1-20. https://doi.org/10.1111/j.1365-2621.2006.tb12401.

Lenart, A. (1996). Osmo-convective drying of fruit and vegetable tissues undergoing osmotic processing. *Drying Technology, 14*, 2-10. https://doi.org/10.1080/07373939608917104.

Lin, T. M., Durance, T. D., & Scaman, C. H. (1998). Characterization of vacuum microwave, air and freeze dried carrot slices. *Food Research International, 4*,111-117. https://doi.org/10.1016/S0963-9969(98)00070-2.

Owureku-Asare, M., Agyei-Amponsah, J., Saalia, F. K., Alfaro, L., Espinoza, L., & Sathivel, S. (2014). Effect of pre-treatment on physicochemical quality characteristics of a dried tomato (*Lycopersicon esculentum*). *African. Journal of Food Science, 8*, 253-259. https://doi.org/ 10.5897/ajfs2014.1156

Papaioannou, E. H., & Karabelas, A. J. (2012). Lycopene recovery from tomato peel under mild conditions assisted by enzymatic pre-treatment and non-ionic surfactants. *Acta Biochimica Polonica, 59*, 71-74.

Periago, M. J., & Garcia-Alonso, J. (2009). Bioactive compounds, folates and antioxidant properties of tomatoes (*Lycopersicum esculentum*) during vine ripening. *International Journal of Food Sciences and Nutrition, 60*(8), 694-708. https://doi.org/10.3109/09637480701833457.

Piga, A., Pinna, I., Ozer, K. B., Agabbio, M., & Aksoy, U. (2004). Hot air dehydration of figs (*Ficus carica L.):* drying kinetics and quality loss. *International Journal of Food Science and Technology, 39*, 793-799. https://doi.org/10.1111/j.1365-2621.2004.00845.

Pizzocaro, F., Torregiani, D., & Gilardi, G. (1993). Inhibition of apple Polyphenoloxidase (PPO) by ascorbic acid, citric acid and sodium chloride. *Journal of Food Processing and preservation, 17*, 21-30. https://doi.org/10.1111/j.1745-4549.1993.tb00223.

Prabhakar, K., & Venkateswara, S. (2014). Dried Foods. *Encyclopedia of Food Microbiology, 1*, 530-537.

Prothon, F., Ahrne, L. M., Funebo, T., Kidman, S., Langton, M., & Sjoholm, I. (2001). Effect of combined osmotic and microwave dehydration of apple on texture, microstructure and rehydration characteristics. *Lebensm Wiss Technology, 34*, 95-101. https://doi.org/10.1006/fstl.2000.0745.

Rajkumar, P., Kulanthaisami, S., Raghavan, G. S. V., Garie´py, Y., & Orsat, V. (2007). Drying kinetics of tomato slices in vacuum assisted solar and open sun drying methods. *Drying Technology, 25*, 1349-1357. https://doi.org/10.1080/07373930701438931.

Ranganna, K. (1986). Handbook of analysis and quality control for fruits and vegetable products. New Delhi: Central Food Technological Research Institute, Mysore. Tata McGraw-Hill Pub. Co.

Sargent, J. A. (1988). Low temperature scanning electron microscopy: Advantages and application. *Scanning Microscopy, 2*, 835-849. http://digitalcommons.usu.edu/foodmicrostructure/vol7/iss2/2.

Singh, R., Mangaraj, S., & Kulkarni, S. D. (2006). Particle-size analysis of tomato powder. *Journal of Food Processing and Preservation, 30*, 98. https://doi.org/10.1111/j.1745-4549.2005.00050.

Stahl, W., & Sies, H. (1996). Lycopene: a biologically important carotenoid for humans? *Archives of Biochemistry and Biophysics, 336*, 1-9.

Tonucci, L., Holten, J. M., Beecher, G. R, Kachik, F., Davis, C. S., & Mulokozi, G. (1995). Carotenoid content of thermally processed tomato based food products. *Journal of Agricultural Food Chemistry, 55*, 1597-1603.

Tortoe, C., & Orchard, J. (2006). Microstructural changes of osmotically dehydrated tissues of apple, banana and potato. *Scanning, 28*, 172-178. https://doi.org/10.1002/sca.4950280306.

Verma, A., Dikshit, S. N., Panigrahi, H. K., & Pandey, N. (2016). Study of physico-chemical composition of fresh tomato and standardization of recipe for tomato juice. *National Academy of Agricultural Science, 34,* 4. https://doi.org/10.1080/23311932.2017.1310596.

Wang, N., & Brennen, J. G. (1991). Moisture sorption isotherm characteristics of potatoes at four temperatures. *Journal of Food Engineering, 14*, 269-287. https://doi.org/10.1016/0260-8774(91)90018-N.

Zogzas, N. P., Maroulis, Z. B., & Marinos-Kouris, D. (1994). Densities, shrinkages and porosity of some vegetables during hot-air drying. *Drying Technology, 12*, 1653-1666. https://doi.org/10.1080/07373937.2015.1036289.

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Development of Gluten-Free Egg Pasta based on Amaranth, Maize and Sorghum

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Abstract

Due to increase of the population affected by Coeliac disease and awareness of consumers about the relationship between food and health, the production of cereal products from raw materials other than wheat is of interest. The aim of the work was to produce good quality gluten-free spaghettis, based on sorghum, amaranth and maize flour. Response surface methodology was applied to determine optimal formulation for the production of pasta. The resulting products of the experimental design were characterized regarding humidity, color, density, water activity, texture (firmness and elasticity), optimal cooking time, cooking weight, and cooking loss. The main trials were compared to two industrials gluten-free spaghetti. The results showed that water activity and cooking weight of the main trials were similar to the industrials pasta, but were really different for the other studied parameters. The optimal formulation was determined in order to obtain pasta with low cooking loss and optimal color and texture firmness. It utilized 12% egg white, 60% maize flour, 30% sorghum flour, 10% amaranth flour, 2.4% guar gum and 36% water. The optimal formulated pasta was evaluated by an untrained consumer panel in a sensorial analysis to show the consumer acceptance. The product presents nutritional benefits, the color was appreciated, but the texture was granular and crumbly which was not liked by the consumers.

Keywords: gluten-free pasta, amaranth, sorghum, maize, cooking loss, texture, color

1. Introduction

Pasta is a traditional Italian cereal-based food that is popular worldwide because of its convenience, versatility, sensory and nutritional value. The term usually refers to unleavened extruded wheat dough, composed simply of flour and water, sometimes egg (Fuad & Prabhasankar, 2010). Yet, the problem with wheat pasta is that the gliadins and glutenins of wheat gluten contain protein sequences toxic to persons with celiac disease (Verdu et al., 2015).

Coeliac disease is a chronic immune-mediated enteropathy triggered by the ingestion of gluten in wheat, rye and barley, in patients who are HLA-DQ2 or HLA-DQ8 positive (Verdu et al., 2015). It is characterized by a strong immune response to certain amino acid sequences found in the prolamin fractions of gluten, resulting in the destruction of the villi in the small intestine and leading to the malabsorption of nutrients, thus adversely affecting all systems of the body (Ciacci et al., 2007). Currently, the only effective treatment for CD is the strict lifelong renunciation of gluten-containing foods (Feighery, 1999). In the United States, about one percent of the population is affected by the Coeliac disease and one person in one hundred people suffer from celiac disease worldwide. Such a rate establishes that CD is the most common food intolerances (Broz & Horne, 2007). And it appears that this disease, and other gluten intolerances are increasing worldwide.

Durum wheat proteins are characterized by a typical viscoelastic behavior, provided by the protein-starch interaction, that allows good networking of the matrix and optimal dough formation during the mixing and extrusion phases. Therefore, the network forming ability of gluten, preventing the dissolution of pasta during cooking, needs to be substituted by other means, in order to achieve products with satisfying quality (Feillet & Dexter, 1996).

The use of gluten-free cereals like maize, millet, sorghum, or even non-grass pseudocereals like amaranth, quinoa, or buckwheat, can be a big challenge for food research and development as well as commercial processing operations. The advantage of using such raw material is that it could improve the nutritional quality of gluten-free foods (Schoenlechner et al., 2010).

However, several studies show that the production of pasta based solely on gluten-free flours is unsuccessful, and additional structuring agents are necessary to obtain extrudable dough. An obvious ingredient to increase the protein content is egg. Eggs are traditionally used in pasta mainly to achieve flavour effects and also to aid the structure formation. Egg proteins facilitate the formation of a tighter protein network, yielding a harder product, both before and after cooking. In addition, the tighter protein network reduces penetration by water and hence starch granule swelling during cooking (Antognelli, 1980).

Amaranth, an ancient grain, contains about thirty percent more protein than cereals such as rice, sorghum and rye. Amaranth is a source of thiamine, niacin, riboflavin, folate, and dietary minerals including calcium, iron, magnesium, phosphorus, zinc, and manganese that are comparable to grain products such as wheat germ, oats, and others. Amaranth flour particularly has an unusually rich source of the essential amino acid, lysine, which is low in other grains (George et al., 2015).

Sorghum is a cereal grain, rich in glucides (~70% of starch), it contains about 12% of proteins, mostly polyunsaturated fatty acids, fibres, minerals and vitamins (B1, B2, B3). The flour produced from sorghum is light in color and has a bland, neutral taste that does not impart unusual colors or flavors to food products. These attributes make it desirable for use in wheat-free food products. However this grain contains also anti-nutrients such as tannins, which reduce the digestibility of the product (FAO, 1995).

Like sorghum, maize is mostly constituted of starch (~72%). This grain contains also proteins (~10%), essential fatty acids (such as linolenic acid), fibers and vitamins (A and E) **(**FAO, 1992**).** This combination of these three grains in the gluten free pasta formulation could add value to the final product and limit the negatives aspects of each grain individually.

To the authors' knowledge, no publications exist on the production of pasta from a combination of amaranth, maize and sorghum. The aim of this study was to develop amaranth, maize and sorghum pasta of similar quality to wheat pasta. A response surface methodology was applied to determine the optimal formulation in a minimal number of experimental trials. The gluten free pastas produced during this study were characterized regarding various physical properties, functional properties, and sensorial analysis.

2. Material and Methods

2.1 Raw Materials

The amaranth used in the experiment was organic whole grain amaranth of variety Amaranthus Hypochiondriacus (11.7% moisture) and was donated to Iowa State University by Mark & Marice Jones (4498 Rd. 167 Oshkosh, NE 69154, United States). The sorghum (6.47% moisture) was supplied by Woodland Ingredients (Waukegan, IL 60087, United States) and the Maize flour (4.34% moisture) by Premium White Corn (Honeyville, Utah, 84314, United States). The brand of the emulsifier, guar gum, added to the pasta formulation was Bob's Red Mill and was bought in Hyvee (Ames, IA, United States). The eggs were grade A large eggs and were purchased in Hyvee (Ames, IA, United States).

2.2 Pasta Manufacture

A small-scale standardized laboratory procedure was used for pasta manufacture. The sorghum and amaranth grains were firstly milled into flour in Magic Bullet blender (Model MB1001). Then all dried components of the formula (maize flour, sorghum flour, amaranth flour, and guar gum) were mixed in a Kitchen Aid mixer (Model KSM75WH) at low speed until a uniform mix was achieved. Whole liquid eggs and water were added and mixed at low speed until the ''dough'' had an adequate consistency for lamination. The dough was at the end kneaded by hand and put into the Stand-Mixer Pasta Extruder Attachment of the Kitchen Aid. The pasta were arranged on trays and dried at room temperature for 24 hours. They were then packed in plastic bags (Ziploc) to protect them from the humidity.

2.3 Experimental Design

Preliminary trials (Table 1) were conducted to study the flour behaviors, the impact of whole or egg whites, and the average level of water needed for the pasta manufacture. All formulations were manufactured in duplicate.

Table 1. Formulations of the Pre-Trials

	Trials									
Ingredients	1	2	3	4	5	6	7	8	9	10
Sorghum flour (%)	30	50	40	10	20	40	20	20	25	20
Amaranth flour (%)	20	20	30	20	10	10	30	30	25	40
Maize flour (%)	50	30	30	70	70	50	50	50	50	40
Egg white (%)	12	13	-	12	-	-	12	-	12	13
Whole egg (%)	-	-	20	-	12	-	-	12	-	-
Water (%)	40	35	30	45	40	37	35	35	32	33
Emulsifier (%)	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2

Some difficulties occurred during the extrusion of pasta containing more than 30% of sorghum flour. The best and easiest extrudable pasta were those with a large percentage of maize flour.

According to the comparison between the pre trials 7 and 8 it was noticeable that the pasta with egg white were of better quality than pasta with whole egg. Consequently, the main trials were manufactured using only egg white.

Dough moisture is generally recognized to have a major influence on pasta quality. High dough moisture toughened the dough, which adhered to the screw of the pasta machine and the produced pasta were very sticky and therefore showed very low texture firmness. Too low dough moisture resulted in noodles, which showed surface cracks. After these pre trials it was decided to work with dough moisture levels between 33 and 39%.

Response surface methodology was then used to evaluate the effect of the independent variables (egg white amount, amaranth and sorghum ratio, level of water, and emulsifier) on the dependent variables (firmness, color, density and cooking loss). Hereupon, optimum ingredient levels could be determined. A Doehlert design (Table 2) was developed featuring variations in the addition of egg white (ranging from 0 to 24 % based on 100 % maize sorghum and amaranth flour), sorghum and amaranth flour (ranging from 5% sorghum/35% amaranth to 35% sorghum/ 5% amaranth, using a constant level in each sample of 60% of maize flour), levels of water (ranging from 33 to 39 % based on 100 % maize sorghum and amaranth flour), and emulsifier (ranging from 0 to 2,4 % based on 100 % flour). The upper and lower limits of these levels were selected based on preliminary trials conducted. A total of 21 trials were carried out, with duplicates formulated for each treatment. The response of each of the investigated parameters was analyzed by fitting quadratic models to the data with least square regression in order to identify significant ($p<0.05$) effects of the variations in ingredient levels on the responses. Nine-dimensional graphs for the models were used to visualize overall trends. Commercial pastas (Barilla and Royal Quinoa) were also analyzed and served as the baseline comparisons.

Table 2. Formulations of the Main Pasta Trials

Trial/Sample	% Egg White	% Sorghum /Amaranth	% Water	% Guar Gum
1	12	20/20	36	1.2
2	24	20/20	36	1.2
3	18	35/5	36	1.2
4	18	25/15	39	1.2
5	18	25/15	37	2.4
6	0	20/20	36	1.2
7	6	5/35	36	1.2
8	6	15/25	33	1.2
9	6	15/25	35	0
10	18	5/35	36	1.2
11	18	15/25	33	1.2
12	18	15/25	35	0
13	6	35/5	36	1.2
14	12	30/10	33	1.2
15	12	30/10	35	0
16	6	25/15	39	1.2
17	12	10/30	39	1.2
18	12	20/20	38	0
19	6	25/15	37	2.4
20	12	10/30	37	2.4
21	12	20/20	34	2.4

2.4 Determination of Physical Properties

Each of the following properties were measured in duplicate or triplicate, and were carried out using approved and published methods. All properties were compared to control samples, which included commercial Barilla and Royal Quinoa Pasta.

Moisture content

The moisture content of flours and pasta were determined according to the AACC (American Association of Cereal Chemists) 44-19. Two grams of flour or dry pasta were placed into a laboratory oven (Heratherm General Protocol Ovens, ThermoFisher Scientific, Waltham, Massachusetts, United States) at 135°C for two hours. The samples were then weighed and the percentage of moisture and volatile matter (MVM) were calculated using the following equation:

$$\text{MVM}= \frac{\text{loss of moisture (g)}}{\text{initial weigh of the sample (g)}} *100 \quad (1)$$

Color analysis

A Chroma meter (CR-400, Konica Minolta, Ramsey, New Jersey, United States) was used to study the color of the pasta, according to the Reflectance Colorimeter Method 14-22 of AACC. The L* value quantify the brightness, a* the redness and b* the yellowness. ΔE value is dependent of L*, a* and b* and is used to determine if there is a significant color difference between the samples.

Unit density

According to Rosentrater et al. (2005), the density of the pasta was calculated using the following equation:

$$\text{density (g/cm}^3\text{)}= \frac{\text{Weight of the pasta (g)}}{\text{Volume of the pasta (cm}^3\text{)}} \quad (2)$$

The volume was calculated, assuming the pasta to be a cylinder: $V = \pi * r^2 * l$. The diameter (d = r/2) and the length (l) were measured using an electronic digital caliper (Digital Caliper, Fisher Scientific, Pittsburgh, Pennsylvania, United States).

Water Activity

The water activity was measured for each sample of the main trials pasta with a water activity meter (3TE, Aqua Lab, Washington, United States).

Texture

Dried pasta texture measurements were carried out with an Autograph (Model AGS-J, Shimadzu Scientific Instruments, 8052 Reeder Street, Lenexa, KS, United States). Data were evaluated using the Trapezium Software. Tests were carried out at 20°C on all the 21 samples, in triplicate, and on the two reference pasta. The autograph evaluated the maximum stress (N/m²), maximum strain (%), the force (N) applied and the stroke (mm). The speed was adjusted to 1mm/min.

With the raw data (stroke and force), the stress and the strain were calculated and the graph stress dependent of the strain were plot.

$$\sigma = \frac{F*L}{\pi*r\text{^}3} \qquad \varepsilon = \frac{6*D*d}{L^2} \tag{3}$$

Where σ is the stress (N/m²), L (mm) is the length of the area where the pasta is placed on the fixture, r (mm) is the radius of the pasta, ε (%) is the strain, D (mm) is the stroke of the fixture, and d (mm) the diameter of the pasta. Strain is "deformation of a solid due to stress", and stress is force per unit area. The toughness and the Young's Modulus were then determined with the graph Stress = f(Strain). The toughness is the area under the curve and these values were calculated using the trapezium method (Fuad & Prabhasankar, 2010), and the Young Modulus corresponds to the slope of the breaking straight line.

2.5 Determination of Functional Properties

Cooking quality of the manufactured pasta was evaluated using official methods 66-50 and 66-51 of the American Association of Cereal Chemists (AACC, 2000). Optimum cooking time, weight gain by pasta, and solid lost during cooking were evaluated.

Cooking Time

Optimum cooking time for pasta was the time required for the opaque central core of the noodle to disappear when squeezed gently between two glass plates (Approved Method 66-50, AACC, 2000)**.** In a 500 ml beaker about 300 ml of water were heated until boiling. Ten g noodles were put into the boiling water (no salt addition) without stopping the water from boiling. Every 30 s, one noodle was taken out and pressed between two glass plates. At the time when a white core could no longer be seen the cooking time was reached. This time was noted as the cooking time and used for the following evaluations. As the cooking time depends very much on the noodle formulation and processing conditions, it had to be determined for each formulation.

Cooking Weight

Cooking weight is defined as the weight gain of the noodles during cooking and indicates the amount of water that is absorbed and is therefore an index for the swelling ability of the noodles. According to the AACC Approved Method 66-50, 10 g dry spaghetti sample were cooked at the optimum cooking time and were drained and then weighted. The cooking weight was calculated and given in % using the following equation:

$$\%\ \text{cooking weight} = \frac{\text{MCS-MRS}}{\text{MRS}} * 100 \tag{4}$$

Where MCS is the mass of cooked sample (g) and MRS is the mass of raw sample (g).

Cooking Loss

Dry matter losses during cooking were determined by AACC Approved Method 66-50. Pasta samples (10 g) were cooked to optimum time in 300 mL of distilled water in a beaker, rinsed in a stream of cold water for 30 s and drained. Instead of collecting cooking and rinse water in the Approved Method, the pasta were dried in the oven at 50°C during 48 hours and then weighted. The percentage of cooking loss was calculate with following equation:

$$\%\ \text{cooking loss} = \frac{\text{mass of the cooking loss (g)}}{\text{mass of raw pasta sample } \mathbf{(g)}} * 100 \tag{5}$$

Where the mass of cooking loss is the difference of weight between the raw pasta sample and the pasta after cooking and drying.

2.6 Sensory Evaluation

Pasta made with the optimal formulation were evaluated by a consumer panel. They were made one week before the sensory analysis and kept in plastic bags at refrigeration temperatures (4-6 °C) until the date of the analysis. They were compared to Barilla and Royal Quinoa Pasta. Pasta samples were cooked at the optimal cooking time strained, rinsed, and cooled in water at 20°C. Before testing pasta were placed in plastic cups for evaluation.

Samples were evaluated for the degree of liking for the color, odor, taste, texture (mouth), and overall liking. Participants were instructed to rinse with water (20 °C) before they began testing and between samples. Participants were briefed on the objective of the study without revealing any information that might have compromised the validity of the test. Ten healthy adults participated in the study. All participants had consumed pasta before. Rating were collected using a 5-hedonic scale where 1 = "dislike extremely" and 5= "like extremely". The mid-point of the scale 3= neither like nor dislike. Participants were asked to complete paper ballots. They were also asked which pasta they preferred between the three without sauce and with tomato sauce, to see if they will be able to appreciate the pasta with sauce.

3. Results and Discussion

To evaluate the results of each analysis, the samples were compared to two industrial pastas: Barilla spaghetti, made with rice and maize flour, and Royal Quinoa spaghetti, made with quinoa flour.

3.1 Physical Properties

Pasta moisture

The pasta moisture is the amount of water content in the pasta. It was measured using the 44-19 AACC method. Results are shown in Figure 1.

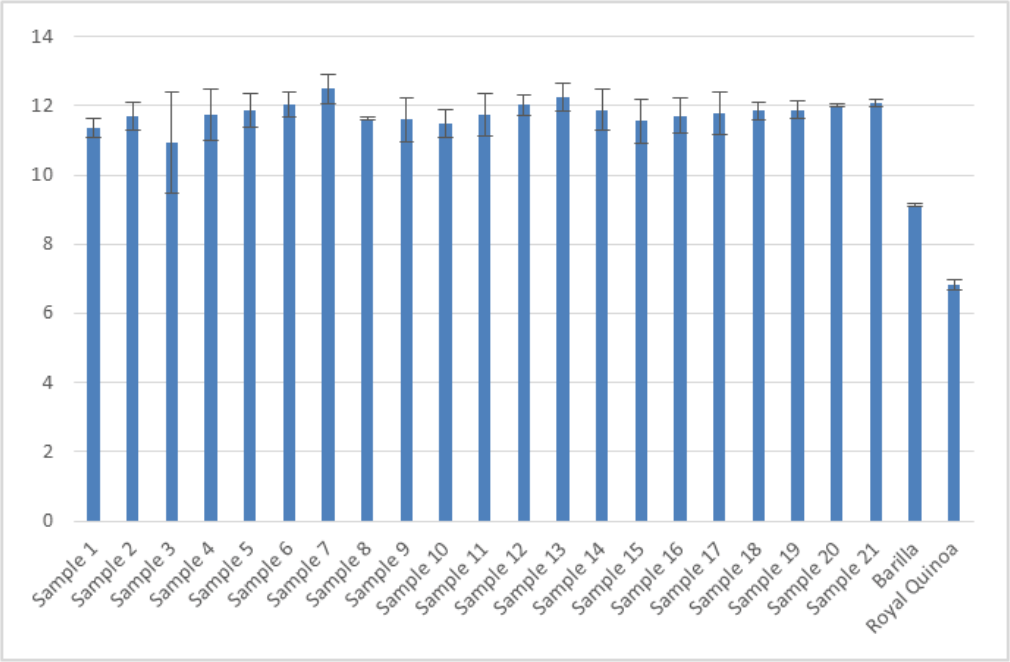

Figure 1. Moisture Content of the Trial Pastas and the Reference Pastas (+/- 1 SD)

The moisture content in Barilla and Royal Quinoa pasta were lower than in the 21 sample. The main trials were dried at room temperature for 24 hours, but the drying technology of industrial pasta is certainly different, explaining the difference between them. It is well known today that the high temperature drying technologies have a positive effect on the pasta quality (Novaro et al, 1993). Indeed, a high temperature drying could improve the firmness of the spaghetti and reduce the cooking loss (Dexter et al, 1983).

Pasta color

Color of dry spaghetti is an important quality factor for U.S. consumers. It is one of the first criterion selection, which will incite the consumer to buy, or not the product. Multigrain breads are darker and coarser than white breads, yet the market for these bread loaves has grown and expanded. Multigrain pastas may have the advantage of being perceived by a section of the population as a pasta variety related to wellness (Rayas-Duarte et al., 1996).

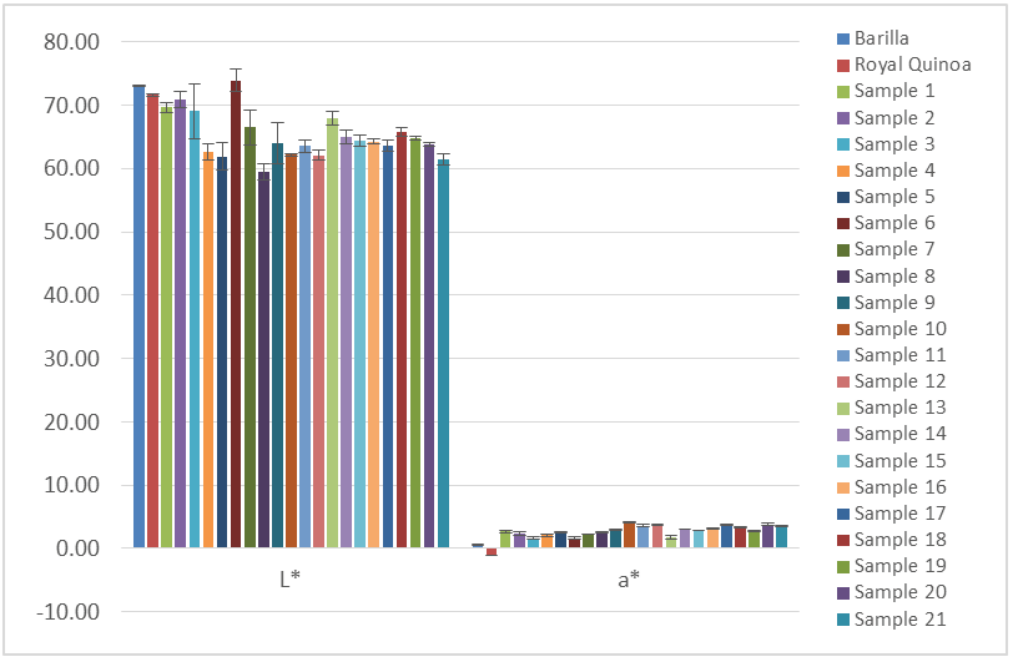

Figure 2. L* and a* Values of the Trial Pastas and the Reference Pastas (+/- 1 SD)

The L* value (Figure 2), brightness, of the references was similar to the L* value of the samples 1, 2, 3, 6 and 13 and in the same order of value of the other samples. The a* value (Figure 2) , redness, was much lower for the references compared to the trials.

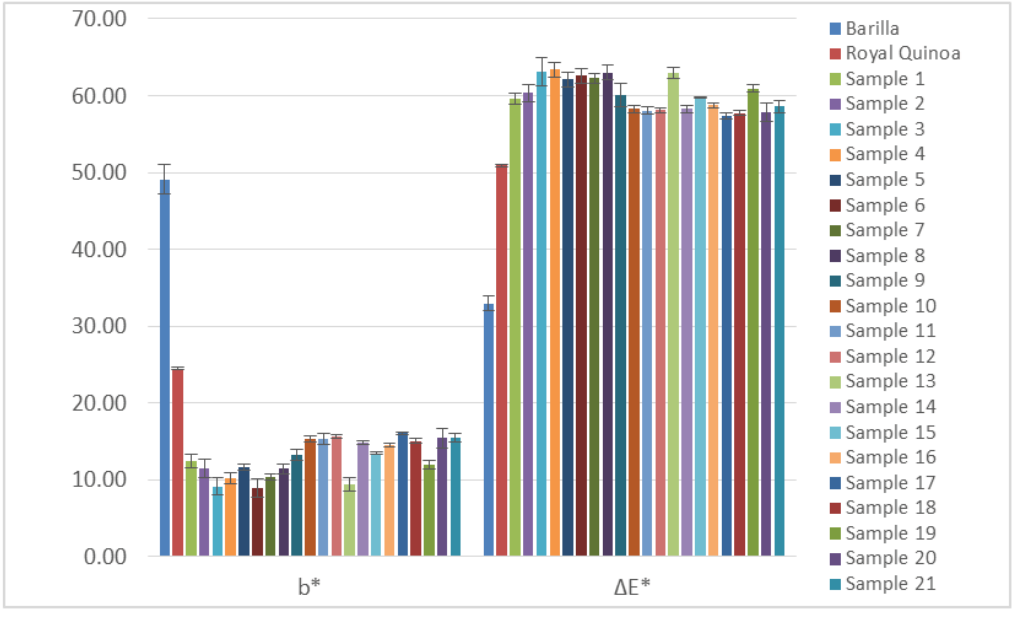

Figure 3. b* and ΔE* Value of the Trial Pastas and the Reference Pastas (+/- 1 SD)

The b* value (Figure3), greenness, of the references was really different from the trial values. These results are explained by the fact that Barilla pasta were much more yellow than the Royal Quinoa Pasta and the trials. This difference of b* explains the difference of color between the samples and the references.

The ΔE value (Figure 3) was calculated using L*, a* and b* values. Studying the previous graph, there was no significant difference between all the trials: they had almost the same color. However comparing the Barilla pasta to the trials, there was a major color difference, but this difference as less important between the Royal Quinoa pasta and the trials. A photo of the main trials pasta and the industrials pasta is presented in Figure 7.

The analysis of variance for color properties of pastas is presented in Table 3. The predictive models developed for color (L*, a* and b*) were considered adequate because they possessed a non-significant lack of fit and had satisfactory levels of R^2, and model significance.

Table 3. Analysis of Variance for Color Properties of the Pastas

Response	Source	Df	Sum of squares	F value	p value
Color L*	Model	4	33.044	3.4493	0.01281
	Residual	6	14.370		
R^2 = 0.805	Lack of fit	6	14.370		
	Pure error	0	0.0000		
Color a*	Model	4	4.4006	14.2159	0.00507
	Residual	6	0.4643		
R^2 = 0.861	Lack of fit	6	0.4643		
	Pure error	0	0.0000		
Color b*	Model	4	28.318	9.1665	0.00442
	Residual	6	4.634		
R^2 = 0.867	Lack of fit	6	4.634		
	Pure error	0	0.0000		

The significant variables affecting the L* parameter wereX2 (ratio sorghum/amaranth), and the interaction X2:X3 (with X3 the amount of water). a* and b* parameters were affected by the amount of egg whites (X1), the sorghum/amaranth ratio (X2) and the interaction (X1:X2) between these two factors. Surface plots were generated for the b* response to describe the quality response of these parameters (Figures 4, 5 and 6).

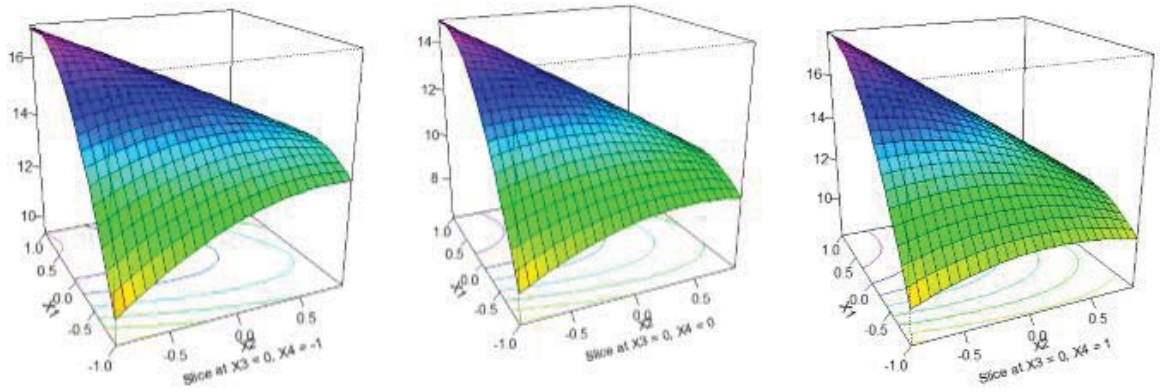

Figure 4. Effect of Egg White Level, Sorghum/Amaranth Ratio and Emulsifier Level on Color b* Parameter (at a Water Level of 36 %)

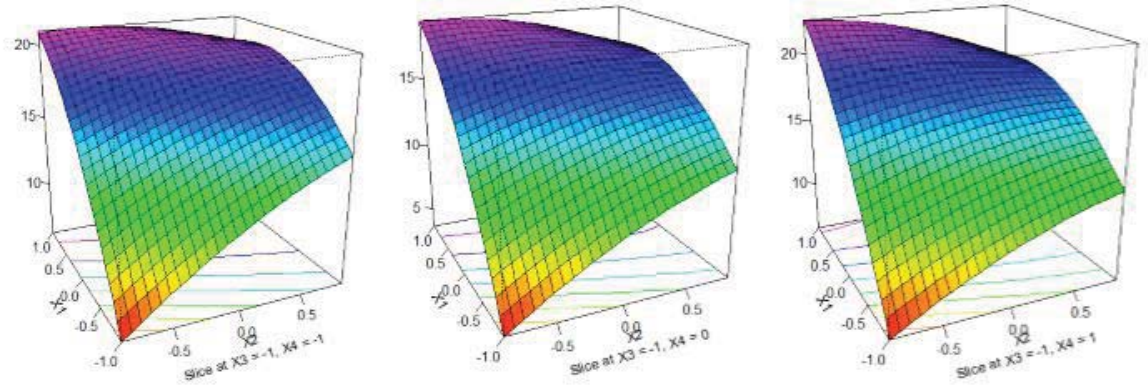

Figure 5. Effect of Egg White Level, Sorghum/Amaranth Ratio and Emulsifier Level on Color b* Parameter (at a Water Level of 33 %)

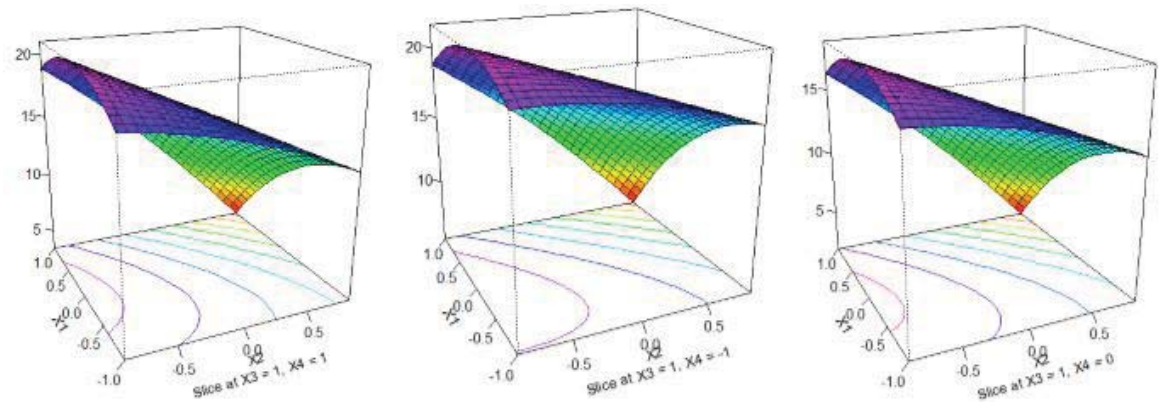

Figure 6. Effect of Egg White Level, Sorghum/Amaranth Ratio and Emulsifier Level on Color b* Parameter (at a Water Level of 39 %)

According to the surface plots, the amount of water has a significant effect on the b* parameter whereas the emulsifier level has none. The maximal b* value was close to Barilla and Royal Quinoa values. b* value was maximal with a level of water of 33%, a egg white level of 24% and a sorghum/amaranth ratio of 5/35%. It was also maximal with a level of water of 39%, a egg white level of 12% and a sorghum/amaranth ratio of 5/35%.

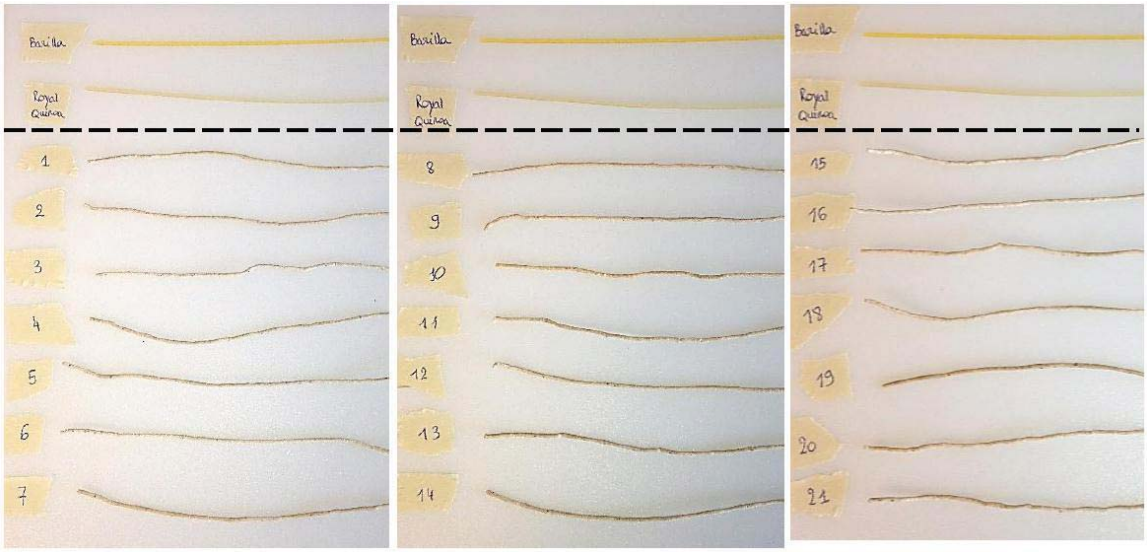

Figure 7. Photos of the Trial Pastas and the Two Reference Pastas (Barilla and Royal Quinoa)

Unit density

The pasta structure compactness is determinate during the pasta making process. Indeed, the density depends of the type of pasta (spaghetti, lasagna, etc.) and of the process used. Fardet et al. (1998) found that extruded pasta can be denser and their starch/protein network is tighter than in lasagna.

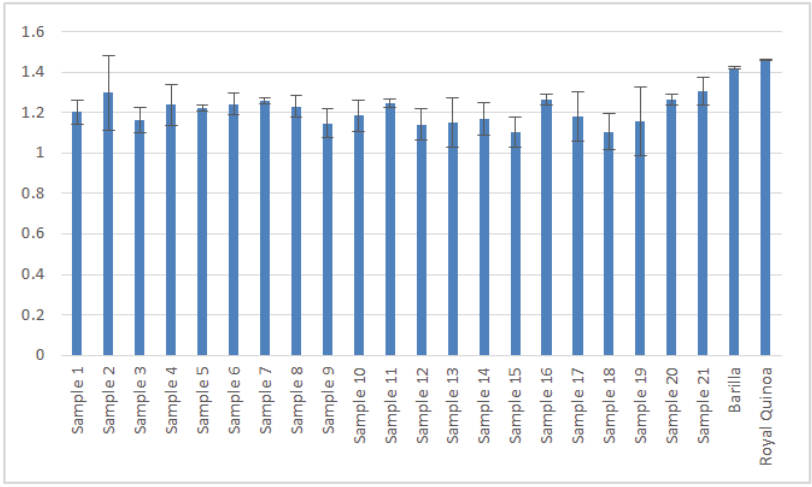

Figure 8. Unit Density of the Trial Pastas and the Reference Pastas (+/- 1 SD)

According to Figure 8, the density of Barilla and Royal Quinoa pasta was higher than the density of the trial pastas. It was more than 1400 g/cm^3 for the references and include between 1105 and 1306 g/cm^3 for the samples. This was certainly due to the difference of extrusion process. Indeed, the pastas were extruded using a Kitchen Aid Stand-Mixer Pasta Extruder Attachment and the references were extruded with industrial extruders.

The analysis of variance for density of pastas is presented in Table 4. The predictive model developed for density was not considered adequate because of the unsatisfactory level of R^2. Even though the model was not significant, explanatory analysis of data was performed in order to verify the tendency of this parameter.

Table 4. Analysis of Variance for Density of Pastas

Response	Source	Df	Sum of squares	F value	p value
Density	Model	4	0.0331	4.9014	0.1252
	Residual	6	0.0101		
$R^2 = 0.525$	Lack of fit	6	0.0101		
	Pure error	0	0.0000		

The analysis of variance determined that the sorghum/amaranth ratio, and the emulsifier amount had a significant effect on the density. Surface plots (not presented here) showed that pasta density was higher with a sorghum/amaranth ratio of 5/35, emulsifier level of 2.4%, egg white level of 24% and water level of 33%.

Water activity

The water activity is equivalent to the amount of reactive water available. It is a major parameter used to predict and control the food safety/stability during the transformation process and the storage (Palmade, 2015).

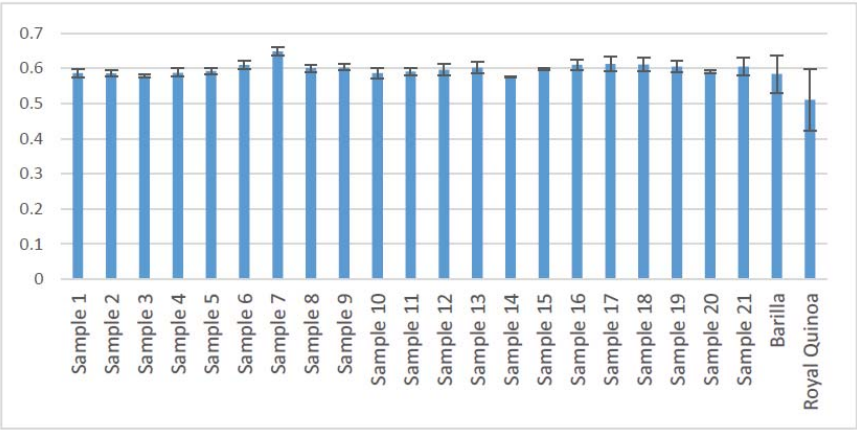

Figure 9. Water Activity (a_w) of the Trial Pastas and the Reference Pastas (+/- 1 SD).

As shown in Figure 9, the water activity varied between 0.57 and 0.65 for the trial pastas and the references. These values ensure the microbiologically safety of the products. Indeed, the microorganisms were unable to multiply at a water activity lower than 0.5 and only some yeasts can proliferate at an a_w of 0.6 (Palmade, 2015).

The analysis of variance for water activity of pastas is presented in Table 5. The predictive model developed for water activity was acceptable but not satisfactory because of the low level of R^2. Even though the model was not significant, explanatory analysis of data was performed in order to verify the tendency of this parameter. The model predicted that the egg white level and the sorghum/amaranth ratio were the two factors which have a significant effect on aw. The response surface models were plots and it was found that the lowest water activity was obtained with a maximal amount of egg white (24%), a minimal ratio of sorghum/amaranth (5/35%) and a level of water included between 33 and 36%.

Table 5. Analysis of Variance for the Water Activity of Pastas

Response	Source	Df	Sum of squares	F value	p value
Water activity	Model	4	0.0028	5.4366	0.0091
	Residual	15	0.0019		
$R^2 = 0.478$	Lack of fit	15	0.0019		
	Pure error	0	0.0000		

Texture

Texture is defined as the sensory response and the reaction of the food structure after a force action (Szczesniak, 1987). This is a critical parameter in the product acceptance by the consumers (Moskowitz & Drake, 1972). Factors involved in pasta texture are the quality and quantity of proteins, the drying process, and the cooking parameters (cooking water, water absorption) (Matsuo et al., 1972; Manthey et al., 2004; Cunin et al, 1995). Indeed, the cooking water and the drying temperature impact the pasta adhesion (Smewing, 1997). Guan & Seib also reported that the extrusion process affect the raw and cooked pasta texture (Guan, & Seib, 1994). Several researchers have adopted firmness of raw pasta and stickiness of cooked pasta as main quality parameters for pasta (Raina et al., 2005). In this study, the firmness of raw pasta was analyzed.

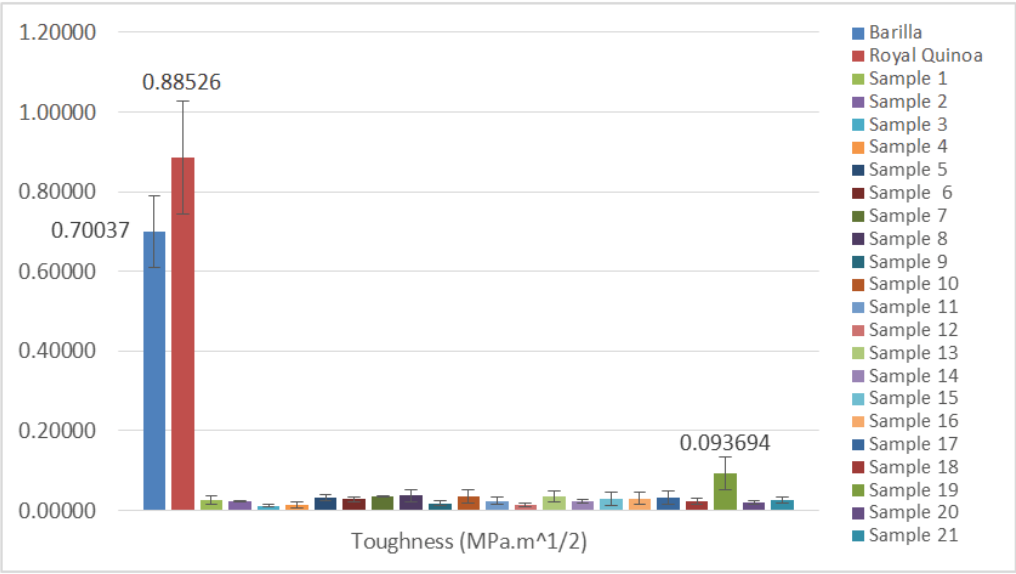

Figure 10. Toughness of the Trial Pastas and the Reference Pastas (+/- 1 SD)

The standard toughness value for classical pasta is 0.478 $MPa/m^{1/2}$ (Guinea et al., 2004). The toughness value of industrial pasta Barilla and Royal Quinoa was close to this standard value (Figure 10), but the firmness values of the main trials were lower (Figure 11). Only the value of the 19th sample was a little higher, but was still too low. This means that all the sample were quite breakable, which will impact the consumer acceptance.

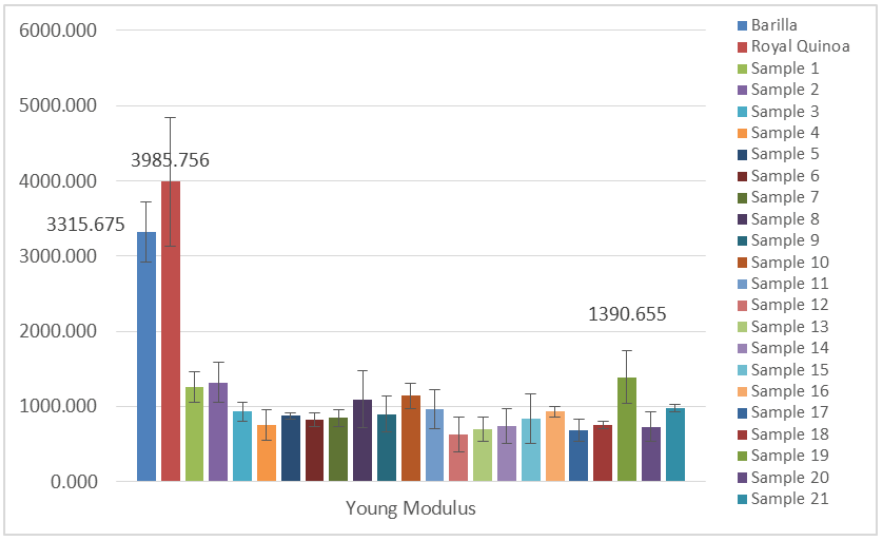

Figure 11. Young's Modulus of the Trial Pastas and the Reference Pastas (+/- 1 SD)

The young modulus is defined as the rate at which a deformed material returns to its original shape after a stressing force is removed. The young modulus of the reference pasta was higher than the value of the main trials. These results were correlated to the toughness value.

To study the factors involved in pasta texture, an analysis of variance (ANOVA) was carried out. Results are shown in Table 6.

Table 6. Analysis of Variance for Texture of Pastas

Response	Source	Df	Sum of squares	F value	p value
Firmness	Model	4	0.0019	2.5126	0.0878
	Residual	13	0.0024		
R^2 = 0.320	Lack of fit	12	0.0024		
	Pure error	0	0.0000		
Elasticity	Model	4	129625	0.7240	0.4476
	Residual	12	537136		
R^2 = 0.023	Lack of fit	12	537136		
	Pure error	0	0		

The predictive model developed for young modulus (elasticity) was really too low and was not suitable to be analyzed. The predictive model for toughness was not adequate either because of the low value of R^2, even though, explanatory analysis of data was performed and surface plots (Figures 12, 13, 14) were generated to describe the quality response of the egg white and emulsifier level, which were the two parameters that had significant effects on toughness.

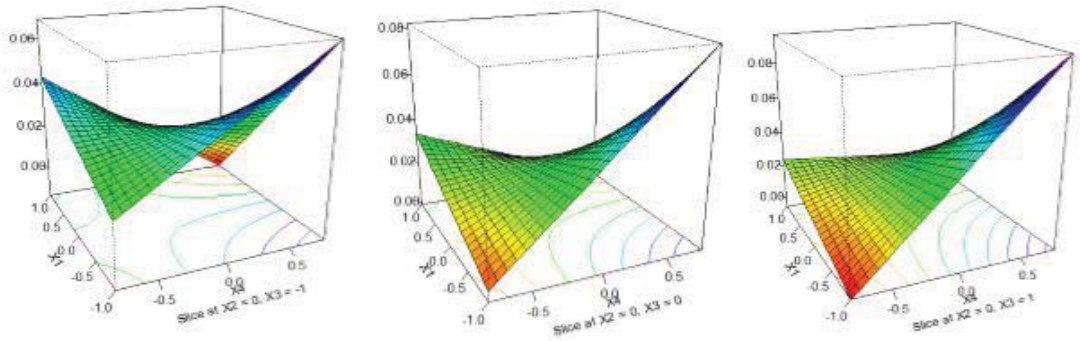

Figure 12. Effect of Egg White Level, Emulsifier Level and Water Level on Toughness (at a Sorghum/Amaranth Ratio of 20/20)

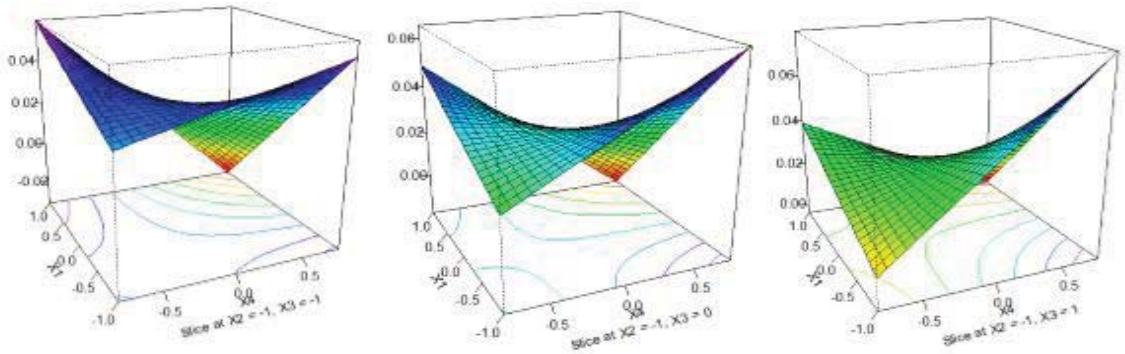

Figure 13. Effect of Egg White Level, Emulsifier Level and Water Level on Toughness (at a Sorghum/Amaranth Ratio of 5/35)

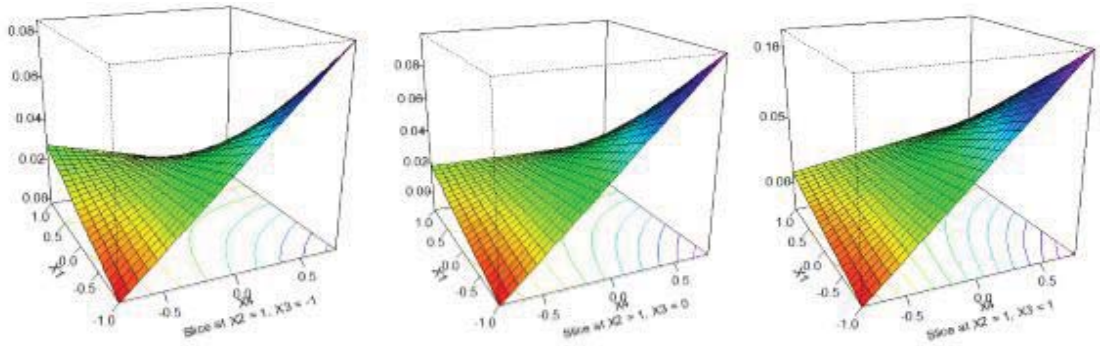

Figure 14. Effect of Egg White Level, Emulsifier Level and Water Level on Toughness (at a Sorghum/Amaranth Ratio of 35/5)

According to the surface response plots, sorghum / amaranth ratio has a significant effect on the pasta firmness. The level of water has an effect too, but less significant. A maximal toughness value is critical to have strong pasta. This maximal value was obtained with a maximal emulsifier level (2.4%) and water level (39%), a minimal egg white amount (0%) and a sorghum/ amaranth ratio of 35/5.

3.2 Functional Properties

During cooking, pasta undergoes complex modifications caused by heating and water uptake that determine different effects both at the macroscopic and molecular levels. In traditional pasta made with durum wheat semolina, cooking induces starch gelatinization with partial disappearance of the crystalline zones of amylopectin, the leaching of amylose together with other soluble substances into the cooking water, and the contemporaneous coagulation of the gluten network.

A completely different situation occurs in gluten-free pasta: the viscoelastic gluten network is absent, and the intensive heating applied during the different technological steps involved in pasta production has given rise to important modifications to the starch organization, leading to the creation of a new structure formed by retrograded or partially gelatinized and retrograded starch; during cooking, the extent of starch gelatinization increases and, at the same time, a denaturation of the proteins contained in the different raw materials takes place (Lucisano et al., 2012).

Cooking time

Optimum cooking time is the time needed to cook the entire core of the pasta. It is related to density of extruded spaghetti. Indeed, it has been reported that low pasta density are related to short optimum cooking time. Spaghetti with low density have a less tightly proteins network, which provide a path for water absorption into pasta, which resulted in shorter cooking times (Manthey et al., 2004). The optimal cooking time for the 21 samples was between 7 and 8.5 minutes, whereas it was 14 minutes for Barilla pasta and 17.5 minutes for Royal Quinoa pasta. There was a significant difference between the samples and the references, probably due to the difference of density. But these cooking time were satisfactory because a study reported that too long cooking

time damaged the spaghetti quality especially for gluten free pasta (Grzybowski & Donnelly, 1979).

Table 7. Analysis of Variance for Optimal Cooking Time of Pastas

Response	Source	Df	Sum of squares	F value	p value
Cooking time	Model	4	1.8000	3.2727	0.1744
	Residual	6	0.8250		
$R^2 = 0.45$	Lack of fit	6	0.8250		
	Pure error	0	0.0000		

The predictive model (Table 7) developed for optimal cooking time was acceptable. Explanatory analysis of data was performed and it was found that emulsifier level was the only parameter that had a significant effect on the cooking time. The higher the level of guar gum, the longer the optimal cooking time.

Cooking weight

Another important measured index is pasta water uptake (Figure 15), which depends on the weakness of starch granules and is related to the amount of starch damage (Lucisano et al., 2012). It is related to the cooking time too: longer cooking times induced an even more marked weight increase.

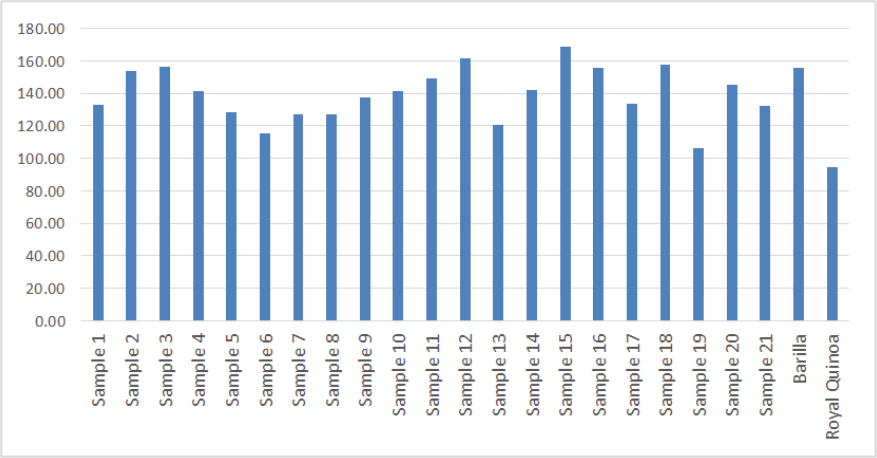

Figure 15. Water Absorption (%) in the Trial Pastas and the Reference Pastas (+/- 1 SD)

The water absorption of Barilla pasta was almost equal to the 2, 3, 16 and 18 samples. On the other hand, the water absorption of Royal Quinoa pasta was the lowest and was probably due to the difference of composition. Indeed a study found that the water absorption in pasta is related to the kind of flour which is used (Adekunle Ayo, 2007). The predictive models developed for water absorption (Table 8) was considered adequate because it possessed a non-significant lack of fit and had satisfactory levels of R^2.

Table 8. Analysis of Variance for Water Absorption of the Pastas

Response	Source	Df	Sum of squares	F value	p value
Water absorption	Model	4	3274.7	12.796	0.00243
	Residual	10	639.8		
$R^2 = 0.751$	Lack of fit	10	639.8		
	Pure error	0	0.0000		

The significant variables affecting this response were the egg white and emulsifier level, and the interaction egg white amount and water level. A maximal water absorption was linked to a maximal amount of egg white (2.4 %) and a minimal emulsifier level.

Cooking loss

During the cooking, some parts of the noodles dissolve in the water: soluble solids leach out from the pasta and pass into the cooking water. This phenomenon proceeds to different extents in relation to the structural compactness of the sample. The better the protein network is developed, the smaller the cooking loss. The loss of

solids, in fact, traditionally represents a measure of pasta quality, expressing its resistance to disintegration upon boiling: low amounts of solids into the cooking water indicate a high pasta cooking quality (Pagani et al., 2007). The cooking loss can also be increased by the composition of altering the cooking water: the higher the calcium and magnesium in the water, the more the spaghetti will be sticky and the cooking loss high (Dexter et al., 1983). However, unlike durum wheat pasta, due to the lack of gluten, the characteristics of gluten free pasta in general depend heavily upon the functional properties of the starch: it undergoes different heat treatments during processing, and often functions as the only structural network of the final gluten free product (Lucisano et al., 2012).

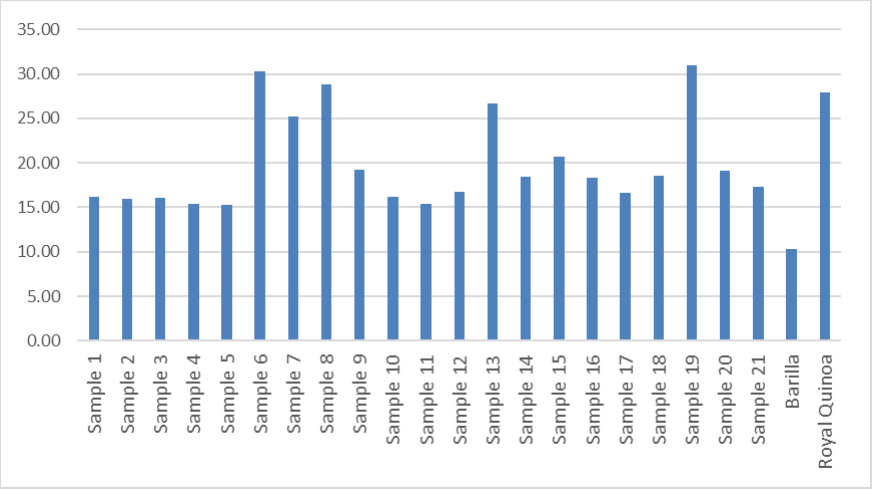

Figure 16. Cooking Loss (%) in the Trial Pastas and the Reference Pastas (+/- 1 SD)

The lowest cooking loss (Figure 16) was associated with the Barilla pasta. The Royal Quinoa pasta had a very high percentage of cooking loss. The 1, 2, 3, 4, 5 and 11 samples lost less matter during cooking compared to all the other experimental design samples.

The analysis of variance for cooking loss of pastas is presented in Table 9. The predictive model developed for cooking loss was considered adequate because it possessed a non-significant lack of fit and had satisfactory levels of R^2, and model significance.

Table 9. Analysis of Variance for Cooking Loss of Pastas

Response	Source	Df	Sum of squares	F value	p value
Cooking loss	Model	4	363.3	12.6088	0.0299
	Residual	6	43.22		
$R^2 = 0.733$	Lack of fit	6	43.22		
	Pure error	0	0.000		

The significant variables affecting the cooking loss parameter were the amount of egg white and the interaction between the amount of egg white and the emulsifier level. Surface plots were generated to describe the quality response of these parameters. According to the plots (Figures 17, 18 and 19), sorghum/ amaranth ratio has no significant effect on cooking loss, whereas water level has a great effect. As mentioned previously, good quality pasta are pasta with low cooking loss. In order to reduce as much as possible the cooking loss, it is required to have a maximal egg white and emulsifier level and an amount of water included between 33 and 36%. The effect of emulsifier on cooking loss was previously demonstrated by Nazarov. He found that mono- and diglycerides of fatty acids form complexes with amylose, thereby preventing the passage of starch into the cooking water, reducing cooking loss and stickiness (Nazarov, 1977).

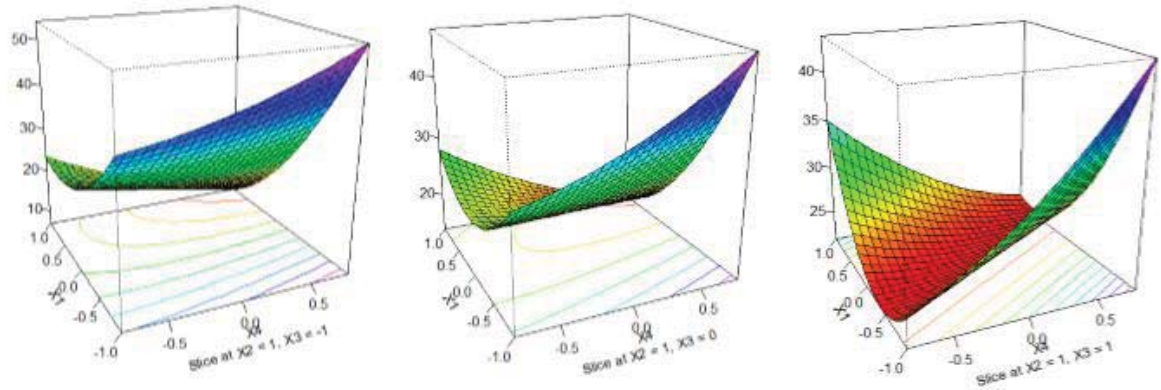

Figure 17. Effect of Egg White Level, Emulsifier Level and Water Level on Cooking Loss (at a Sorghum/Amaranth Ratio of 20/20)

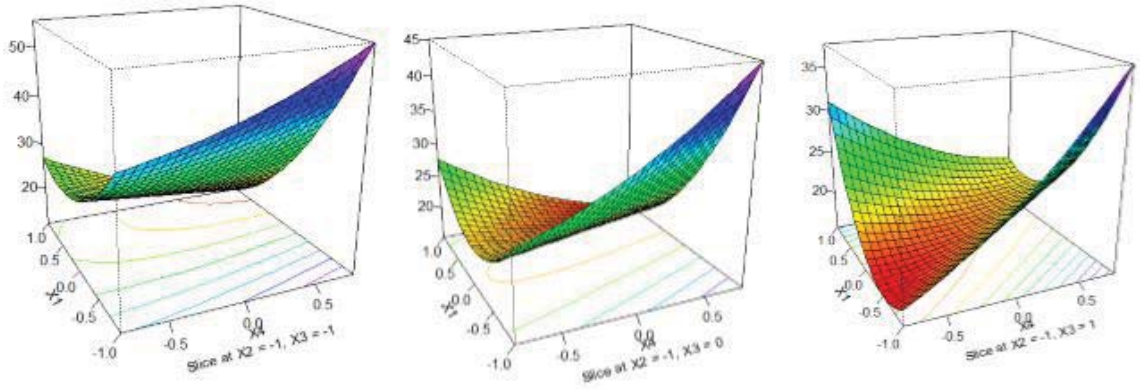

Figure 18. Effect of Egg White Level, Emulsifier Level and Water Level on Cooking Loss (at a Sorghum/Amaranth Ratio of 5/35)

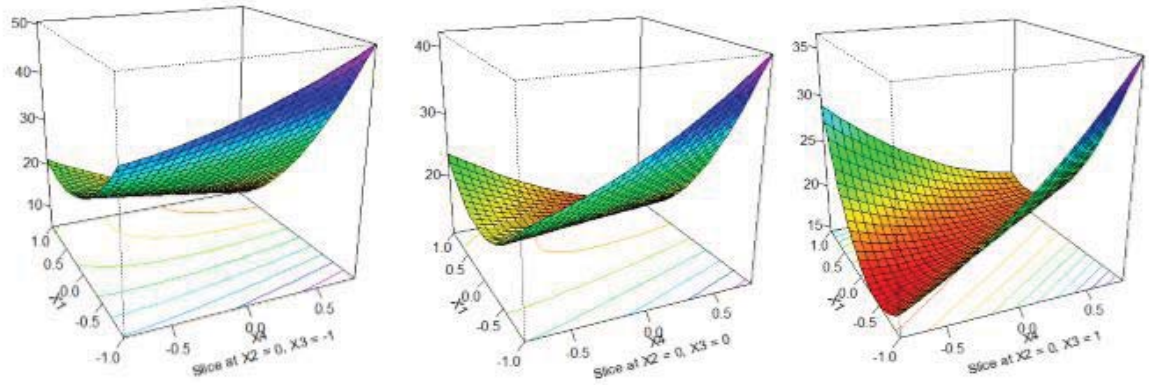

Figure 19. Effect of Egg White level, Emulsifier Level and Water Level on Cooking Loss (at a Sorghum/Amaranth Ratio of 35/5)

The cooking loss can also be reduced if the drying conditions are modified. Manthey et al. (2004) reported that high temperature drying strengthens the gluten matrix, which protects starch granules from rupturing during cooking. Indeed, ultrahigh temperature drying denatures proteins associated with the gluten matrix, which subsequently protects starch granules from rupturing during cooking. Furthermore, it reduces water permeability and causes small changes in the packing and arrangement of starch granules, contributing to decreased cooking loss and increased cooked firmness (Vansteelandt & Delcour, 1998).

3.3 Determination of the Optimal Formulation

The optimal formulation was determined using the best formulation of the major parameter such as cooking loss, firmness, color and density. As shown in Table 10, the egg white level for the optimal formulation was **12 %** to have an intermediate value between cooking loss and toughness. Sorghum/amaranth ratio selected was **30/10** to diversify the flour, the water level choose was **36 %** because beyond this level, the cooking losses were too high, and the best emulsifier level for the majority of analyses were **2.4 %**. After the optimal formulation was manufactured into pasta, it was then subjected to the same physical and quality analyses, conducted in triplicate, and the results are shown below in Table 11.

Table 10. Optimum Formulation of Each Parameter

	Egg white level	Sorghum/ amaranth ratio	Water level	Guar gum level
Cooking loss	24 %	No effect	33 % 36 %	2.4 %
Toughness	0 %	35/5	No effect	2.4 %
b*	12 %	5/35	39 %	2.4 %
a*	24 %	35/5	39 %	No effect
L*	24 %	35/5	33 %	1.2 %
Density	24 %	5/35	33 %	2.4 %

Table 11. Results of Analysis for the Optimal Pastas (+/- 1 SD)

Moisture content (%)		12.63 ± 0.23
Water activity		0.549 ± 0.003
Density (g/cm^3)		1.27 ± 0.05
Color	L*	60.40 ± 0.25
	a*	3.56 ± 0.12
	b*	15.58 ± 0.45
	ΔE	58.79 ± 0.48
Optimal cooking time (min)		9 ± 0
Cooking loss (%)		17.37 ± 0.55
Water absorption (%)		147.85 ± 2.48
Toughness (MPa/m$^{1/2}$)		0.056 ± 0.02
Young modulus		1321.8 ± 389.9

The value of density, water activity, color, water absorption and optimal cooking time of the optimal pasta were as good as expected. However, the cooking loss was acceptable but could be lower, and the texture was too breakable. The problem was that the cooking losses were the lowest when the egg white level was maximum (24% or more) while the firmness was maximal when the egg white level was null (0%). Thus there was a contradiction between the two major parameters. A compromise was chosen in the optimal formulation (12 % egg white), which explain the no optimal value of toughness and cooking loss could be achieved.

3.4 Sensory Analysis

For all consumer parameters (Figure 20), except odor, optimal pastas had lower scores than Barilla and Royal Quinoa Pasta. The two most important parameters have the lower scores: taste was marked 2.6/5 and the texture 2.5/5. The panelists were also asked to explain why they liked or disliked the pastas. Among the comments, it was often said that the texture was too granular and crumbly. The appearance was correct, the color similar to whole pasta, and the odor was pleasant. But the texture was the major reason for low acceptance of the pastas.

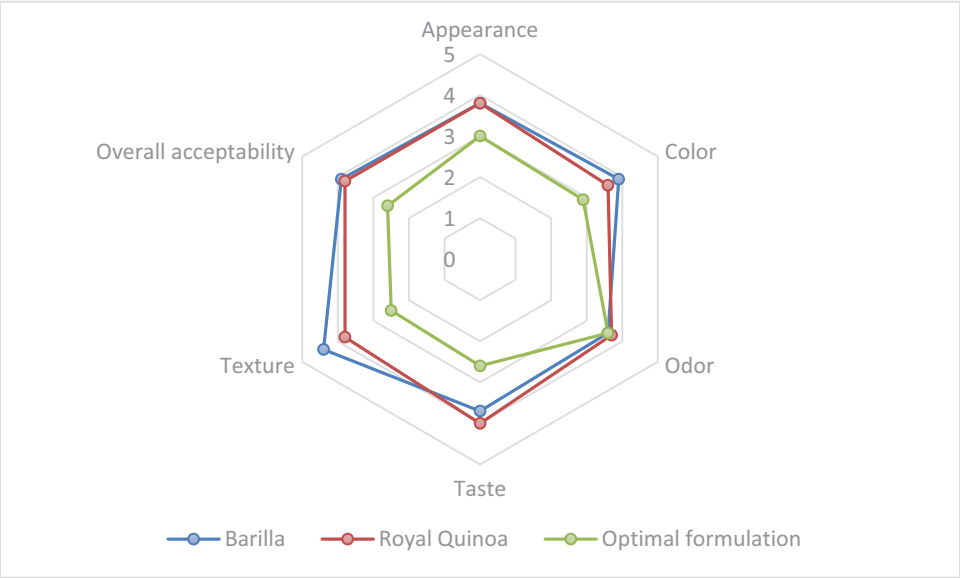

Figure 20. Sensory Analysis Results

In the second part, the panelists classified in order of preference the three different pastas, without sauce and with sauce (Tables 12 and 13).

Table 12. Results of the Ranking Test Without Sauce

	1	2	3	R
Barilla	0 %	40 %	60 %	26
Royal Quinoa	10 %	60 %	30 %	22
Optimal pasta	90 %	0 %	10 %	12

Table 13. Results of the Ranking Test with Sauce

	1	2	3	R
Barilla	0 %	30 %	70 %	27
Royal Quinoa	10 %	70 %	20 %	21
Optimal pasta	90 %	0 %	10 %	12

It appeared that 60 % of the panelist liked the Barilla pasta most, without sauce, and 70 % like the Barilla pasta most, with sauce. Only 10 % of the judges prefer the formulated pasta with and without sauce.

In order to know if the products were classified significantly differently or not, a Friedman test was applied. The Fr value was calculated as following:

$$Fr = \frac{12*(R\ barilla^2+R\ royal\ quinoa^2+R\ optimal\ pasta^2)}{n.k.(k+1)} - 3*n*(k+1) \quad (6)$$

With R the sum of the ranks, n le number of judge (10) and k the number of products tested (3)

$$Fr_{\text{test without sauce}} = 10.4 \text{ and } Fr_{\text{test with sauce}} = 11.4$$

According to the Friedman Table, with k =3 and n =10, **Fo = 9.6** with a risk of 1% that the difference was not significant.

For the two different tests, without and with sauce, Fr>Fo, so the hypothesis Ho (the products are not classified differently) was rejected and the three kinds of pastas were classified significantly differently. The consumers preferred Barilla industrial pasta.

4. Conclusions

In this study, response surface methodology, using Doehlert experimental design, was successfully used to identify the optimal formulation for egg pasta from maize, sorghum and amaranth flours. Varied trials were

carried out on the 21 experimental design samples. Results were compared to two commercial gluten free pastas which were used as reference.

Water activity, density, water absorption and density of main trials were shown to be comparable to the industrial gluten-free pasta. The values of the other studied parameters were significantly different from the references. The experimental design pastas were breakable, their optimal cooking time was lower, the color was different and the cooking losses were higher. Analysis of variance (ANOVA) showed which effect had the different factors (egg white, water, emulsifier and flours) on the different parameters (e.g., cooking loss, density, water activity).

The moisture contents for experimental pastas were higher than the reference pastas. None of the factors had an effect on it, according to analysis of variance, but impacted by the drying process. Previous studies showed that the drying process had major effects on pastas quality. A high temperature drying increases firmness and reduces cooking loss.

The optimal formulation for our trials was chosen according to the response surface plots, in order to develop good quality pastas, with low cooking loss, high firmness, similar color as the references, and high density. Optimal conditions were **12 %** egg white, **30%/10%** sorghum/amaranth ratio, **36 %** water, and **2.4%** emulsifier. However, this led to low firmness and cooking loss. A way to increase the pastas firmness, other than formulation changes, could be to use a different extrusion process.

Finally, sensory aspects of the final product were analyzed by a consumer panel to determine if the product could be appreciated by consumers or not. The overall acceptability was scored as 2.6/5 for our formulated pastas. Our products presented good nutritional values, color was appreciated, but texture was too granular and crumbly, which did the consumers not like. Barilla pasta, made with rice and maize flour, was the most appreciated by the panelists according to the ranking test. The Friedman test showed that these pastas were classified significantly differently.

Indeed, while we were able to produce viable pastas from our formulations, further work is needed before they can be implemented commercially. Texture and flavor must be improved. These should be targets for the next stages of research.

References

AACC International (2000). Method 66-50 Pasta and Noodle Cooking Quality - Firmness Method. In: *Approved Methods of Analysis*, 9th Ed. AACC International: St. Paul, MN, USA.

AACC International (2000). Method 44-19 Moisture-Air-Oven Methods. In: *Approved Methods of Analysis,* 9th Ed. AACC International: St. Paul, MN, USA.

AACC International (2000). Method 14-22 Color of Pasta-Reflectance Colorimeter Method. In: *Approved Methods of Analysis*, 9th Ed. AACC International: St. Paul, MN, USA.

Adekunle Ayo, J. (2007). The effect of amaranth grain flour on the quality of bread. *International Journal of Food Properties, 4*(2), 341-351.

Antognelli, C. (1980). The manufacture and applications of pasta as a food and as a food ingredient. *J Food Technology, 15*(2), 125-145.

Broz, R., & Horne, T. (2007). Gluten Free Product Development. *CFW Column Product Development*, 52(3).

Ciacci, C., Maiuri, L., Caporaso, N., Bucci, C., Del Giudice, L., Rita Massardo, D., Pontieri, P., Di Fonzo, N., Bean, R., Iorger, B., & Londei, M. (2007). Celiac disease: *In vitro* and *in vivo* safety and palatability of wheat-free sorghum food products. *Clinical Nutrition, 26*(6), 799-805.

Cunin, C., Handschin, S., Walther, P., & Escher, F. (1995). Structural changes in starch during cooking of durum wheat pasta, *Lebensm.-Wiss. Technol., 28*, 323-329.

Dexter, J. E., Matsuo, R. R., & Morgan, B. C. (1983). Spaghetti Stickiness: Some Factors Influencing Stickiness and Relationship to Other Cooking Quality Characteristics. *Journal of Food Science, 48*(5), 1545-1551.

FAO. (1992). Le maïs dans la nutrition humaine, Produit par le département de l'agriculture, France. Retrieved from http://www.fao.org/docrep/t0395f/T0395F03.htm.

FAO. (1995). Le sorgho et les mils dans la nutrition humaine, Produit par le département de l'agriculture, France. Retrieved from http://www.fao.org/docrep/t0818f/T0818F08.htm.

Fardet, A., Hoebler, C., Baldwin, P. M., Bouchet, B., Gallant, D. J., & Barry, J. L. (1998). Involvement of the protein network in the in vitro degradation of starch from spaghetti and lasagna: a microscopic and enzymic

study. J*ournal of Cereal Science, 27*, 133-145.

Feillet, P., & Dexter, J. E. (1996). Quality requirements of durum wheat for semolina milling and pasta production, *Monograph on Pasta and Noodle Technology*, Saint Paul, MN; USA: AACC - American Association of Cereal Chemists, 95-131. Retrieved from http://prodinra.inra.fr/record/137270.

Fuad, T., & Prabhasankar, P. (2010). Role of ingredients in pasta product quality: a review on recent developments. *Critical Reviews in Food Science and Nutrition, 50*, 787-798.

Inglett, G. E., Chen, D., & Liu, S. X. (2015) Antioxidant activities of selective gluten free ancient grains. *Food and Nutrition Sciences, 6*, 612-621.

Guan, F., & Seib, P. A. (1994). Instrumental probe and method to measure stickiness of cooked spaghetti and noodles. *Cereal Chemistry, 71*, 330-335.

Guinea, G. V., Rojo, F. J., & Elices, M. (2004). Brittle failure of dry spaghetti, *Engineering Failure Analysis, 11*(5), 705-714.

Grzybowski, R. A., & Donnelly, B. J. (1979). Cooking properties of spaghetti: factors affecting cooking quality. *Journal of Agricultural and Food Chemistry, 27*(2), 380-384.

Lebaud, M. P. (2018). Méthode des trapèzes (p.2-3), Intégration mathématique. Université de Rennes. Retrieved from https://perso.univ-rennes1.fr/marie-pierre.lebaud/agint/ecrit/analyse-reelle/integration-numerique/X-int-num.pdf

Lucisano, M., Cappa, C., Fongaro, L., & Mariotti, M. (2012). Characterisation of gluten-free pasta through conventional and innovative methods: Evaluation of the cooking behaviour. *Journal of Cereal Science, 56*(3), 667-675.

Manthey, F. A., Yalla, S. R., Dick, T. J., & Badaruddin, M. (2004). Extrusion properties and cooking quality of spaghetti containing buckwheat bran flour. *Cereal Chemistry, 81*(2), 232-236.

Matsuo, R. R., Bradley, J. W., & Irvine, G. N. (1972). Effect of protein on the cooking quality of spaghetti. *Cereal Chemistry, 49*, 707-711.

Moskowitz, H. R., & Drake, B. (1972). Psychophysical measurement of texture. *Journal of Texture Studies, 3*, 135-145.

Nazarov, N. (1977). Technology and equipment for the food industry. *Food Industry Press*, 214.

Novaro, P., D'Egidio, M. G., Mariani, B. M., & Nardi, S. (1993). Combined effect of protein content and high-temperature drying systems on pasta cooking quality. *Cereal Chemistry, 70*(6), 716-719.

Pagani, M. A., Lucisano, M., & Mariotti, M. (2007). Traditional Italian products from wheat and other starchy flours. *Handbook of Food Products Manufacturing*, Chap. 17.

Palmade, L. (2015). Water in food, Engineering School course notes. University of Montpellier, France.

Raina, C. S., Singh, S., Bawa, A. S., & Saxena, D. C. (2005). Textural characteristics of pasta made from rice flour supplemented with proteins and hydrocolloids. *Journal of Texture Studies, 36*(4), 402-420.

Rayas-Duarte, P., Mock, C. M., & Satterleei, L. D. (1996). Quality of spaghetti containing buckwheat, amaranth, and lupin flours. *Cereal Chemistry, 73*(3), 381-387.

Schoenlechner, R., Drausinger, J., Ottenschlaeger, V., Jurackova, K., & Berghofer, E. (2010). Functional properties of gluten-free pasta produced from amaranth, quinoa and buckwheat. *Plant Foods Human Nutrition, 65*(4), 339-349.

Smewing, J. (1997). Analysing the texture of pasta for quality control. *Cereal Food World, 42,* 8-13.

Szczesniak, A. S. (1987). Correlating sensory with instrumental texture measurement - An overview of the recent development. *Journal of Texture Studies, 18*, 1-15.

The engineering toolbox, modulus of elasticity or Young's Modulus - and tensile modulus for common materials. Retrieved from http://www.engineeringtoolbox.com/young-modulus-d_417.html

Vansteelandt, J., & Delcour, J. A. (1998). Physical behavior of durum wheat starch during industrial pasta processing. *Journal of Agricultural and Food Chemistry, 46*, 2499-2503.

Verdu, E. F., Galipeau, H. J., & Jabri, B. (2015). Novel players in Coeliac disease pathogenesis: role of the gut microbiota. *Nature Reviews Gastroenterology & Hepatology, 12*, 497-506.

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Purification and Partial Characterization of Melanoidins Fractions from Toasted Oak Heartwood, Comparison with Melanoidins from Roasted Coffee

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Abstract

During the cooking, processing, and storage of food products, a whole range of browning reactions occurs, initiated by the reaction of a carbohydrate with a compound possessing a free amino group. Melanoidins formed, influence food quality, mainly their colour, their flavour, and their antioxidant activities. Melanoidins are complex Maillard reaction products. We developed a method to isolate coffee melanoidins and melanoidins from toasted oak wood. We noted that coffee is richer in melanoidin compounds than oak wood. We presented a partial characterization of melanoidins fractions from toasted oak heartwood, and a comparison with melanoidins from roasted coffee. Mass spectra of the fractions isolated from toasted oak wood indicate the presence of pentose and hexose-based oligosaccharides with different degrees of polymerisation. The presence of the oligosaccharide moieties, as well as their degradation products found in the oak wood melanoidins, supports the postulated carbohydrate-based origin of melanoidins.

Keywords: oak wood, melanoidins, LC/MS, NMR, IR

1. Introduction

During the cooking, processing, and storage of food products, a whole range of browning reactions occurs, initiated by the reaction of a carbohydrate with a compound possessing a free amino group. Melanoidins occur in many stored and processed foods. They influence food quality, mainly owing to their colour, their flavour, their antioxidant activities (Manzocco et al., 2001; Morales et al., 2005; Lindenmeier et al., 2002; Charles-Bernard et al., 2005; Ames, 1998; Delgado-Andrade & Morales, 2005; Delgado-Andrade et al., 2005; Wang et al., 2011; Borrelli et al., 2002; Andriot et al., 2004). Melanoidins are present in several food products, such as coffee, roasted malt, breakfast cereals, bread…

Melanoidins are complex Maillard reaction products. Melanoidins are formed from cyclizations, dehydrations, retroaldolizations, rearrangements from nitrogen compounds and sugars, isomerizations, and condensations of low molecular weight Maillard reaction products. Due to the high reactivity of the intermediates, a complex polymerization takes place, resulting in brown-colored high molecular weight melanoidins. The composition of melanoidin chemical structures is relatively unknown, however, due to the complexity of the products that are generated in the reaction (Bekedam et al., 2008; Kim & Lee, 2009). Based on model systems, different suggestions have been made for melanoidins structures (Tressl et al., 1998; Hofmann, 1988; Cämmerer et al., 2002; Nunes & Coimbra, 2007). However, their complexity and structures depend on the nature and number of possible reactants and the reaction conditions. Therefore the Maillard reaction in foods and the structures of the resulting melanoidins are presumed to be much more complex than in model systems, and different melanoidin structures may coexist (Adams et al., 2005). Different hypotheses have been formulated on the structural backbone of melanoidins. A first hypothesis states that a melanoidin skeleton is constituted mainly from sugar degradation products, polymerized through aldol-type condensations and probably branched via amino

compounds (Kato & Tsuchida, 1981; Yaylayan & Kaminsky; 1998; Cämmerer & Kroh; 1995). Tressl et al. proposed a complex macromolecular structure consisting of repeating units of furans and pyrroles, linked by polycondensation reactions. Hofmann et al. identified low-molecular weight chromophores and postulated the generation of melanoidin-type colorants by a cross-linking reaction between these low molecular weight substances and noncolored high molecular weight biopolymers, such as proteins.

One of the strategies in melanoidin characterization is the chemical or thermal degradation of melanoidins,[18-19] followed by the identification of the decomposition products formed, giving information on structural domains of the melanoidin network. In addition, thermal destruction of melanoidins leads to the formation of volatiles that contribute to the development of aroma in roasted food systems (Kuntcheva et al., 1998; Adams et al., 2005; Adams et al., 2003).

In coffee matrix the amount of melanoidins is often represented 25% of the dry matter. During toasting of oak wood used in making barrels, melanoidins are formed. The aim of this study was to develop a method to isolate coffee melanoidins and melanoidins from toasted oak wood. We compared melanoidins from coffee and from toasted oak wood by different analytical techniques: absorption spectra, IR, viscosity, headspace/GC/MS, elemental analysis, LC/ESI/MS, NMR.

2. Material and Methods

2.1 Materials

Different toasted oak wood were tested: the first one was a no toasted oak wood, and the others, oak wood with different degree of toasting.

2.2 Isolation of Coffee Melanoidins

According to the technique described by Hofmann et al., coffee (10 g) was extracted with hot pure water (80-90°C) until no coloured material could be extracted. The aqueous phase was extracted twice with dichloromethane (200 ml) to eliminate lipids and then freeze-dried. The obtained fraction was the Total Fraction Melanoidins coffee (TFMC).

We used HPLC/DAD technique in order to separate purified compounds. The compounds were collected and the different purified fractions were dried and frozen.

2.3 Isolation of Oak Wood Melanoidins

According to Hofmann et al. (2001) in the case of isolation of coffee melanoidins, oak wood (10 g) was extracted with hot pure water (80-90°C) until no coloured material could be extracted. The aqueous phase was extracted twice with dichloromethane (200 ml) to eliminate lipids and then freeze-dried. The obtained fraction was the Total Fraction Melanoidins Wood (TFMW).

We used different analytical techniques in order to separate purified compounds. First technique consisted to optimize the HPLC/DAD conditions. The compounds were collected and the different purified fractions were dried and frozen. The second one consisted to optimize low pressure gel chromatography: choice of gel, solvents, flow in order to separe and collected purify fractions; we used UV at 280 nm for detection. Fractions were obtained in quantity more important than in the case of liquid chromatography.

2.4 HPLC/DAD Analysis

HPLC/UV-Visible analyses were performed with a Waters separation module system, a Waters UV-Visible detector, and Millenium32 chromatography manager software. UV-visible spectra were recorded at 280 nm and 260 nm. The column was a reverse-phase Sunfire C18 (5 µm packing, 250 x 5 mm i.d.) protected with a guard column of the same material; solvent A, water/formic acid (98:2, v/v); solvent B, acetonitrile/water/formic acid (80:18:2, v/v). The column was placed at ambient temperature (T=21°C). The elution program was performed at a constant flow of 1 ml/min, passing from 5 to 30% of B in 40 min., and then rising to 40% of B in 10 min., and finally to 100% of B in 5 min., followed by washing and re-equilibrating the column during 15 min. The injection volume was 20 µl.

2.5 Semi-preparative HPLC

The fractions were collected and purified by semi preparative HPLC. Analyses were performed with a Waters separation module system, a Waters UV-Visible detector, and Millenium^{32} chromatography manager software. UV-visible spectra were recorded at 280 nm and 260 nm. The column was a reverse-phase Sunfire Prep C18 (5 µm packing, 250 x 10 mm i.d.) protected with a guard column of the same material; solvent A, water/formic acid (98:2, v/v); solvent B, acetonitrile/water/formic acid (80:18:2, v/v). The column was placed at ambient

temperature (T=21°C). The elution program was performed at a constant flow of 3 ml/min, passing from 5 to 30% of B in 40 min., and then rising to 40% of B in 10 min., and finally to 100% of B in 5 min., followed by washing and re-equilibrating the column during 15 min. The injection volume was 200l.

2.6 Low Pressure Gel Chromatography

Separation was performer using low pressure TSK HW-40(S) gel chromatography with 100% H_2O and then 100% MeOH as eluents. Flow rate: 0,8 mL/min; column : 27±2 cm length and 2,5 cm diameter.

2.7 LC/ESI/MS Analysis

LC/ESI/MS, LC/ESI/MS/MS and ESI/HR/MS were performed on a Q-Star™ instrument using an electrospray ionization source in negative-ion mode. Ion spray voltage was selected at 4.5 KV, the capillary temperature was selected at 275°C, and the source temperature was selected at 400°C. The values of sheath gas flow rate were: nebulisation gas=2.85 l/min, turbo gas=4.8 l/min, curtain gas=1.48 l/min. For MS/MS measurements, the energy collision and the gas collision were respectively –30 eV and 5. Isolation window was 1 uma. For HR/MS analysis, we used an internal calibration using PPG. The resolution obtained was 12 000.

The column was a reverse-phase Interchim C18 (10 μm packing, 250 x 4.7 mm i.d.) protected with a guard column of the same material; solvent A, water/acetic acid (98:2, v/v); solvent B, acetonitrile/water/acetic acid (80:18:2, v/v). The column was placed at ambient temperature (T=21°C). The elution program was performed at a constant flow of 1 ml/min using the LC inlet and splitter outlet connection permitting to reduce the flow at 0.5 ml/min, passing from 5 to 30% of B in 40 min., and then rising to 40% of B in 10 min., and finally to 100% of B in 5 min., followed by washing and re-equilibrating the column during 15 min. The injection volume was 20 μl.

2.8 Absorption Spectra

Spectrophotometric measurements were performed using an Anthelie Secomam™ spectrophotometer and UV-Visible spectra were recorded with a spectrophotometer fitted with a quartz cell (1 cm). The wine-like samples were diluted (1/20) and then their spectra measurements were taken.

2.9 IR

The Spectrum was recorded with a Nicolet iS50 FT-IR Fourier Transform Infrared Spectrometer (Thermo Scientific, Madison, WI) equipped with a DTGS/KBr detector, KBr beamsplitter and an iS50-ATR diamond accessory. Each spectrum is obtained from the accumulation of 500 scans using an aperture of 150 at 45° of incidence from 4000 to 400 cm-1 with a 4 cm-1 resolution, and an Happ-Genzel apodisation. Then spectrum was treated for ATR correction, thereafter a small offset was applied for baseline. Attributions were done taking in account the formula $C_{24}H_{49}O_{23}N$, references, tables and softwares (Omnic, KnowItAll).

2.10 Headspace/GC/MS

The total fraction of melanoidins (TFMC and TFMW) obtained from toasted oak wood and coffee were heated at 250°C (10 min), and the produced volatiles were analyzed by SPME-GC-MS. For the analysis of the SPME extracts a Thermo coupled with, and a HP5-MS column (30m x 0.25 mm i.d., 0.25 μm) was used. Working conditions were as follows: injector, 250°C; transfer line, 250°C; oven temperature, start 40°C and hold 2 min, programmed from 40 to 120°C at 4°C/min and from 120 to 240°C at 30°C/min, hold 2 min; carrier gas (He), 1.2 ml/min; splitless; ionization EI, 70 eV; acquisition parameters, scanned m/z 40-600. Compounds were identified by comparison of their mass spectra and retention times with those of reference compounds and by comparison with the NIST Mass Spectral Library. When only MS data were available, identities were considered to be tentative.

2.11 NMR Experiments

NMR experiments were performed at 298 K using a Bruker Avance 800 MHz spectrometer equipped with a 5 mm TCI (^1H / ^{13}C / ^{15}N/ ^2H) cryoprobe with Z-gradients and ATM accessory. All ^1H NMR and ^{13}C chemical shifts are given with respect to tretramethylsilane in D_2O as an external reference.

^1H NMR spectra was acquired with water suppression using excitation sculpting (pulsed field gradients applied for 3 ms). 1D ^1H spectra was acquired with 8 transients were typically collected at frequency of 800.23 MHz with a flip angle of 90° (7.5 μs), a spectral width of 10000 Hz, acquisition time of 1.64 s and 32 K data points. Data were processed with multiplication prior to Fourier transformation by an exponential function (line broadening was 0.3 Hz) and with zero filling.

^{13}C NMR spectra. 1D ^{13}C spectra was acquired with power gated decoupling using 30° flip angle, spectral widths of 48 kHz consisting of 64 K data points, acquisition time of 0.68 s. Data were processed with multiplication

prior to Fourier transformation by an exponential function (line broadening was 2 Hz) and with zero filling.

2.12 Elemental Analysis

The analyzes were performed on an elemental analyzer Thermo Finnigan Flash EA 2000 equipped with an autosampler of 32 samples and a chromatographic column. The system is managed by the Eager 300 software Metered elements C, H, N, S and O. The results are provided with an absolute accuracy of ± 0.2% and are valid for a minimum of two tests.

The combustion of the sample takes place at high temperature (940°C) in the presence of tungstic anhydride (WO 3) in a stream of oxygen for a very short (15s). This decomposition gives CO_2, H_2O, SO_2, NO_2, nitrogen oxides are reduced to N2 (nitrogen) with copper. The entire system is swept by a stream of helium.

For oxygen, pyrolysis of samples were performed under a stream of helium and gas chromatography with a gas-solid stationary phase compound formed CO. Because of the filling system of the reaction tube, the dosage of oxygen can be achieved with the samples containing fluorine.

3. Results

3.1 Isolation and Purification of Melanoidins

Isolation of melanoidin fraction in the case of coffee: yield = 2, 5 g from 10 g of powder (25% of dry matter) and in the case of toasted oak wood : yield = 0,5 g from 10 g of powder (5% of dry matter).

We noted first the difference of yield in the two cases. Coffee matrix was richer in melanoidins than toasted oak wood, it is the most abundant substrate, the percentage of melanoidins was more important because coffee is more roasted than toasted oak wood.

We developed an analytical HPLC method in order to analyze the total fraction composition from coffee and toasted oak wood. HPLC/DAD technique (Figures 1 and 2) permits us to well separate several peaks which present the same spectrum UV-Visible with a maximum of absorbance at 260 nm, specific of melanoidin spectrum. Each peak was collected by semi-preparative HPLC. In the case of roasted coffee we obtained seven fractions: G1, G2, G3, G4, G5, G6, G7, see Figure 1. At the end of the HPLC chromatogram, the peak of polymers and apolar substances is low. In the case of toasted oak wood, we obtained four fractions: F1, F2, F3, F4, see Figure 2. The peak of polymers and apolar substances is more important. HPLC chromatograms show the excellent purity of each product collected nearly 100% (Figures 1 and 2, Table 1).

Table 1. Proportions of each purified fraction from coffee and toasted oak wood melanoidin total fractions

Coffee melanoidin fraction		***Toasted oak wood melanoidin fraction***	
Fractions	**Weight (mg)**	**Fractions**	**Weight (mg)**
Fractions obtained by semi-preparative HPLC		**Fractions obtained by semi-preparative HPLC**	
G1	2	F1	1.2
G2	1.8	F2	1.9
G3	2.2	F3	2.3
G4	1.9	F4	1.4
G5	0.6	**Fractions obtained by low pressure chromatography**	
G6	0.4	P1	6
G7	0.5	P2	7.5
		P3	5
		P4	13
		P5	15
		P6	11
		P7	10

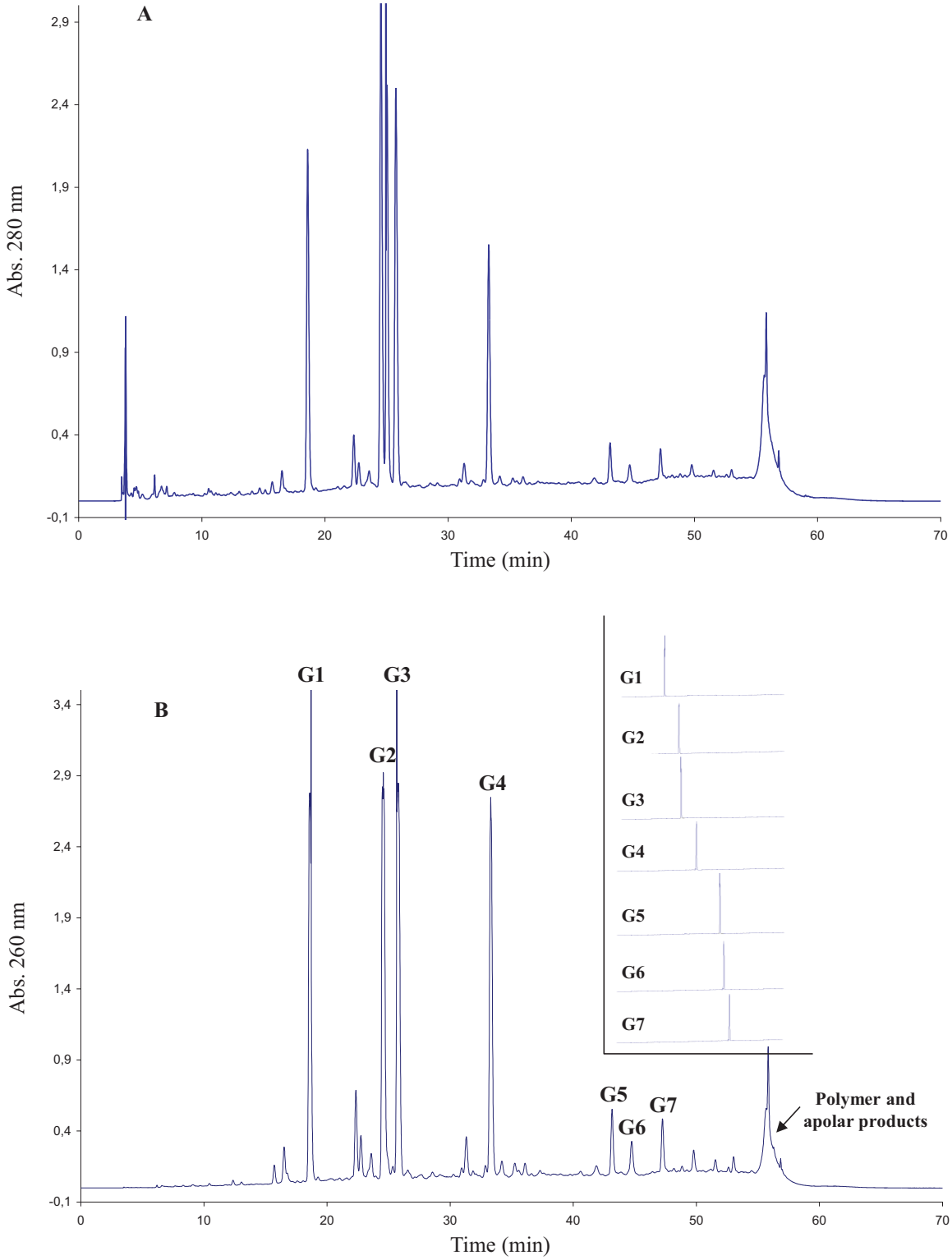

A
Abs. 280 nm
2,9
2,4
1,9
1,4
0,9
0,4
-0,1
0
10
20
30
40
50
60
70
Time (min)
B
Abs. 260 nm
3,4
2,9
2,4
1,9
1,4
0,9
0,4
-0,1
G1
G2
G3
G4
G5
G6
G7
Polymer and
apolar products
Time (min)

Figure 1.

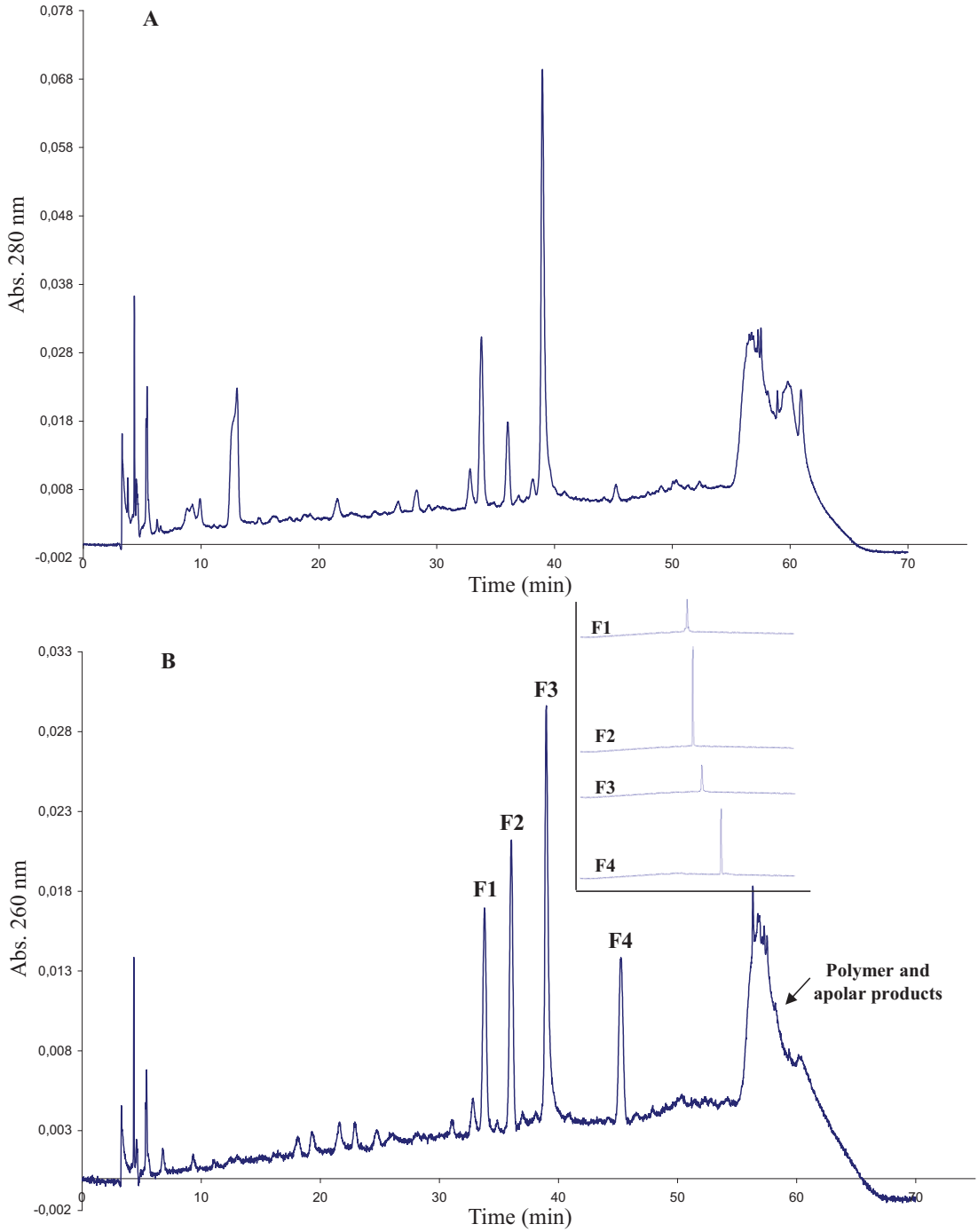


Figure 2.

In the case of toasted oak wood, we developed a second method using low pressure gel chromatography to obtain higher quantitatively product and to separate the polymer form. The different fractions, obtained and detected by UV detector at 280 nm, were collected manually: P1, P2, P3, P4, P5, P6, P7 were the obtained fractions (Figure 3, Table 1). HPLC chromatograms show the excellent purity of each product collected nearly 100%. We retained the second method in order to obtain pure fractions from TFMW, we separated thanks to this technique the polymer form no fractionated by HPLC column.

The fractions P were analyzed by different analytical techniques: LC/ESI-MS, IR and NMR.

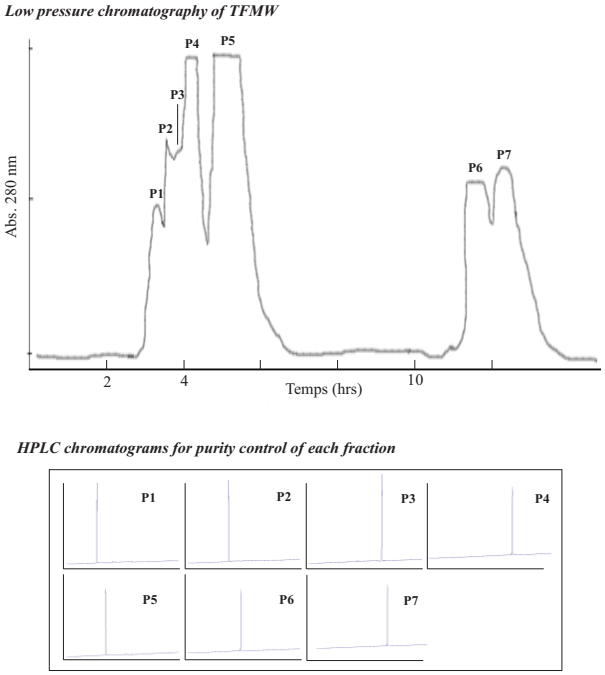


Figure 3.

3.2 Thermal Degradation Studies

The total fraction of melanodins (TFMC and TFMW) obtained respectively from coffee and toasted oak wood were heated at 250°C (10 min) and the produced volatiles were analyzed by SPME-GC-MS (Figures 4 and 5). To this purpose, firstly we subjected the isolated melanoidins from oak wood to thermal destruction at 250°C in an inert atmosphere. The especially selected high temperature was to ensure a sufficient degree of conversion of the melanoidin molecules and to provide us with better information relating to their ability to generate volatile products.

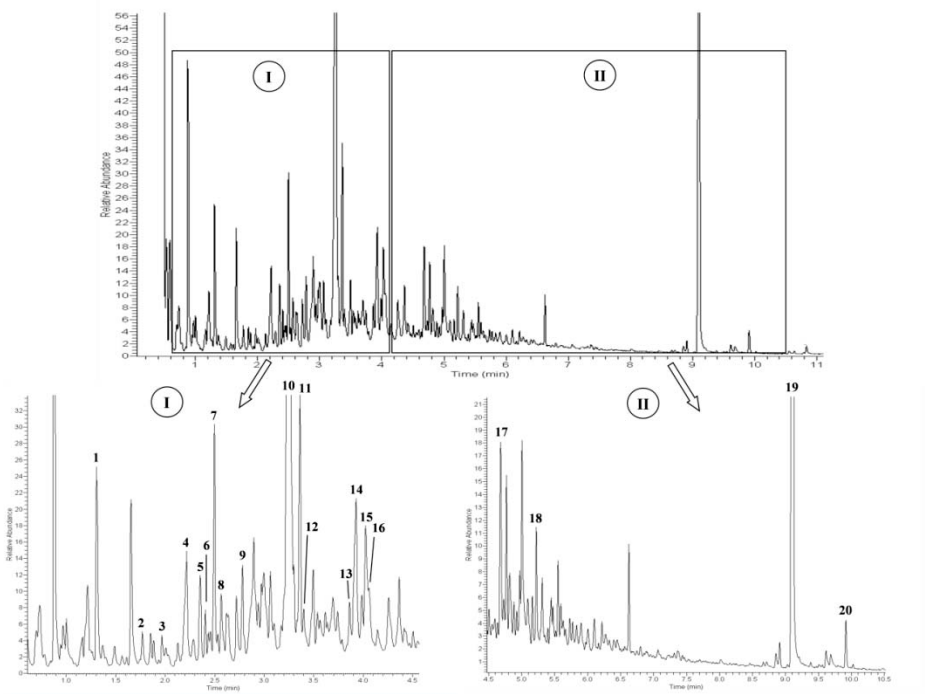


Figure 4.

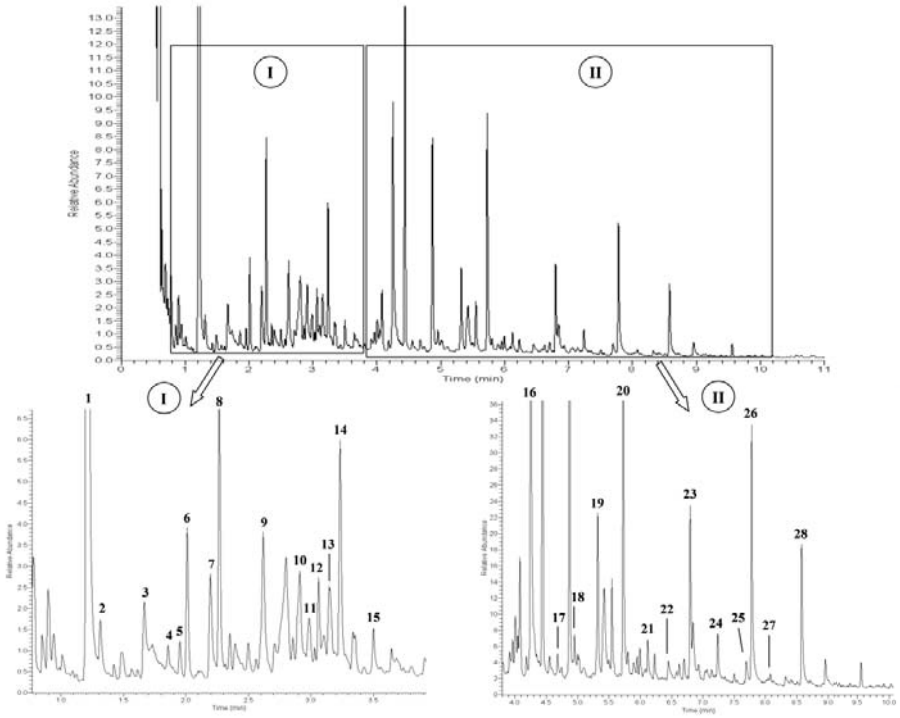


Figure 5.

In the case of coffee, the data are given in Table 2; as can be seen the mixture contains 20 components. The furan group is represented in eight compounds, the total amount being 60%. The highest content is that of 2(3H)-furanone, 5-acetyldihydro-, and 5-hydroxymethyldihydrofuran-2-one. Alicyclic derivatives were found (12%) as well as maltol, see Table 2.

Table 2. Data for the thermal degradation products of melanoidins from coffee

N°	Tr (min)	Molecular peak	Compound
1	1.30	98	2-Furanmethanol
2	1.77	106	Pyrazine, ethenyl-
3	1.85	107	Pyridine, 3-ethyl
4	2.22	102	3-Hydroxydihydro-2(3H)-furanone
5	2.35	95	1H-Pyrrole-2-carboxaldehyde
6	2.41	120	Pyrazine, 2-ethenyl-6-methyl
7	2.50	112	2-Cyclopenten-1-one, 2-hydroxy-3-methyl
8	2.57	130	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl,
9	2.78	109	Ethanone, 1-(1H-pyrrol-2-yl)-
10	3.23	126	Maltol
11	3.36	128	2(3H)-Furanone, 5-acetyldihydro-
12	3.40	109	3-Pyridinol, 2-methyl
13	3.87	153	Imidazole, 2-amino-5-[(2-carboxy)vinyl]
14	3.93	116	5-Hydroxymethyldihydrofuran-2-one
15	4.02	110	Pyrocatechol
16	4.06	133	Pyridine,2-methyl-5-(1-methylethenyl)-
17	4.68	142	2(3H)-Furanone, 3-acetyldihydro-3-methyl
18	5.22	156	2(3H)-Furanone, dihydro-5-pentyl
19	5.32	194	Caffeine
20	9.91	210	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)

The headspace profil of the oak wood melanoidins (Figure 5) consisted mainly of furans (74% of the total GC peak area), a small amount of pyrroles and pyrazines, see Table 3. Furfural, maltol, and isomaltol, which are important compounds in the headspace of heated oak wood melanoidins, are typical caramelization products of sugars.

Table 3. Data for the thermal degradation products of melanoidins from toasted oak wood

N°	Tr (min)	Molecular peak	Compound
1	1.22	96	Furfural
2	1.31	98	2-Furanmethanol
3	1.67	84	Cyclopentanone
4	1.86	112	2,5-Furandione, 3-methyl
5	1.95	139	1H-Imidazole-4-ethanamine, N, 5-dimethyl-
6	2.01	110	5-Methyl furfural
7	2.20	102	3-Hydroxydihydro-2(3H)-furanone
8	2.27	143	Oxazolidine, 2,2-diethyl-3-methyl
9	2.62	153	Imidazole, 2-amino-5-[(2-carboxy) vinyl]
10	2.91	112	2-Furancarboxylic acid
11	2.99	111	2-Furanmethanamine, N-methyl
12	3.06	169	Pyrrolidizine-3-one, ethyl ether
13	3.15	271	(+)-S-Phenethanamine, 1-methyl-N-vanillyl
14	3.23	126	Maltol
15	3.50	144	4H-Pyran-4_one, 2,3-dihydro-3,5-dihydroxy-6-methyl
16	4.25	126	5-(Hydroxymethyl) furfural
17	4.68	152	2,6-Dihydroxyacetophenone
18	4.95	168	2-Furancarboxaldehyde, 5-[(acetyloxy)methyl]
19	5.32	154	Phenol 2,6-dimethoxy
20	5.73	152	Vanillin
21	6.12	223	2,5-Dimethoxy-4-ethylamphetamine
22	6.23	166	Phenol, 2-methoxy-4-propyl
23	6.80	180	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)
24	7.24	141	Pyrrolidine, 2-butyl-1-methyl
25	7.69	156	N-Methyl-3-hyrdoxymethylpyrrolidin-2-one
26	7.78	182	Benzaldehyde, 4-hydroxy-3,5-dimethoxy
27	8.08	194	Phenol, 2,6-dimethoxy-4-(2-propenyl)-
28	8.58	210	2-Pentanone, 1-(2,4,6-trihydroxyphenyl)

In Figures 6 and 7, some representative structures of compounds identified in the headspace of heated melanoidins respectively from TFMC and TFMW are depicted.

From the results presented it can be seen that during manufacturing process destruction of melanoidins is likely to take place, producing volatile components which take part in the formation of the flavour complex in the finished product.

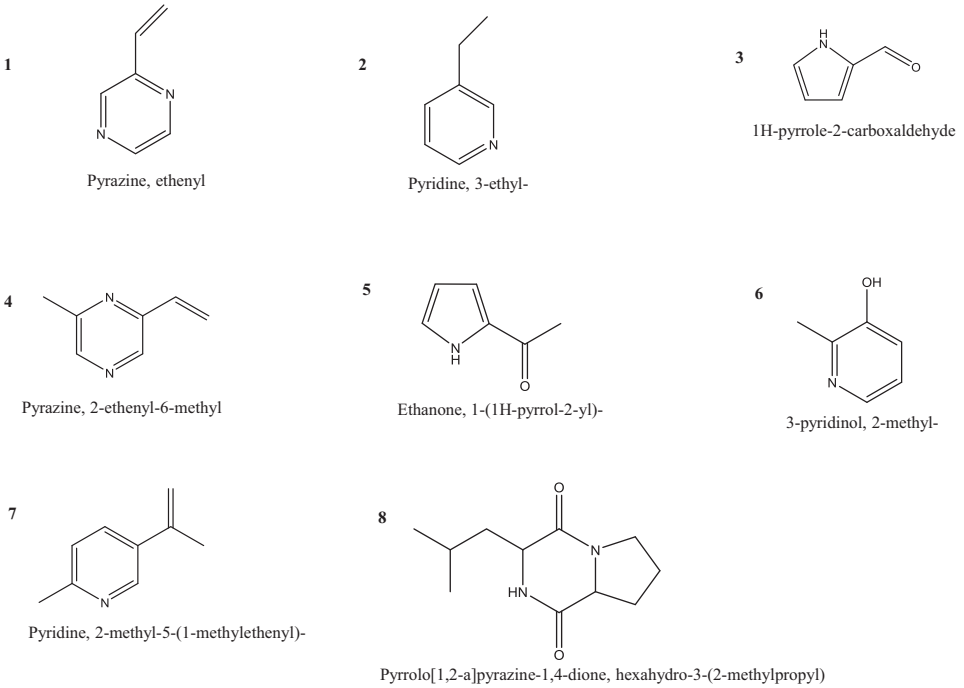


Figure 6.

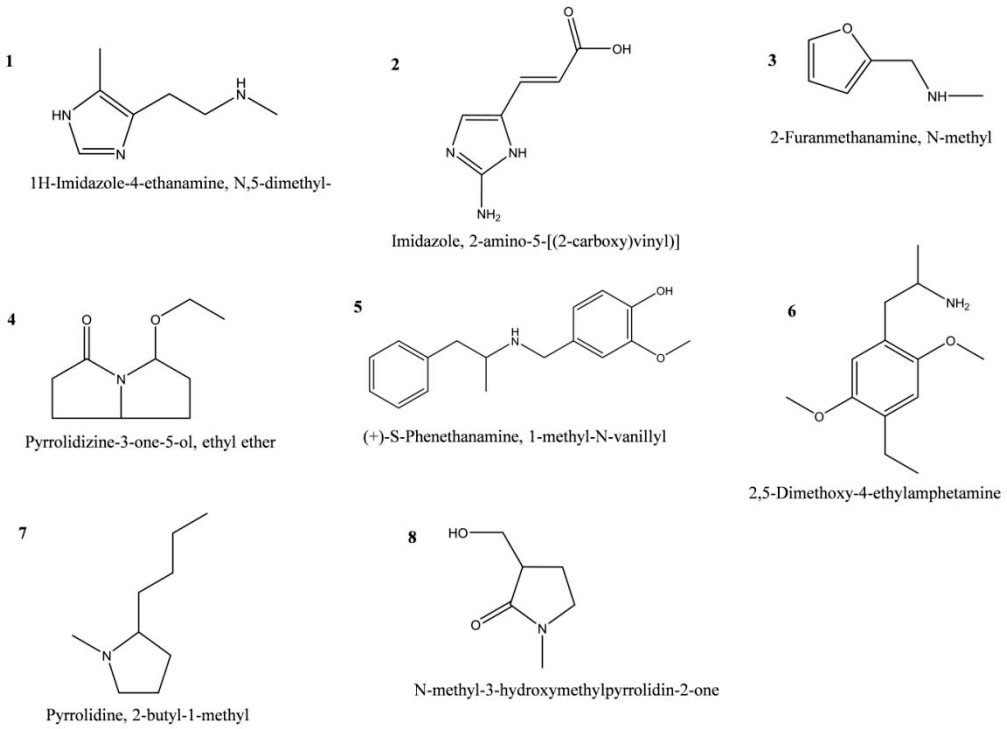


Figure 7.

3.3 Characteristics of the Melanoidins Isolated from TFM

3.3.1 Elemental Analysis (C, H, O, N) and Viscosimetry

The chemical investigations in our study revealed that coffee and toasted oak wood melanodins have different elemental (CHON) compositions and similar viscosity values, see Table 4. Coffee melanoidin fraction (TFMC) contain 2,65% of nitrogen, 42,15% of carbon, 5,10% hydrogen, and 50,10% of oxygen. Toasted oak wood melanoidin fraction (TFMW) contain 1,95% of nitrogen, 39,95% of carbon, 6,86% hydrogen, and 50,96% of oxygen, and the formula for their ratio is $C_{24}H_{49}O_{23}N$. These data are similar to those obtained for the quantitative element composition of known model and food melanodins (Cämmerer & Kroh, 1995; Margarita et al., 1996). The percentage of nitrogen is more important in the case of coffee fraction; it was easily explain by the fact that coffee contained a large majority of melanoidins.

Table 4. Elemental compositions of coffee melanodin fraction and toasted oak wood melanoidin fraction

Coffee melanoidin fraction (TFMC)				
Component Name	**Average**	**Std. Deviation**	**% Relative S.D.**	**Variance**
Nitrogen	2.65	0.1089904	4.1385	0.0119
Carbon	42.15	0.9364315	2.2227	0.8769
Hydrogen	5.1	0.03153798	0.6288	0.001
Oxygen	50.1	0.9804534	1.336	0.9134
Toasted oak wood melanoidin fraction (TFMW)				
Component Name	**Average**	**Std. Deviation**	**% Relative S.D.**	**Variance**
Nitrogen	1.95	0.0802004	3.04531	0.0087
Carbon	39.95	0.8875548	2.1067	0.8311
Hydrogen	6.86	0.04242167	0.8457	0.001
Oxygen	50.96	0.9867877	1.465	0.9078

Viscosity of each fraction is around 1,466 mPa.s in the case of oak wood and 1,545 mPa.s in the case of coffee. The viscosity in each case varied according to the temperature (Table 5). More temperature increased and more viscosity decreased and inversely.

Table 5. Viscosty measurements from coffee and toasted oak wood fractions

Coffee melanoidin fraction (TFMC)			
Fractions	**Viscosity at 15°C (mPa.s)**	**Viscosity at 20°C (mPa.s)**	**Viscosity at 25°C (mPa.s)**
G1	1.631	1.545	1.52
G2	1.632	1.547	1.521
G3	1.632	1.545	1.525
G4	1.632	1.544	1.524
G5	1.631	1.546	1.525
G6	1.631	1.545	1.526
G7	1.635	1.544	1.52
Toasted oak wood melanoidin fraction (TFMW)			
Fractions	**Viscosity at 15°C (mPa.s)**	**Viscosity at 20°C (mPa.s)**	**Viscosity at 25°C (mPa.s)**
P1	1.51	1.466	1.435
P2	1.511	1.467	1.434
P3	1.515	1.467	1.433
P4	1.511	1.465	1.435
P5	1.511	1.465	1.433
P6	1.51	1.466	1.434
P7	1.51	1.466	1.433

3.3.2 UV-Visible and IR Spectra

The UV and IR spectra are characteristics of melanoidins (Figures 8, 9 and 10). The absorption maximum at about 260 nm is broad; this is characteristic of polymer compounds containing a large number of chromophores which a different nature.

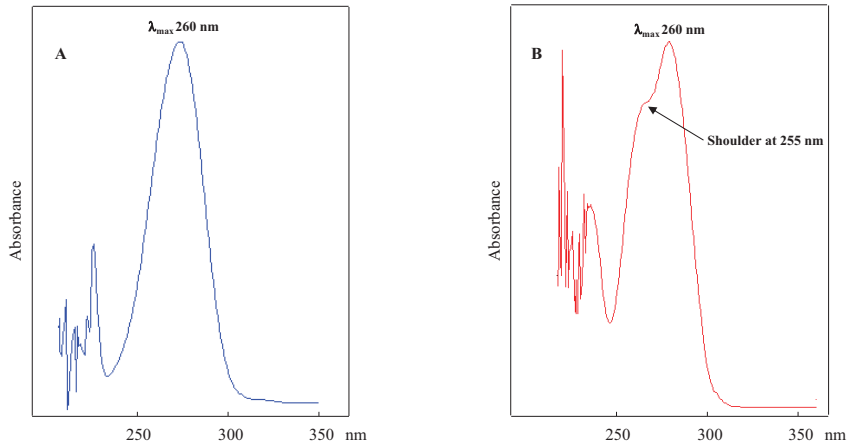


Figure 8.

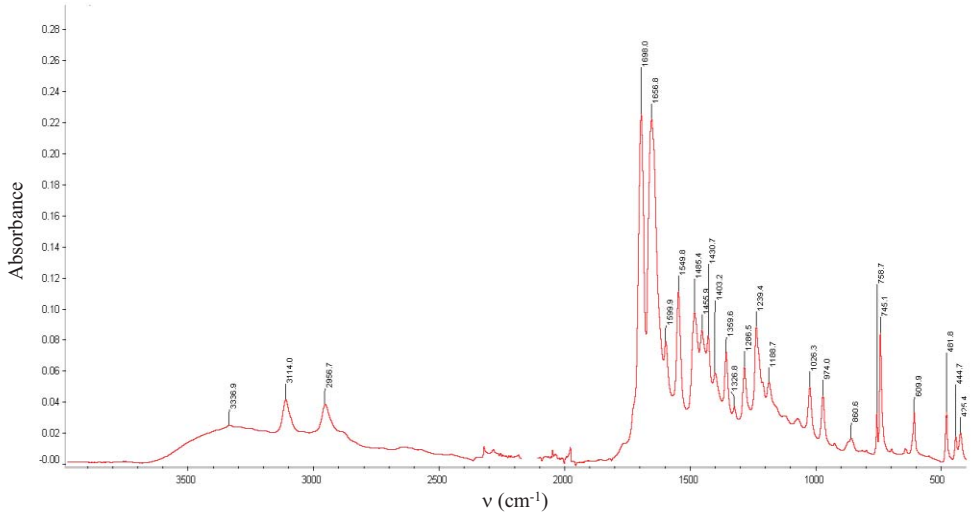


Figure 9.

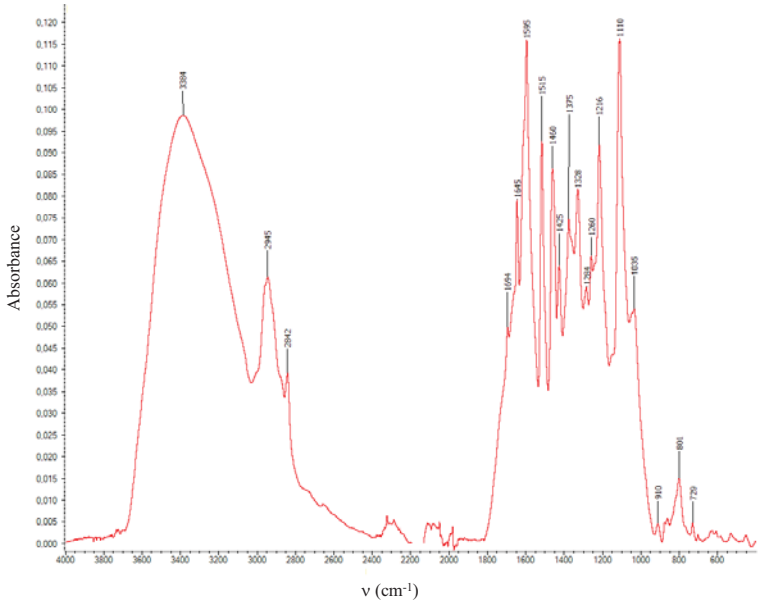


Figure 10.

The IR spectra (Figure 10) of the different fractions : P1, P2, P3, P4, P5, P6, P7 are similar in the case of toasted oak wood, and they are typical of polymeric compounds with spreading bands. In the same way the IR spectra (Figure 9) of the different fractions: G1, G2, G3, G4, G5, G6, G7 in the case of coffee are similar and present the same characteristic bands.

Nevertheless some characteristic absorptions are observed which allow assignment to group frequencies. Characteristic frequencies in the case of oak wood (Table 6) are observed for C-OH groups at 1035 cm^{-1}, C=O (-COOH) groups at 1700 cm^{-1}, C=N and C=C groups at 1644 cm^{-1}. The band at 2946 cm^{-1} can be attributed to the hydrogen stretching vibrations in CH_2 and CH_3 groups, the broad band at 3382 cm^{-1} to the –O-H and –N-H stretching. The same bands are observed in the case of coffee, see Table 7.

Absorptions are in agreement with the general hypothetical melanoidin structure presented in different studies (Cämmerer & Kroh, 1995; Poirier et al., 2000; Rubinsztain et al., 1986; Rubinsztain et al., 1986).

Table 6. Assigments of the bands in infrared spectra of toasted oak wood melanoidin fraction

Frequencies, n (cm^{-1})	Intensities		Assignment
3382	vs		n O-H, n N-H
3100-3500	mw		polymeric H-bonding (O-H)
2946	s	Strongly coupled modes	n_{AS} C-H (CH_3, CH_2)
2842	m		n_S C-H (CH_2, O-CH_3)
1644	m		n C=C, n C=N, n C=O
1596	vs		n C=C aromatic
1516	s		n C=C (para substitued aromatic)
1461	s		d CH_2 aliphatic
1426	m		d CH_2 aliphatic
1375	m		d -CH_3, d -C-O-H (phenol)
1329	m		n_{AS} C-O-C
1217	s		n C-O-H (phenol), n C-N
1112	vs		d >C-O-H (secondary alcohol), d C-O-CH_3, n C-N aliphatic
1035	m		n C-O, n C-N, n C-C skeletal
802	m		d_{op} aromatic, d C=C-H alcanes

w, wide; m, medium; ms, medium strong; s, strong; vs, very strong
ν: stretching
ν_S: symetric stretching
ν_{AS}: asymetric stretching
δ: bending
δ_{op}: bending out of plane

Table 7. Assigments of the bands in infrared spectra of coffee melanoidin fraction

Frequencies, n (cm^{-1})	Intensities	Assignment
3337	w	n O-H
3100-3500	s	polymeric H-bonding (O-H)
3114	ms	n_{AS} CH_3
2957	ms	n_S CH_3
1698	vs	n C=O
1658	vs	n C=O, n C=C
1600	s	n C=C, n C=N
1550	s	n N-CH_3, d ring, CH_3 rock
1485	s	d_S CH_3, n CN, d CH_3
1456	s	d_S CH_3
1431	s	d_S CH_3, n CN, d CH
1403	ms	n C-N in imidazole ring
1360	s	n_S NCH_3 + n CN + d CH_3
1287	ms	n NCH_3, n C-N & n C-C in rings
1239	s	d C-H, g CH_3 rock, n C-N
1189	ms	d C-H bend, n CN, r CH_3
1026	ms	CH_3 rock (in plane), n C-N
974	ms	d_{ip} pyrimidine ring, r CH_3
745	vs	n N-CH_3, d_{ip} imidazole ring, t CH_3
610	s	d_{op} imidazole ring (t NCN)
482	ms	d_{ip} pyrimidine ring, d CNC

w, wide; m, medium; ms, medium strong; s, strong; vs, very strong
ν: stretching
ν_S: symetric stretching
ν_{AS}: asymetric stretching
δ_S: symetric bending
δ_{ip}: bending in plane
δ_{op}: bending out of plane
ω: wagging
τ: torsion
r: rocking

3.3.3 LC/ESI/MS Analysis

Carbohydrates are the most important substrates in the Maillard reaction and melanoidin formation. Both, monosaccharide degradation products and intact sugars play a role in the formation of the melanoidin backone (ref). Therefore, to obtain further insight into the carbohydrate composition of the melanoidins, the isolated melanoidins fractions (P) from TFMW low pressure gel chromatography were analysed using LC/ESI/MS.

Mass spectrum of Fraction P1, obtained by electrospray ionisation in the negative ion mode, was obtained (Figure 11).

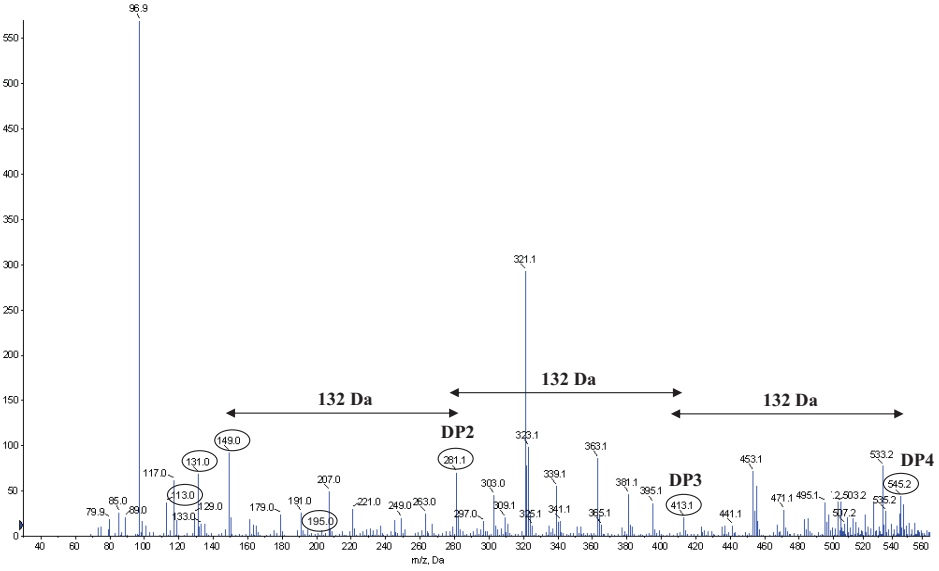


Figure 11.

The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 545, m/z 413, m/z 281, m/z 149 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation (DP) from DP2 (m/z 281) to DP4 (m/z 545). Mass spectrum showed fragment ions, specifically m/z 113, 131, 149, and 195, that are characteristic of the fragmentation pattern of pentoses. The fragmentation patterns of monosaccharides, disaccharides, trisaccharides and oligosaccharides have been studied by Verardo, Duse, and Callae and Brudzynski et al. using LC/ESI tandem mass spectrometry negative ion mode. The examination of mass spectra in negative ESI/MS^n of the fragmentation patterns of pentoses by those authors produced a list of fragmentation ions identical to those found in the mass spectrum of the Fraction P1.

Mass spectrum of Fraction P2, obtained by electrospray ionisation in the negative ion mode, was obtained. The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 545, m/z 413, 281, 149 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation (DP) from DP2 (m/z 281) to DP4 (m/z 545). Mass spectrum showed fragment ions, specifically m/z 113, 131, 149, and 195, that are characteristic of the fragmentation pattern of pentoses.

Mass spectrum of Fraction P3, obtained by electrospray ionisation in the negative ion mode, was obtained. The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 789, 657, 525, 393, 261 and 129 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation (DP) from DP2 (m/z 261) to DP5 (m/z 789). Mass spectrum showed fragment ions, specifically m/z 113, 131, 149, and 195, that are characteristic of the fragmentation pattern of pentoses.

Mass spectrum of Fraction P4, obtained by electrospray ionisation in the negative ion mode, was obtained. The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 373, 241, 109 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation (DP) from DP2 (m/z 241) to DP3 (m/z 373). Mass spectrum showed fragment ions, specifically m/z 113, 131, 149, and 195, that are characteristic of the fragmentation pattern of pentoses. Mass spectrum showed fragment ions, specifically m/z 89, 113, 143, 161, and 179, that are characteristic of the fragmentation pattern of hexoses.

Mass spectrum of Fraction P5, obtained by electrospray ionisation in the negative ion mode, was obtained (Figure 12).

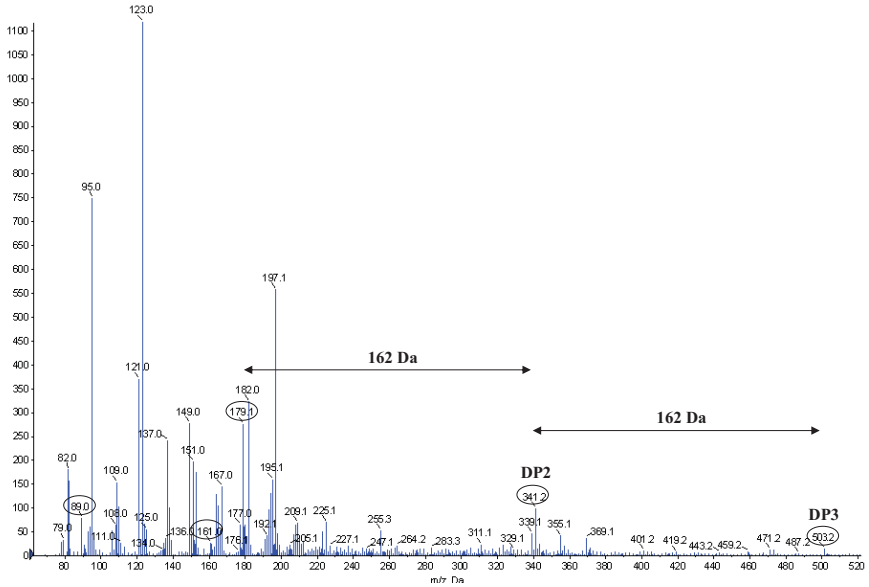


Figure 12.

The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 503, 341, 179 formed a series differing by 162 amu. The 162 amu corresponds to anhydrous hexose and therefore the above ions

indicate the degree of hexose polymerisation (DP) from DP2 (m/z 341) to DP3 (m/z 503). Mass spectrum showed fragment ions, specifically m/z 89, 113, 143, 161, and 179, that are characteristic of the fragmentation pattern of hexoses.

Mass spectrum of Fraction P6, obtained by electrospray ionisation in the negative ion mode, was obtained. The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 581, 317 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation: DP2.

Mass spectrum of Fraction P7, obtained by electrospray ionisation in the negative ion mode, was obtained. The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 551, 419 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation (DP) from DP2 (m/z 281) to DP4 (m/z 545). Mass spectrum showed fragment ions, specifically m/z 113, 131, 149, and 195, that are characteristic of the fragmentation pattern of pentoses.

Mass spectra of the fractions isolated from TFMW indicate the presence of pentose and hexose-based oligosaccharides with different degrees of polymerisation. The presence of the oligosaccharide moieties, as well as their degradation products found in the oak wood melanoidins, supports the postulated carbohydrate-based origin of melanoidins.

In the case of TFMC, the fractions G were analysed using LC/ESI/MS. Mass spectra of fractions G1, G2, G3 were presented respectively on Figures 13, 14, 15. Fractions G3, G4, G5, G6 and G7 presented the same mass spectrum.

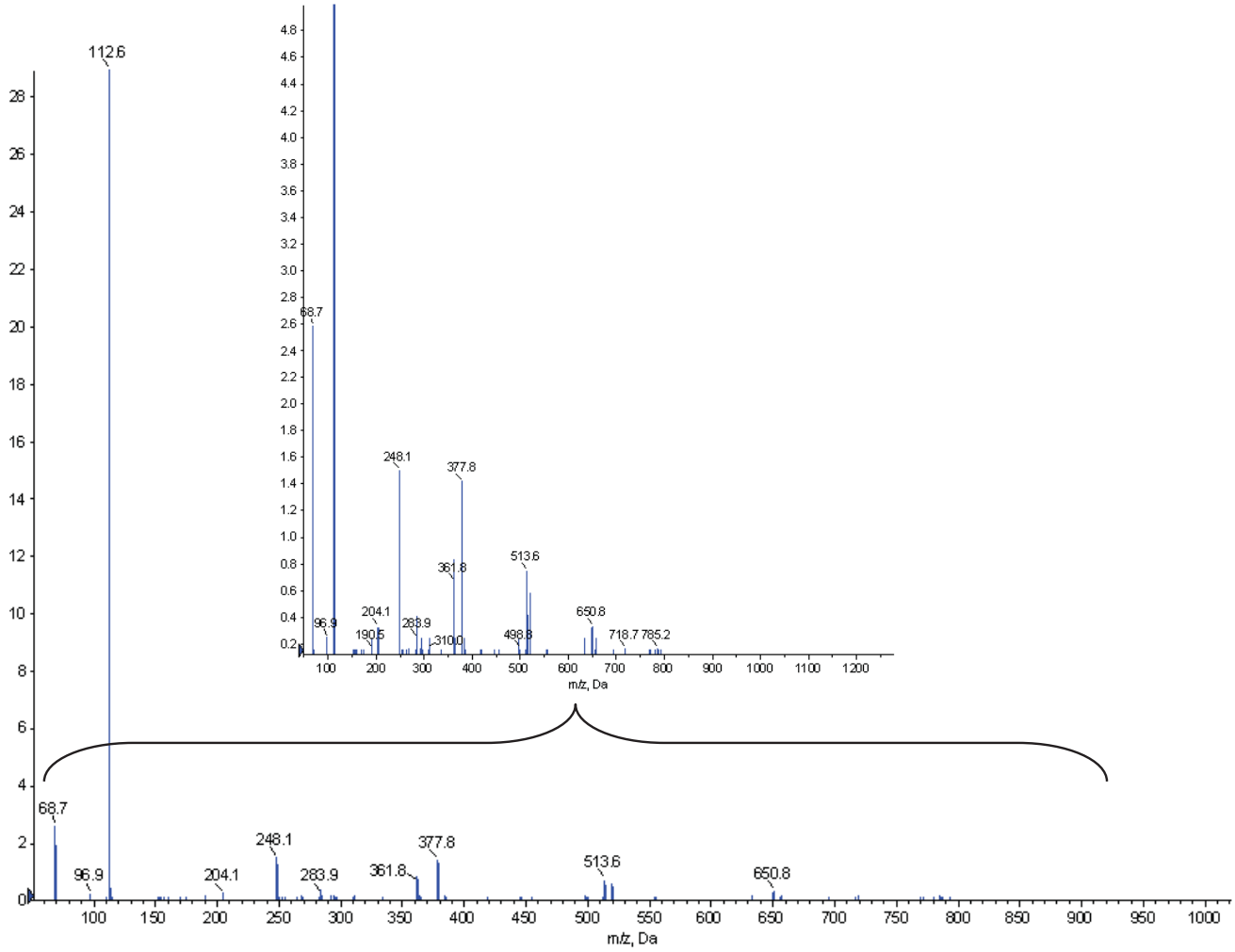


Figure 13.

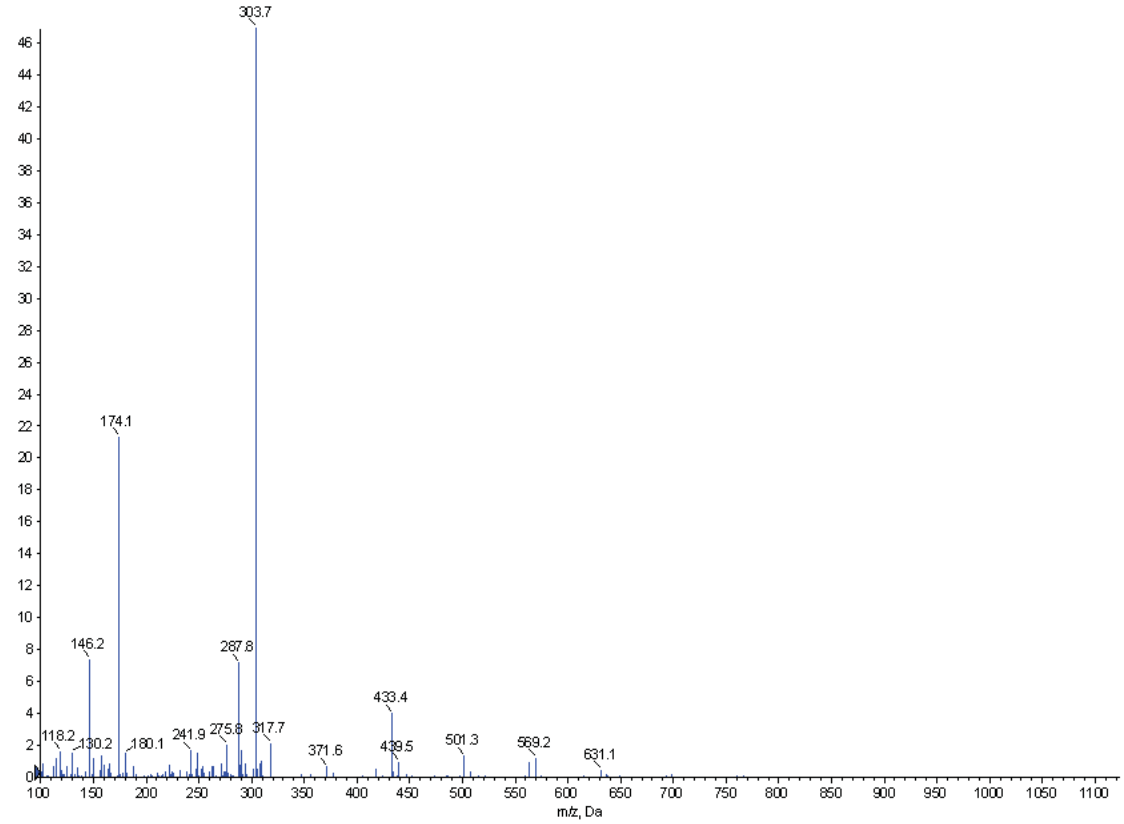


Figure 14.

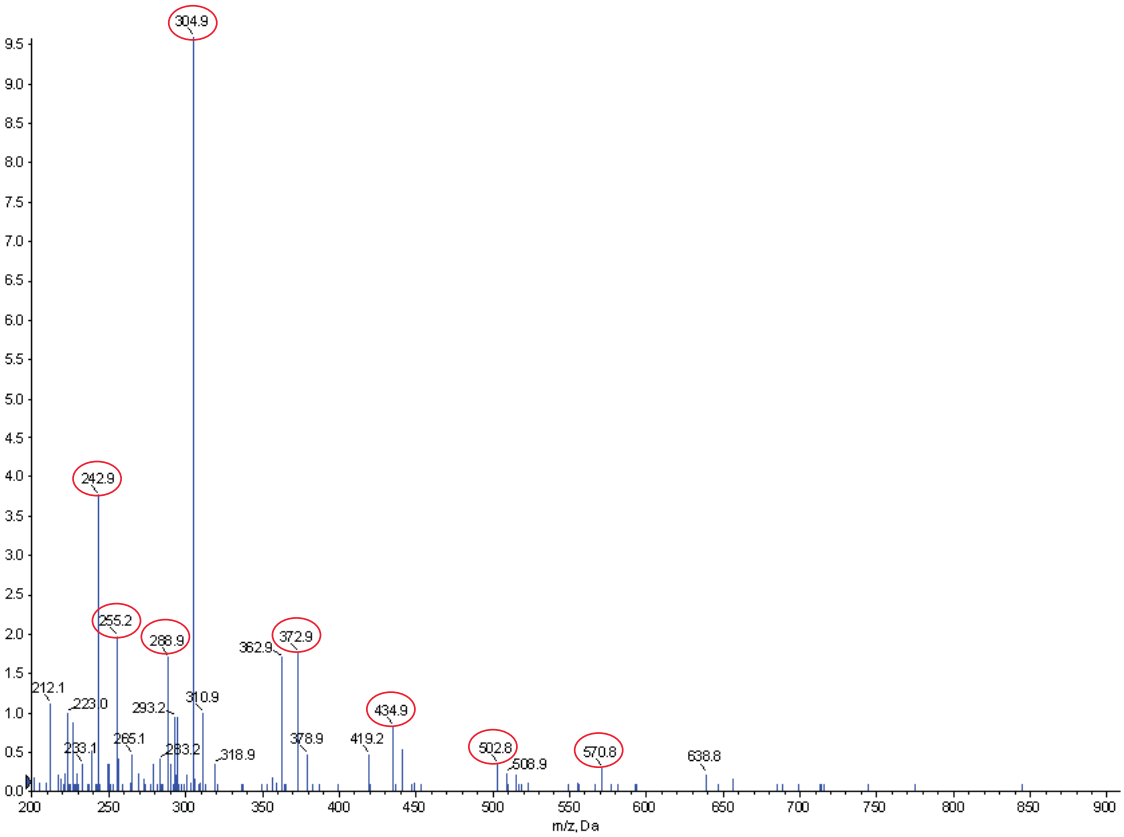


Figure 15.

3.4 Structure of Melanoidin Polymer

Melanoidins are high molecular weight amino-carbonyl compounds. In general, the separation of melanoidins from food and other biological samples is very difficult and therefore, most of the chemical and biological studies about melanoidins have done on model melanoidins. Although the chemical structure of melanoidins is not understood clearly, some part of the chemical structure of model melanodins have been elucidated by NMR techniques (Allard et al., 1997; Fang & Schmidt-Rohr, 2009; Gniechwitz et al., 2008).

Figure 16 presented ^{1}H NMR spectra of each P purified fractions from toasted oak wood.

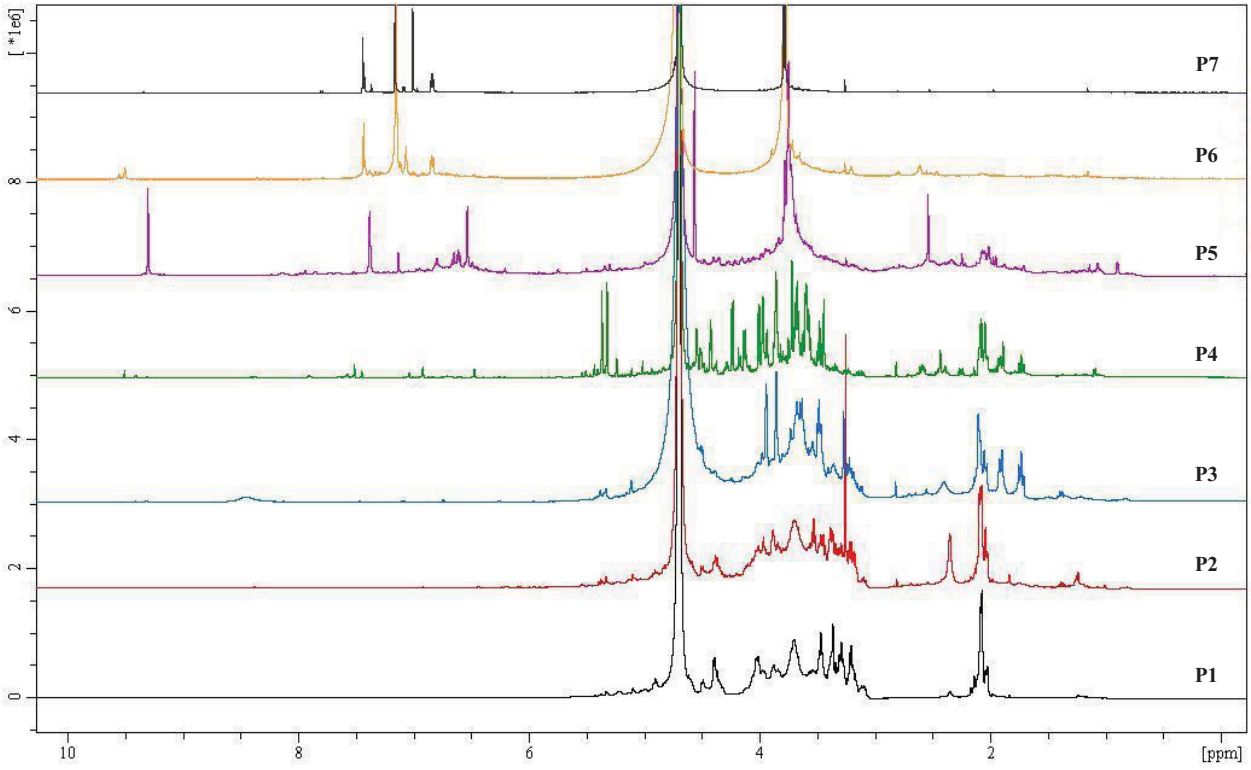
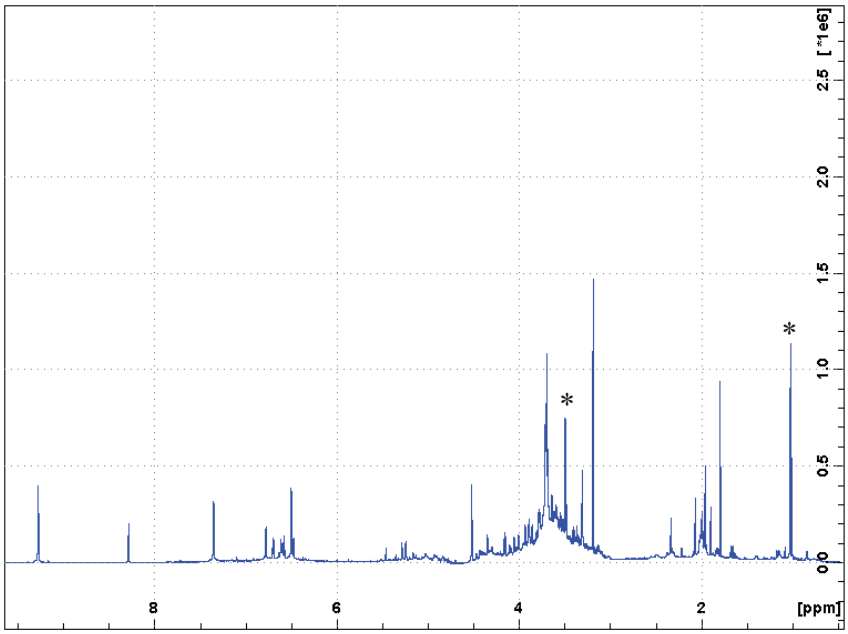


Figure 16.

Figures 17 and 18 presented respectively ^{1}H NMR and ^{13}C spectra of P4 fraction with experimental data. ^{1}H - ^{13}C correlations were obtained with normal 2D techniques HSQC and HMBC (Table 8).

Table 8. Assignments of the ^{1}H and ^{13}C chemical shifts in NMR spectra of toasted oak wood melanoidin fraction

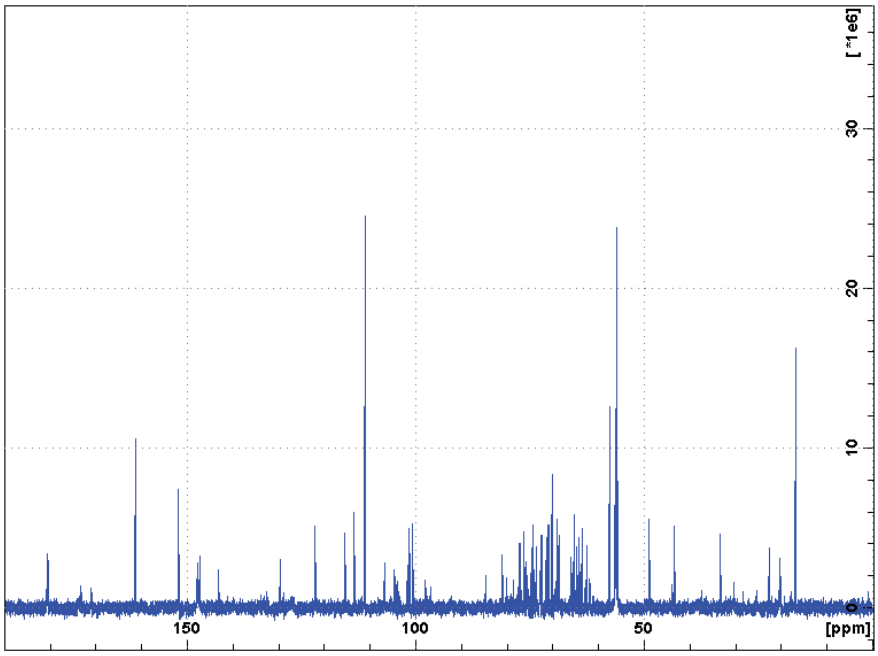
	^{1}H (δ, ppm)	^{13}C (δ, ppm)	Groups or functions
1	9.28	180.4	Aldehyde >C=O
2	8.29	_	
3	_	162<δ<150	Heteroaromatic >C=N
4	6.0<δ<7.5	101.6<δ<122	Alkene, aromatic >C=C<
5	5.0<δ<5.5	91.9<δ<104.5	Anomeric proton and carbon
6	3.0<δ<5.0	62.4<δ<101.5	Sugar ring
7	0.5<δ<2.5	17.6<δ<37.4	



Experimental data

^{1}H NMR (D_2O, 800.23 MHz) δ 9.28 (s, 1H), 8.29, 7.35 (d, 1H, J = 3 Hz), 6.78 (s, 1H), 6.70 (d, 0.5 H, J = 5.5 Hz), 6.64 (br, 0.2 H), 6.61 (t, J = 5.3 Hz), 6.58 (s), 6.57 (s), 6.50 (d, J = 3.1 Hz), 6.48 (br), 5.46 (s), 5.35-5.0 (complex signals, 1H), 4.52 (s), 4.50-3.0 (complex signals, 40 H), 2.34 (s), 2.28-1.55 (complex signals, 6H), 1.50-1.09 (complex signals, 3H)

Figure 17.



Experimental data

^{13}C NMR (D_2O, 201.22 MHz) δ 180.4, 161.2, 151.7, 147.5, 147.1, 143.1, 129.5, 121.8, 115.3, 113.3, 110.9, 106.6, 104.5, 104.3, 104.1, 103.8, 103.7, 103.5, 101.6, 101.3, 101.1, 100.5, 97.8, 96.5, 84.4, 80.9, 79.9, 78.5, 77.6, 77.1, 76.1, 75.6, 75.4, 74.7, 74.5, 74.4, 74.1, 73.4, 72.6, 72.3, 72.1, 71.3, 71.1, 70.8, 70.6, 70.0, 69.9, 69.1, 68.8, 68.5, 65.8, 65.1, 64.5, 64.1, 63.4, 62.3, 61.8, 57.3, 56.1, 55.9, 55.7, 48.7, 43.7, 43.3, 33.1, 22.5, 20.1, 16.7.

Figure 18.

These correlations permitted to observe and confirm some groups and functions: aldehyde >C=O, heteroaromatic >C=N-, alkene, aromatic >C=C<, anomeric proton and carbon, and sugar rings. These results were in correlation with those obtained in IR and ESI/MS.

The determination of the exact chemical structure of these compounds will be achieved by using other classical battery of 1D and 2D NMR structural experiments.

4. Discussion

We studied the extractible forms of melanoidins produced by toasting oak heartwood and coffee beans. For these two different sources of melanoidins, maillard reactions represent an important transformation of the raw material, improving the quality of products and develop a large part of these aromatic interest and diversity. Because coffee melanoidins are well documented, we used it as a model to develop our specific method on the toasted oak heartwood melanoidins.

We noted that the quantities of melanoidins in coffee and oak wood are very different. Coffee is richer in melanoidin compounds than oak wood. This is probably due to the roasting step that is different in the two cases. The roasting process, in the case of coffee, is more favorable to the production of melanoidins than the toasting process in the case of oak wood. The roasting temperatures and the toasting temperatures are comparable but the times in each case is different.

Specialists situate “roasting zone” between 185 and 240°C, the optimum being between 210 and 230°C. Above of these temperatures starts over-roasting. The time of the roasting is usually 12 to 15 minutes. The temperature roasting and its conduct have considerable influence on the qualities of coffee.

In the case of oak wood, we noted the presence of a larger amount of melanoidin polymers probably in relation with the heating time (± 50-55 min). The time of roasting coffee is around 15 to 20 min in the case of traditional roasting. This may explain the lower extraction because more the degree of polymerization increase and more the extractability decrease. So in oak wood, the extractible melanoidins are less present than in the case of coffee; this may also explain the difference in content melanoidins in both matrices.

In addition of the total amount of melanoidins, the polymeric status of both melanoidins are different.

After the maillard reaction, inducing the formation of melanoidins, the thermic treatment permitted the degradation of melanoidins, source of various aroma : vanilla for vanillin, almond for furfural, toasted almond for 5-methyl furfural, toasted for maltol.

The Pyrolysis GC of coffee melanoidins product less of various compounds, about 20, against 28 for Pyrolysis/GC of oak wood melanoidins. It exist probably a relation between degree of polymerization and number of molecules product and identify by pyrolysis technique.

Pyrolysis is the decomposition of an organic compound by a significant increase in its temperature (450°C) to obtain other products. At this high temperature, polymerized melanoidins, more present in oak wood melanoidins, are decomposed and produce more simple organic compounds.

From measurements of the viscosity of the solution for different concentrations of dissolved polymer, one can calculate the viscosity-average molecular weight (ref).

The viscosity of the melanoidins oak wood (1,46 ±0.08) is less than that of coffee melanoidins (1,54 ±0.08), while oak wood present higher molecular weight melanoidins.

Oak wood melanoidins contain less nitrogen (1,9%) than coffee melanoidins (2,6%). Green coffee contain from 1 to 3% total nitrogen, engaged in various combinations, the main ones are proteins and alkaloids (ref). Amino acids in general and certain amino acids containing sulfur (cystine and methionine especially), contained in the coffee proteins play an important role in the formation of the roasted coffee aroma. Among the amino acids that make up green coffee proteins, some of them, such as arginine, cysteine, lysine and serine, showed a high decrease in their amount during roasting (ref review). Oak wood contain less than 1% total nitrogen. And toasting process have also an impact on the degradation compounds.

We noted that the absorption maximum is about 260 nm, characteristic of nucleic acid absorbance. Nucleic acids are macromolecules with nitrogen groups, that is to say relatively large complex molecules. They enter in the family biomolecules as they are of great importance in the kingdom of life.

The nucleic acids consist of a chain of nucleotides linked by phosphodiester bonds. Nucleotides always consist of two basic components: a sugar (ose with 5 carbons or pentose) and nucleic base or nitrogenous base. This corroborates the observations made by LC/ESI/MS technique. Mass spectra of the different fractions in the

case of oak wood, show the presence of pentoses linked with another element. We proposed a hypothetic structure with a degree of pentose polymerization, DP=5.

The IR spectra present some characteristic frequencies and bands similar in the case of oak wood melanoidins and coffee melanoidins : C-OH groups, C=O (-COOH) groups, C=N and C=C groups, band corresponding to the hydrogen stretching in CH_2 and CH_3 groups, band corresponding to the –O-H and –N-H stretching.

In the case of coffee melanoidins, fractions didn't present frequencies at 800 cm^{-1} (aromatic bending) and 1110 cm^{-1} (C-N aliphatic stretching, C-O-CH_3 bending), but presented supplementary frequencies at : 745 (N-CH_3 stretching), 974 (bending pyrimidine ring), 1287 (N-CH_3, C-N stretching), 1485 (CH_3 symetric bending, C-N stretching), 1698 cm^{-1} (C=O stretching), with strong intensities.

Acknowledgment

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References

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., & Lerici, C. R. (2001). Review of nonenzymatic browning and antioxidant capacity in processed foods. *Trends Food Science Technololy, 11*(9-10), 340-346. https://doi.org/10.1016/S0924-2244(01)00014-0

Morales, F. J., Fernandes-Fraguas, C., & Jimenez-Perez, S. (2005). Iron-binding ability of melanoidins from food and model systems. *Food Chemistry, 90*(4), 821-827. https://doi.org/10.1016/j.foodchem.2004.05.030

Lindenmeier, M., Faist, V., & Hofmann, T. (2002). Structural and functional characterization of pronyl-lysine, a novel protein modification in bread crust melanoidins showing in vitro antioxidative and phase I/II enzyme modulating activity. *Journal of Agricultural and Food Chemistry, 50*(24), 6997-7006. https://doi.org/10.1021/jf020618n

Charles-Bernard, M., Kraehenbuehl, K., Rytz, A., & Roberts, D. D. (2005). Interactions between volatile and nonvolatile coffee components.1. Screening of nonvolatile components. *Journal of Agricultural and Food Chemistry, 53*(11), 4417-4425. https://doi.org/10.1021/jf048021q

Ames, J. M. (1998). Applications of the Maillard reaction in the food industry. *Food Chemistry, 62*(4), 431-439. https://doi.org/10.1016/S0308-8146(98)00078-8

Delgado-Andrade, C., & Morales, F. J. (2005). Unraveling the contribution of melanoidins to the antioxidant activity of coffee brews. *Journal of Agricultural and Food Chemistry, 53*(5), 1403-1407. https://doi.org/10.1021/jf048500p

Delgado-Andrade, C., Rufian-Henares, J. A., Morales, F. J. (2005). Assessing the antioxidant activity of melanoidins from coffee brews by different antioxidant methods. *Journal of Agricultural and Food Chemistry, 53*(20), 7832-7836. https://doi.org/10.1021/jf0512353

Wang, H. Y., Yao, W. R., & Qian, H. (2011). Melanoidins produced by the Maillard reaction: Structure and biological activity. *Food Chemistry, 128*(3), 573-584. https://doi.org/10.1016/j.foodchem.2011.03.075

Borrelli, C., R., Visconti, A., Mennella, C., Anese, M., & Fogliano, V. (2002). Chemical characterization and antioxidant properties of coffee melanoidins. *Journal of Agricultural and Food Chemistry, 50*(22), 6527-6533. https://doi.org/10.1021/jf025686o

Andriot, I., Le Quéré, J-L., & Guichard, E. (2004). Interactions between coffee melanoidins and flavour compounds : impact of freeze-drying (method and time) and roasting degree of coffee on melanoidins retention capacity. *Food Chemistry, 85*(2), 289-294. https://doi.org/10.1016/j.foodchem.2003.07.007

Bekedam, E. K., Roos, E., Schols, H. A., Van Boekel, M. A. J. S., & Smit, G. (2008). Low molecular weight melanoidins in coffee brew. *Journal of Agricultural and Food Chemistry, 56*(11), 4060-4067. https://doi.org/10.1021/jf8001894

Kim, J. S., & Lee, Y. S. (2009). Enolization and racemization reactions of glucose and fructose on heating with amino-acid enantiomers and the formation of melanoidins as a result of the Maillard reaction. *Amino Acids,* 36, 465. http://doi.org/10.1007/s00726-008-0104-z

Tressl, R., Wondrak T., G., & Garbe, L. A. (1998). Pentoses and hexoses as sources of new melanoidin-like maillard polymers. *Journal of Agricultural and Food Chemistry, 46*(5), 1765-1776. https://doi.org/10.1021/jf970973r

Hofmann, T. (1998). Studies on melanoidin-type colorants generated from the Maillard reaction of protein-bound lysine and furan-2-carboxaldehyde – chemical characterization of a red coloured domaine. *Z Lebensm Untersuch Forsch.* https://doi.org/10.1007/s002170050253

Cämmerer, B., Jalyschko, W., & Kroh W. L. (2002). Intact carbohydrate structures as part of the melanoidin skeleton. *Journal of Agricultural and Food Chemistry, 50*(7), 2083-2087. https://doi.org/10.1021/jf011106w

Nunes, F., M., & Coimbra, M. A. (2007). Melanoidins from coffee infusions. Fractionation, chemical characterization, and effect of the degree of roast. *Journal of Agricultural and Food Chemistry, 55*(10), 3967-3977. https://doi.org/10.1021/jf063735h

Adams, A., Borrelli, R. C., Fogliano, V., & De Kimpe, N. (2005). Thermal degradation studies of food melanoidins. *Journal of Agricultural and Food Chemistry, 53*(10), 4136-4142. https://doi.org/10.1021/jf047903m

Kato, H., & Tsuchida, H. (1981). Estimation of melanoidin structure by pyrolysis and oxidation. *Prog. Food Nutr., 5*, 147-156.

Yaylayan, V. A., & Kaminsky, E. (1998). Isolation and structural analysis of Maillard polymers: caramel and melanoidin formation in glycine/glucose model system. *Food Chemistry, 63*(1), 25-31. https://doi.org/10.1016/S0308-8146(97)00237-9

Cämmerer, B., & Kroh W. L. (1995). Investigation of the influence of reaction conditions on the elementary composition of melanoidins. *Food Chemistry, 53*(1), 55-59. https://doi.org/10.1016/0308-8146(95)95786-6

Hofmann, T., Czerny, M., Calligaris, S., & Schieberle, P. (2001). Model studies on the influence of coffee melanoidins on flavor volatiles of coffee beverages. *Journal of Agricultural and Food Chemistry, 49*(5), 2382-2386. https://doi.org/10.1021/jf0012042

Kuntcheva, M., Rogacheva, S., & Obretenov, T. (1998). Model melanoidins as a source of thermally generated aroma. *Nauchn. Tr.-Vissh Inst. Khranit. Vkusova Promst, Plovdiv, 43*, 427-430.

Adams, A., Borrelli, R. C., Fogliano, V., & De Kimpe, N. (2005). Thermal degradation studies of food melanoidins. *Journal of Agricultural and Food Chemistry, 53*(10), 4136-4142. https://doi.org/10.1021/jf047903m

Adams, A., Abbaspour Tehrani, K., Kersiene, M., Venskutonis, R., & De Kimpe, N. (2003). Characterization of model melanoidins by the thermal degradation profile. *Journal of Agricultural and Food Chemistry, 51*(15), 4338-4343. https://doi.org/10.1021/jf0340254

Margarita, J., Kuntcheva J., M., Tzvetan, D., & Obretenov, D. T. (1996). Isolation and characterization of melanoidins from beer *Z. Lebensm Unters Forsch, 202*, 238-243.

Poirier, N., Derenne, S., Rouzaud, J-N., Largeau, C., Mariotti, A., Balesdent, J., & Maquet, J. (2000). Chemical structure and sources of the macromolecular, resistant, organic fraction isolated from a forest soil (Lacadée, south-west France). *Organic Geochemistry, 31*(9), 813-827. https://doi.org/10.1016/S0146-6380(00)00067-X

Rubinsztain, Y., Yariv, S., Ioselis, P., Aizenshtat, Z., & Ikan, R. (1986). Characterization of melanoidins by IR spectroscopy – I. Galactose-glycine melanoidins. *Organic Geochemistry.* https://doi.org/10.1016/0146-6380(86)90101-4

Rubinsztain, Y., Yariv, S., Ioselis, P., Aizenshtat, Z., & Ikan, R. (1986). Characterization of melanoidins by IR spectroscopy – I.I. Melanoidins of galactose with arginine, isoleucine, lysine and valine. *Organic Geochemistry, 9*(6), 371-374. https://doi.org/10.1016/0146-6380(86)90118-X

Verardo, G., Duse, I., & Callea, A. (2009). Analysis of underivatized oligosaccharides by liquid chromatography/electrospray ionization tandem mass spectrometry with post-column addition of formic acid. *Rapid Communications in Mass Spectrometry, 23*(11), 1607-1618. https://doi.org/10.1002/rcm.4047

Brudzynski, K., & Miotto, D. (2011). Honey melanoidins: Analysis of the compositions of the high molecular weight melanoidins exhibiting radical-scavenging activity. *Food Chemistry, 127*(3), 1023-1030. https://doi.org/10.1016/j.foodchem.2011.01.075

Allard, B., Templier, J., & Largeau, C. (1997). Artifactual origin of mycobacterial bacteran. Formation of melanoidin-like artefact macromolecular material during the usual isolation process. *Organic Geochemistry, 26*(11-12), 691-703. https://doi.org/10.1016/S0146-6380(97)00037-5

Fang, X., & Schmidt-Rohr, K. (2009). Fate of the amino acid in glucose-glycine melanoidins investigated by Solid-Sate Nuclear Magnetic Resonance (NMR). *Journal of Agricultural and Food Chemistry, 57*(22), 10701-1071. https://doi.org/10.1021/jf9020587

Gniechwitz, D., Reichardt, N., Ralph, J., Blaut, M., Steinhart, H., & Bunzel, M. (2008). Isolation and characterization of a coffee melanoidin fraction. *Journal of the Science and Food Chemistry, 88*(12), 2153-2160. https://doi.org/10.1002/jsfa.3327

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Effects of Boiling and Smoking on the Proximate Composition and Oil Quality of a Commercially Important Freshwater Fish (*Chrysichthys nigrodigitatus*) from Nkam River in Cameroon

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Abstract

The effects of boiling and smoking on the proximate composition and lipids quality of a freshwater fish (*Chrysichthys nigrodigitatus)* collected from Nkam River in Cameroon were investigated. Fresh fish was filled, boiled or smoked and then, aliquoted for analyses. One portion was dried at 50 °C and ground into flour for the proximate composition determination. The other portion was served for lipids extraction using the Bligh and Dyer method. The extracted oil was characterized physico-chemically using chemical indexes and GC/FID. Moisture, protein, ash and lipid contents of raw fish were 80.67±4.18, 64.42±1.51, 10.90±0.42 and 22.06±5.40 % dm respectively. The changes in dry matter, protein and ash contents were found to be statistically significant ($P<0.05$) after smoking. The most important mineral of this fish was the Potassium (7017.54-8771.93 mg/kg). Except the calcium and phosphorus contents which decreased with the treatments, the amount of the other detected minerals was significantly increased. It was also found that these technological treatments significantly increased ($P<0.05$) the free fatty acids and hydroperoxides formation in oil, while it decreased its iodine value. The fatty acid profile of untreated and treated fish revealed her richness in Palmitic (22.91-34.76%), Oleic (12.83-23.55%), Stearic (11.29-14.81%), Linoleic (LA) (ω6) (2.83-6.75%), Arachidonic (ω6) (2.50-6.64%) and Docosahexaenoic (DHA) (ω3) (1.56-12.31%) acids. The ratio PUFA/SFA of raw fish (0.47) was severely reduced by the smoking (0.26) while it increases after boiling (0.61). This fish contain appreciable levels of Omega-6 PUFA, suggesting that it could be used as a source of healthy diet for human beings. These findings may also be beneficial for the fish industry, nutritionists and researchers who were striving to improve the nutritive value, processing and marketing of selected fish species.

Keywords: *Chrysichthys nigrodigitatus*, chemical composition, fatty acids, fresh fish, smoking, boiling, Nkam river

1. Introduction

Fishes are very important animal proteins source which can be exploited for preventing food insecurity in both rural and urban areas of Cameroon. They serve as feeds for livestock, poultry and carnivorous fish. Apart from being the richest source of high quality protein, vitamins and essential minerals, they are virtually a unique and good source of n-3 and n-6 long-chain poly-unsaturated fatty acids (PUFAs). The nutritional importance of seafood, fats and oils has increased substantially because of their health benefit effects (Azamand & Ali, 2004). Fish containing high n-3/n-6 PUFAs ratios is important for human health and it has been proven that regular fish consumption reduces the risk of cardiovascular diseases, enhances neurodevelopment of infants, leads to improvement in learning ability and prevents cancers (Suzuki *et al*., 1998; Mozaffarian *et al.,* 2005). PUFAs are also essential in maintaining the functions of living cell membranes. Moreover, they are useful in making

prostaglandins which regulate many body processes, notably the inflammation and blood clotting. Fish fats are equally needed to absorb fat soluble vitamins A, D, E and K from food and for regulating body cholesterol metabolism (Connor, 2000; Kris-Etherton *et al*., 2003). Clinical results of an epidemiological research suggest that, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, found only in fish and seafood, possess extremely beneficial properties for human coronary artery disease prevention. Additionally, fish oil helps in preventing brain aging and Alzheimer's disease (Conner, 1997). Many health experts recommend the consumption of seafood 2 or 3 times a week. This is mostly recommended for pregnant women, children and elderly people (Krauss *et al.,* 2000). Consumption of EPA and DHA may reduce the risk of mortality due to cardiovascular diseases (Conner, 1997; Krauss *et al.,* 2000). Although the hydrographic network of Cameroon is wide with an abundant and diversified aquatic fauna (Vivien, 1991), very few studies on their nutritional quality have been addressed in the literature. *Chrysichthys nigrodigitatus* is a species of fish in the family of claroteidae (Hopkins et al., 2007). This freshwater fish is of great commercial value at Yabassi and is interesting for consumption after boiling or smoking. However, very few studies have been done in Cameroon from this species. Previous works on this species include Tenyang *et al.* (2016) and Mouokeu *et al.* (2018). The first studied the proximal, mineral and fatty acid composition of raw *C. nigrodigitatus,* from the Maga Lake in Far North region. The second analyzed the chemical composition and antibacterial activity of oil of *C. nigrodigitatus* from Nkam River at Yabassi, extracted hot by pressing and maceration in hexane. Furthermore, no study made mention of the effects of boiling and smoking generally applied on their nutritional potential and the quality of their lipids. These effects are not yet been reported and remain unknown in this species of fish. Studies of nutrient intake from fish in relation to health are frequently carried out with data obtained from raw food. But, chemical contents in raw fish tissue might not provide explicit information on the nutritive value of the species after technological treatments. The objective of this study is to assess the influences of smoking and boiling on the proximate composition and fat quality of *C. nigrodigitatus* from Nkam River at Yabassi.

2. Material and Methods

2.1 Sample Preparation and Cooking

Fish samples *(Chryschsthys nigrodigitatus)* were obtained from the landing stages of the Nkam River at Yabassi-Cameroon, located at Titina: 4° 45' North latitude and 9° 97' East Longitude, former River Port 4° 27' North Latitude and 9° 57' East Longitude and Bodiman 4° 24' North latitude and 9° 53' East longitude. The fishes were harvested, transferred into ice containing boxes and transported to the laboratory. Identification was made by ichthyologists of the Laboratory of Ecosystem and Fisheries Resources of the Institute of Fisheries and Aquatic Sciences.

2.2 Boiling Procedures

Fresh fishes were washed with tap water to remove adhering blood and slime. They were prepared using a handling process, i.e. eviscerating, beheading, washing, and then cooked by boiling. The fishes were dipped into boiling water at the ratio of 1:1.5 (w/v) for 20 minutes at 98°C (water temperature).

2.3 Smoking Procedures

The traditional heat smoking methods was used in the "banda chorhor" room for smoking. Firewood was set up in the combustion chamber and then lighted. The smoking temperature was monitored in the smoking chamber using a thermometer. Fish samples were then placed on the mesh in the kiln after weighing. The burning wood was adjusted continuously to maintain the required temperature in the chamber during the three smoking periods. During the pre-drying phase, the temperature was kept low between 32 and 50 °C for 2 h. During cooking-smoking, the temperature was between 60 and 80 °C for 2h30minutes and in the final phase smoking-drying, the temperature was decreased and maintained between 50 and 60 ° C for 2h30minutes.

2.4 Sample Collection

After boiling or smoking, the bones of fishes were removed; meanwhile the fresh samples were filled in parallel. These samples of raw, boiled and smoked fish were then aliquoted for analysis. After evaluating their moisture content, a portion of the aliquots was dried at 50 °C and ground into flour in which proximal and mineral composition has been determined. For lipids analysis, the extraction was done in the other portion using a mixture of solvent chloroform, methanol, according to the Bligh and Dyer (1959) method.

2.5 Proximate Composition Analysis

The moisture content of *C. nigrodigitatus* was determined by drying the meat in an electric air dried oven at 105°C, until a constant weight was obtained as described by AOAC method (AOAC, 1990). Crude protein (CP) content was calculated by converting the nitrogen content determined via Kjeldahl's method (6.25xN). Fat (F)

was determined by the method described by the AOAC (1990) using the Soxhlet system apparatus, with a non-polar solvent hexane. Ash (A) content was evaluated by dry-aching in a furnace at 550°C for 24 h (AOAC, 1990). Total carbohydrates (C) were determined by subtracting the sum of % F, % CP and % A contents from 100 g of fish dry matter (Onyeike *et al.*, 2000). Gross energy value of each sample was calculated, by multiplying the percentage CP, F and total Carbohydrate (C) contents with their respective energy values of 4, 9 and 4 kcal per 100 g of fish dry matter (dm).

2.5.1 Mineral Analysis

For mineral determination, the samples were digested in $HNO_3/HClO_4$ (Pauwels *et al.*, 1992)**.** The elements Fe, Zn and P were determined using a spectrophotometer. While Sodium (Na) and potassium (K) were measured by flame photometry, calcium (Ca) and magnesium (Mg) were measured by titration, using EDTA-complex metric method (AOAC, 2000).

2.5.2 Chemical Analysis for Lipids Quality

The extracted oils of samples were characterized through the assessment of their Iodine (I_2), Peroxide (PV) and free fatty acid (FFA) values as per the AFNOR official methods (AFNOR, 1981).

2.5.3 Fatty Acids Profile of Fish Oil Analysis

Fatty acids profiles of oils samples were investigated following the conversion of their fatty acids into fatty acids methyl esters (FAMEs). Then they were prepared by trans-esterification using 2% of sulfuric acid in methanol (Christie, 1993). The FAMEs were extracted into ethyl acetate and thoroughly washed with water to make them free of acid and dried over anhydrous sodium sulfate. The dried esters were analyzed in Gas chromatography flame ionize detector (GC-FID). The GC-FID analyses were performed with an Agilent (Agilent Technologies, Palo Alto, CA, USA) 7890A series gas chromatograph equipped with a FID detector using a DB-225 capillary column (30 m × 0.25 mm, 0.25 µm of film thickness). The column temperature initially maintained at 160°C for 2 minutes, was subsequently increased to 220°C at 5°C/minutes and maintained for 10 min at 220°C. The carrier gas utilized was nitrogen at a flow rate of 1.5 ml/min. The injector and detector temperatures were maintained at 230 and 250°C, respectively with a split ratio of 50:1. The identification of fatty acid was based on the comparison of retention time with that of standard reference fatty acid methyl esters performed under same conditions.

2.5.4 Statistical Analysis

Completely Randomized Design (CRD) was adopted in carrying out the experiment. Experimental trials on evaluation of the physicochemical properties of each sample were conducted at least three times. One-way analysis of variance (ANOVA) with a level of significance $P< 0.05$ was applied to the different sample values obtained. The differences among the means were characterized by the multiple comparison test of Tukey Kramer. Graph Pad InStat Software Inc (C) 1992-2000 version 3.05, 32 bit for Win 95/NT was used for the data analysis.

3. Results and Discussions

3.1 Proximate composition

Results of the proximate analysis carried out on fresh, smoked and boiled *C. nigrodigitatus* are presented in Table 1. The average values obtained on the raw flesh of this fish were 80.67 % fw for the water content, 64.42 %dm for the crude protein, 22.06 % dm for the crude lipid, 10.90 % dm for the crude ash and 2.62 % dm for the crude carbohydrates. These values differ from the 78.46% fw, 39.73, 30.34, 7.32 and 22.61 % dm found by Tenyang *et al.* (2016) respectively on the same fish species in the Maga Lake in Far North. However, this proximate composition was not far from the one found by Tenyang *et al.,* (2013). These authors found 76.48 % fw, 64.24, 23.02, 10.98 and 1.76 % fw respectively on a catfish *Arius maculatus* from Wouri. It is clear that the flesh of *C. nigrodigitatus* is dominated by water. This result is in agreement with Tenyang *et al.* (2014) and (2016) on the catfish. Also, value of water content of this fish is in the range of 70-85 g / 100 g FW described by Yeannes and Almandos (2003) on the fresh fish. The fat content of our fresh samples is lower than that found by Tenyang *et al.* (2016) on the same species in the Far North-Cameroon. This fat value is similar to that of *A. maculatus* from Wouri (Tenyang et al., 2014) and fall within the range of 17.16 - 39.06 % dm found by Olele (2012) on *C. nigrodigitatus* from Niger River between September and February. Protein of raw *C. nigrodigitatus* of this study is similar to that of *A. maculatus* from Wouri (Tenyang *et al.*, 2014), but remains very high than the same species collected in the Maga Lake in the far North (Tenyang *et al.*, 2016). This value is also very high compared to the range 0.86 - 14.15% found by Olele (2012) on *C. nigrodigitatus* of Niger. The carbohydrate content of our raw sample is very small compared to that found (22.61 %DW) by Tenyang *et al.* (2016) and the range (4.23 - 14.11%) recorded by Olele (2012). The high protein levels recorded is proving that this fish could

be used to enrich the basic food rations and prevent its deficiency-related diseases. To be healthy, the FAO (Food and Agricultural Organization) and WHO (World Health Organization) (2007) recommend a protein intake of 0.66 g/kg of weight body/day. Overall the differences observed in the nutritional composition of *C. nigrodigitatus* could be explained by some variation of the extrinsic factors (geographic areas, quality of water (temperature, salinity and turbidity), quality and availability of food, migration) and intrinsic factors (species, breeding period, age, sex and size at capture) (Ackman, 1990; Argen *et al.*, 1991; Rao *et al.*, 1995 and Sargent *et al.*, 2002).

Table 1. Proximate composition of raw, smoked and cooked *C. nigrodigitatus*

Parameters	Raw	Smoked	Boiled
Moisture (%)	$80.67\pm4.18_{a}$	$15.89\pm0.01_{b}$**	$76.47\pm1.77_{a}$
Dry matter (%)	$19.33\pm4.1_{a}$	$84.11\pm0.01_{b}$**	$23.53\pm1.77_{a}$
Moisture of flour (%)	$7.67\pm0.46_{a}$	$7.47\pm0.34_{a}$	$8.13\pm0.12_{a}$
Dry mater of flour (%)	$92.33\pm0.46_{a}$	$92.53\pm0.34_{a}$	$91.87\pm0.12_{a}$
Ash (%DM)	$10.90\pm0.42_{a}$	$27.45\pm0.31_{b}$**	$11.30\pm3.54_{a}$
Lipides (%DM)	$22.06\pm5.40_{a}$	$26.79\pm2.3_{a}$	$19.08\pm4.6_{a}$
protein (%DM)	$64.42\pm1.51_{a}$	$38.75\pm7.19_{b}$*	$60.27\pm1.11_{a}$
carbohydrates (%DM)	$2.62\pm2.46_{a}$	$7.01\pm3.28_{a}$	$9.35\pm3.10_{a}$
Energy (kcal/100gDM)	$466.70\pm64.48_{a}$	$424.15\pm62.58_{a}$	$450.2\pm58.24_{a}$
Calcium (mg/kg)	$7.92\pm0.22_{a}$	$5.68\pm0.84_{a}$	$5.23\pm0.61_{a}$
Magnesium (mg/kg)	$12.98\pm6.7_{a}$**	$37.12\pm1.13_{b}$	$38.46\pm6.12_{b}$
Sodium (mg/kg)	$923.15\pm0.01_{a}$	$1384.72\pm0.00_{b}$*	$923.15\pm0.01_{a}$
Potassium (mg/kg)	$7017.54\pm0.00_{a}$*	$8187.13\pm1654.05_{b}$	$8771.93\pm827.02_{b}$
Phosphorus (mg/kg)	$406.25\pm44.20_{a}$	$343.75\pm44.20_{a}$	$375\pm0.01_{a}$
Iron (mg/kg)	$1.70\pm0.01_{a}$	$1.20\pm1.17_{a}$	$2.70\pm0.01_{a}$
Zinc (mg/kg)	Trace	Trace	Trace
Na/K	0.13	0.17	0.11

Values are shown as mean ± standard deviation of triplicates. N=3. Within the same line, values with different letters (a,b) are significantly different (*P<0.05; **P<0.01).

The effects of treatments on the proximate composition showed a drastic moisture decrement with the smoking. This dehydration concentrates the dry mater of fish, as well as its nutrient contents. This result is in agreement with those of Kumolu-Johnson *et al.* (2010) who reported the same observations. With respect to the dry matter, while value of ash content significantly (P<0.01) increases with the smoking process, the amount of proteins significantly decreases (P<0.01). The augmentation in ash content of smoked fish could be due to the loss of water during the process as explained in previous studies (Salan *et al.*, 2006)**.** Likewise, the observed statistically significant decrease of proteins amount in the present study during the smoking process, may affect the quality of food as stated in previous studies (Salan *et al.*, 2006). The loss in available lysine may vary from 6-33% at 25 °C to 53-56 % at 40 °C during hot smoking (Dvorak & Vognarova, 1965); 25 % loss of available lysine on the surface and a 12% loss at the center of hot smoked fish (Clifford *et al*., 1980). Akande *et al.* (1998) observed that lysine reduction was directly proportional to the temperature and duration of smoking. However, the present study showed that boiling does not significantly (P>0.05) affect the proximate composition of *C. nigrodigitatus*.

The mineral contents of *C. nigrodigitatus* carried out in fresh, smoked and boiled are also shown in Table 1. In this fish, potassium content was higher (7017.54-8771.93 mg/kg), followed by sodium (923.15-1384.72 mg/kg), phosphorus (343.75-406.25 mg/kg), magnesium (12.98-38.46 mg/kg) and calcium (5.23-7.92 mg/kg). Iron was reported to be the least represented (1.20-2.70mg/kg), while zinc was observed in traces. The ratio Na/K, increases (from 0.13 to 0.17) with smoking and decrease (from 0.13 to 0.11) with boiling. This report is very interesting in nutrition insofar as consumption of foods with high Na/K ratio is often associated with high blood pressure (Liu, 1996). The P, Ca and Mg are the essential components of the bones. Calcium and magnesium plays a significant role in photosynthesis, carbohydrate metabolism, nucleic acids and binding agents of cell walls (Russel, 1973). Calcium assists in teeth development (Brody, 1994). Magnesium is an essential mineral for enzyme activity; like calcium and chloride, magnesium also plays a role in regulating the acid-alkaline balance in the body (Fallon & Enig, 2001). The order of predominance of these minerals from raw *C. nigrodigitatus* is different from the one obtained by Tenyang *et al.* (2016) on the same species collected in the Maga Lake. These last authors had found the order of predominance of *C. nigrodigitatus* minerals as Ca, P, K, Mg, Na, Zn and Fe with respective values of 82938.9, 35394.8, 6117.5, 1797.8, 1072.3, 122.8 and 77.2 mg/kg dm. Thus, *C.*

nigrodigitatus from Nkam River at Yabassi is less rich in minerals than the Maga Lake. The differences could be explained as previously by the variation in environmental factors (geographic areas, quality of water (temperature, salinity), availability of natural food and migration) and intrinsic (species, period of reproduction, age, sex and size at capture) (Ackman, 1990; Argen *et al.*, 1991; Rao *et al.*, 1995 & Sargent *et al.*, 2002).

Processing methods like smoking and boiling significantly ($P<0.05$) and positively affect the content in some minerals. For example, it is clearly noted in the table 1 that, it leads to an increment of potassium (16-25%), magnesium (185-196%) and sodium (50%) content of fish. This corroborates the findings of Akinwumi (2014), who demonstrated that smoking of *Clarias gariepinus* increased its phosphorus, iron and potassium content. Similarly Effiong & Fakunle (2012) observed high values of phosphorous and low iron contents in the three tropical smoked freshwater fishes studied. However, Eyo (2014) reported low phosphorous and iron contents, but high potassium and vitamin C contents in frozen fish. The current study is in contradiction with the report of Gokoglu *et al.*, (2004) in regard to the effect of boiling on minerals contents. These authors observed a significant decrease in minerals Na, K, Mg, P, Zn and Mn after smoking. Flesh of freshwater fish is a particularly valuable source of minerals calcium and phosphorous as well as iron (FAO, 2014).

3.2 Chemical Analyses for Lipids Quality

The table 2 shows the effect of smoking and boiling on the oxidation parameters of *C. nigrodigitatus* oil. From this table, it is clearly observed that compared to extracted oil from raw fish, boiling and smoking have significantly affected the oil quality. The oil extracted from boiled fish has exhibited the highest peroxide and acid values, and the lowest iodine values in comparison to smoked and raw fishes. The index value of acid (10.25% oleic acid which corresponds to 20.4 mg KOH/g) obtained in this work with the raw fish is at the limit (≤ 20 mg KOH/g) granted by the *Codex Alimentarius* for Virgin oils of fish rich in phospholipids. This value is greater than 7.33 mg KOH/g obtained by Mouokeu *et al.* (2018) in *C. nigrodigitatus* dried and macerated into hexane. This difference could be justified by the extraction technique. The increase in acid value observed in fish oil after treatment might be attributed to the hydrolysis of lipid compounds (triglycerides, phospholipids, etc.) due to the effect of heating and water. At high temperature, water can catalyze the hydrolysis of lipids, leading to free fatty acids, which result in the increment of oil acidity and in the susceptibility of the released fatty acids easily oxydable especially if they are unsaturated. These results are in agreement with those of Labuza (1974) who observed an increment of free fatty acids in food during dehydration and storage processes.

Table 2. Oxidation parameters of oil extracted from raw, smoked and boiled *C. nigrodigitatus*

Parameter	Raw	Smoked	Boiled
Acid value (% oleic acid)	$10.25\pm0.01_a$	$18.80\pm0.01_b$	$29.96\pm0.01_c$
Iodine value (g of I_2/100g)	$56.37\pm0.22_a$	$52.66\pm0.01_a$	$51.60\pm4.59_a$
Peroxyde value (meq O_2/Kg)	$22.02\pm4.21_a$	$29.54\pm3.21_{ab}$	$35.00\pm0.01_b$

Values are shown as mean± standard deviation of triplicates. N=3.

In the same line, values with different letters (a, b) are significantly different ($P<0.05$).

The iodine value of the raw samples was 56.37 g I_2 /100 g of oils. This value was lower than 82.64 and 93g I_2 /100g obtained respectively by Mouokeu *et al.* (2018) in the same fish and Tenyang (2015) in *Arius maculatus.* Smoking and boiling have not significantly affected ($P>0.05$) this parameter. These effects are not in the same line as those observed by Tenyang (2015). This author showed a significant decrease in the iodine value of *Arius maculatus* lipids after smoking and boiling. However, this result is in agreement with those of Onyeike and Oguike (2003) who found similar effects during heating of peanut oil. The observed variations could be explained by the inactivation of exogenous lipoxygenase responsible of oxidation (Eymard, 2003) and which depend on intrinsic factors of the samples, mainly, duration and temperature of the heat treatment (Franckel, 1998).

Table 2 also illustrates the changes in peroxide value of the tested fish oil before and after treatments. It is clearly observed that these treatments have significantly increased the peroxide value of oil. The highest value was noted in boiled fish oil samples, showing that boiling might accelerate the primary oxidation of fish oil as compared to smoking. This result is related to the result of acid value, because the same sample has exhibited the highest acid value. It is well known that hydrolysis of lipids leads to free fatty acids, which, if unsaturated, can rapidly undergo oxidation. The highest peroxide value obtained in boiled fish oil sample is contradictory to the observed findings of Tenyang (2015) during cooking by boiling and smoking of *Arius maculatus* fish. The observed increment in peroxide value is associated to the formation of primary oxidation products, mainly

hydroperoxides. In fact, during heating, unsaturated fatty acids of oil can undergo auto oxidation, which can lead to the formation of these compounds. So these parameters strengthen on the primary oxidation status of oil. The peroxide value of the oils extracted from raw *C. nigrodigitatus* was 22.02 meq O_2/kg. This result was greater than 4.49 meq O_2/Kg and smaller than 72 meq O_2/Kg found respectively by Mouokeu *et al.* (2018) in the same fish and Tenyang (2015) in *Arius maculatus* oil.

3.3 Changes in Fatty Acid Profile of Fish Oil during the Process

The fatty acid profiles of raw, smoked and boiled samples of *C. nigrodigitatus* are presented in Table 3.

Table 3. Fatty acid profiles of raw, smoked and boiled fish oils

Fatty acids	Raw	Smoked	Boiled
C10:0, Capric	$0.09\pm0.01_a$	$0.06\pm0.01_b$	$0.10\pm0.01_a$
C11:0, Undecanoate	$0.27\pm0.04_a$	$0.24\pm0.03_a$	$0.85\pm0.11_b$
C12:0, Lauric	$0.66\pm0.02_a$	$1.22\pm0.03_b$	$0.23\pm0.00_c$
C13:0, Tridecanoate	$0.16\pm0.03_a$	$0.33\pm0.04_b$	$0.11\pm0.03_a$
C14:0, Myristic	$2.91\pm0.06_a$	$3.60\pm0.07_b$	$2.05\pm0.06_c$
C15:0, Pentadecanoic	$0.70\pm0.01_a$	$0.75\pm0.04_a$	$1.17\pm0.03_b$
C16:0, Palmitic	$27.61\pm0.50_a$	$34.76\pm0.41_b$	$22.91\pm0.10_c$
C17:0, Heptadecanoic	$1.58\pm0.03_a$	$2.07\pm0.03_b$	$2.58\pm0.01_c$
C18:0, Stearic	$11.29\pm0.21_a$	$12.29\pm0.14_b$	$14.81\pm0.03_c$
C20:0, Arachidic	$0.52\pm0.04_a$	$0.32\pm0.01_b$	$0.68\pm0.02_c$
C21:0, Heneicosanoic	$0.17\pm0.01_a$	$0.16\pm0.09_a$	$0.32\pm0.13_a$
C22:0, Behenic	$0.42\pm0.08_a$	$0.46\pm0.15_a$	$0.91\pm0.32_a$
C23:0, Tricosanoic	$0.87\pm0.07_a$	$0.71\pm0.00_b$	$1.64\pm0.01_c$
C24:0, Lignoceric	$0.18\pm0.02_a$	$0.27\pm0.01_a$	$0.43\pm0.05_b$
C14:1 Myristoleic	$0.09\pm0.03_a$	$0.56\pm0.49_a$	$0.50\pm0.29_a$
C16:1, Palmitoleic	$5.88\pm0.10_a$	$5.68\pm0.06_a$	$3.00\pm0.04_b$
C17:1, cis-10 Heptadecanoic	$0.41\pm0.21_a$	$0.58\pm0.04_a$	$0.67\pm0.05_a$
C18:1, Elaidic			4.17±0.10
C18:1, Oleic	$23.55\pm1.16_a$	$21.15\pm0.25_a$	$12.83\pm0.05_b$
C20:1, cis-11 Eicosenoic	$0.42\pm0.02_a$	$0.29\pm0.05_{ab}$	$0.20\pm0.01_b$
C22:1, Erucic	$0.07\pm0.02_a$	$0.37\pm0.33_a$	
C24:1, Nervonic	0.09±0.04		
C18:2, Linolelaidic	$0.24\pm0.01_a$	$0.20\pm0.04_a$	
C18:2, Linoleic (LA) (ω6)	$6.75\pm0.17_a$	$3.62\pm0.10_b$	$2.83\pm0.10_c$
C20:2, cis-11,14 Eicosadienoic (ω6)	$0.82\pm0.02_a$	$0.46\pm0.02_b$	$0.79\pm0.02_a$
C22:2, cis 13,16 Docosadienoic (ω6)	$0.05\pm0.01_a$	$0.11\pm0.02_a$	
C18:3, γ-linolenic (GLA) (ω6)	$0.56\pm0.06_a$	$0.48\pm0.12_a$	$0.45\pm0.11_a$
C18:3, α-linolenic (ω3)	$1.32\pm0.04_a$	$2.37\pm0.05_b$	$1.47\pm0.04_a$
C20:3, cis-8,11,14 Eicosatrienoic (hGL) (ω6)	$1.43\pm0.05_a$	$1.07\pm0.02_b$	$0.87\pm0.07_b$
C20:3, cis-11,14,17 Eicosatrienoic (ω3)	$0.65\pm0.01_a$	$0.84\pm0.08_b$	$0.54\pm0.05_a$
C20:4, Arachidonic (ω6)	$4.53\pm0.11_a$	$2.50\pm0.10_b$	$6.64\pm0.22_c$
C20:5, Eicosapentaenoic (EPA) (ω3)	$1.41\pm0.02_a$	$0.95\pm0.03_b$	$3.92\pm0.06_c$
C22:6, Docosahexaenoic (DHA) (ω3)	$4.33\pm0.04_a$	$1.56\pm0.00_b$	$12.31\pm0.17_c$
Σ Total Fatty acids	**100**	**100**	**100**
Number of fatty acids	**32**	**31**	**29**
Σ Saturated Fatty acids (SFA)	$\mathbf{47.41\pm1.11_a}$	$\mathbf{57.23\pm1.06_b}$	$\mathbf{48.8\pm0.88_a}$
Σ Unsaturated Fatty acids (UFA)	$\mathbf{52.59\pm2.14_a}$	$\mathbf{42.77\pm1.80_b}$	$\mathbf{51.20\pm1.39_a}$
Σ Monounsaturated fatty acids (MUFA)	$\mathbf{30.51\pm1.59_a}$	$\mathbf{28.63\pm1.21_a}$	$\mathbf{21.37\pm0.55_b}$
Σ Polyunsaturated fatty acids (PUFA)	$\mathbf{22.08\pm0.55_a}$	$\mathbf{14.14\pm0.59_b}$	$\mathbf{29.83\pm0.8_c}$
Σn-3	$\mathbf{7.7\pm0.12_a}$	$\mathbf{5.72\pm0.17_b}$	$\mathbf{18.25\pm0.32_c}$
Σn-6	$\mathbf{14.14\pm0.43_a}$	$\mathbf{8.23\pm0.38_b}$	$\mathbf{11.59\pm0.52_c}$
n-3/n-6	**0.54**	**0.69**	**1.57**
PUFA/SFA	**0.47**	**0.25**	**0.61**

Values are shown as mean± standard deviation of triplicates.

In the same line, values with different letters (a, b) are significantly different ($P<0.05$).

Thirty two fatty acids were identified in raw samples of fish oil *C. nigrodigitatus*. This number is very high compared to 20 and 17 obtained by Mouokeu *et al.* (2018) on oil of the same fish species collected at Yabassi and extracted respectively by press after boiling and maceration into hexane. This number is also higher than 23 obtained by Tenyang *et al.* (2016) on *C. nigrodigitatus* from Lake Maga and 22 recorded by Tenyang *et al.* (2014) on *A. maculatus* from Wouri. These differences could be explained by some extrinsic factor and the extraction method used. The oil of raw fish of this study contains 47.41±1.11, 30.51±1.59, and 22.08±0.55 % of saturated, monounsaturated and polyunsaturated fatty acids respectively. These proportions are also different from those of Tenyang *et al.* (2016) who recorded 43.52, 42.53 and 11.62 percent, respectively in the same fish from Lake Maga and Mouokeu *et al.* (2018) who also registered 50.09 - 51.72; 30.5 - 33.9 and 18.4 - 12.92 % respectively on the same fish species.

The most abundant fatty acids were Palmitic acid (C16:0) and Oleic acid (C18:1) with 27.61±0.50 % and 23.55±1.16 % respectively. These results corroborate with those of previous authors on *C. nigrodigitatus*. Tenyang *et al.* (2016) found an abundance of 22.75 and 32.96% while Mouokeu *et al.* (2018) found 27.5 - 34.0 % and 23.9 - 26.3% of these two fatty acids respectively. Oleic acid is n-9 fatty acids which plays a moderate role in the body. The n-6 fatty acids cannot be synthesized by humans and are therefore considered as essential fatty acids (Watanabe *et al*., 1989; Osborn & Akoh, 2002; Bell & Sargent, 2003). Linoleic (C18:2, ω6), Arachidonic (C20:4, ω6) and Docosahexaenoic (DHA) (C22:6, ω3) acids were in general, the polyunsaturated fatty acids found in the largest percentages of 6.75±0.17 %, 4.53±0.11 % and 4.33±0.04 % respectively. These findings are in line with previous studies conducted in catfish samples (Mouokeu *et al.*, 2018; Tenyang *et al.*, 2016; Tenyang *et al.*, 2013**;** Weber *et al.,* 2002; Sharai *et al., 2002)*. DHA are the fatty acids predominant in n-3 series of *C. nigrodigitatus*. This fatty acid has been considered as important for brain, eyes development and good cardiovascular health (Conner, (1997). *C. nigrodigitatus* had low level of n-3 PUFA when compared to the sum of n-6. However, the value 0.54 recorded on the n-3/n-6 ratio in this study is very high when compared to 0.3 and 0.2 obtained by Weber *et al.* (2002) and Sharai *et al.* (2002) respectively. These differences could be attributed to their diet. Several authors have concluded that FA profiles in fish reflect the diets of the animals (Zenebe *et al.*, 1998a; 1998b, Ahmed *et al*., 2010; Tenyang *et al.*, 2016; Osibona, 2011). Also, feeding of fish depend on the environmental conditions, including temperature, salinity, prey composition and types of metabolism (Sargent *et al*., 2002).

As shown in the table 3, smoking has significantly increased ($P<0.05$) the amount of saturated fatty acids, meanwhile it has significantly reduced ($P<0.05$) the amounts of unsaturated ones (n-3 and n-6 polyunsaturated fatty acids). The increase in saturated fatty acids concerns Lauric, Tridecanoic, Myristic, Palmitic, Heptadecanoic and Stearic acids. Nervonic (C24:1) acid disappeared with this treatment. The decrease of polyunsaturated fatty acids concerns Linoleic, Cis-11, 14-Eicosadienoic, cis-8,11,14-Eicosatrienoic, Arachidonic, Eicosapentaenoic and Docosahexaenoic acids. The ratio PUFA/SFA regressed from 0.47 to 0.25. Similar effects have been recorded in our precedent research in smoking of *Oreochromis niloticus* in the same zone (Djopnang *et al.*, 2017). The decreased of this quality index could be related to the fat oxidation, which was confirmed by the previous results on the indices of acids and peroxides. From this effect, Julie (2002) concluded that Fat oxidation can decrease the level of essential fatty acids in the diet and can lower the overall food quality by introducing free radicals and other oxidized products. It is well known in the literature that a ratio of 0.2 is associated with hypercholesterolemia, while the oils with 0.8 ratios are recommended to prevent cardiovascular diseases (Rahman *et al*., 1995).

Boiling also affects the fatty acid profile. In general, the loss of four fatty acids with boiling is observed: Nervonic, Linolelaidic, Erucic and Docosadienoic (ω6). A new Elaidic acid was generated during cooking. The cooking processes did not significantly influence ($P>0.05$) the total saturated fatty acids, but, a significant ($P<0.05$) decrease in monounsaturated fatty acids is registered. In Contrast, a significant ($P<0.05$) increase in polyunsaturated fatty acids was registered with the increase of n-3 fatty acids. This process increased the nutritional status of n-3 PUFA like the n-3/n-6 and PUFA/SFA ratios at 1.57 and 0.61 respectively, which are necessary for cardiovascular diseases prevention. These results are in agreement with our previous research in boiling of *O. niloticus* (Djopnang *et al.*, 2017). The increase in PUFA concerns Docosahexaenoic (DHA, ω3), Eicosapentaenoic (EPA, ω3), and Arachidonic (ω6), while the decrease of MUFA and PUFA concerns Palmitoleic, Oleic, cis-11 Eicosenoic, Linoleic (LA, ω6) and cis-8,11,14 Eicosatrienoic (hGL, ω6) respectively. The increase in PUFA and different quality oils ratio of *C. nigrodigitatus* could be justified by extraction. Indeed, during extraction, fine grinding of the skin in raw samples was difficult unlike in boiled samples. Boiling has the role of weakening the cell membranes, and in turn facilitates the extraction of oils. The only source of higher fatty acids could reside in the subcutaneous fat deposit. Similar effects were observed by Kaitaranta (1980) on

the *Coregonus albula*.

4. Conclusion

The present study consisted in an evaluation of the influence of smoking and boiling processes of a selected freshwater fish *C. nigrodigitatus* commercialized in Yabassi-Cameroon on the chemical composition and lipids qualities. This fish was found to be a good source of protein, minerals and fatty acids. It is equally rich in essential PUFAs, such as EPA and DHA, which are good for health. Processing by smoking and boiling methods applied to this fish influenced significantly its composition. Smoking concentrates ash value and decreases the amounts of water, protein, PUFA such as Docosahexaenoic (DHA), Eicosapentaenoic (EPA), Arachidonic, cis-8,11,14-Eicosatrienoic (hGL) and cis-11,14-Eicosadienoic and PUFA/SFA ratio. Thus, the drastic decline of this last parameter would indicate that oil of *C. nigrodigitatus* smoked could expose the consumer to cardiovascular diseases. Both processes led to an increase of potassium, sodium and magnesium. Lipid oxidation was revealed by the increase of free fatty acids and peroxide value. Moreover, boiling improved the values of the ratio Omega-3/Omega-6 and PUFA/AGS, which upgrade their nutritional status. Another advantage of this treatment resides in the fact that nutrients lost in the cooking water could be recovered by consuming its juice. Thus, boiling would be the best way for their nutritional valorization.

Conflict of Interests

The authors declare to do not have any conflict of interest.

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References

Ackman, R. G. (1990). Seafood lipids and fatty acids. *Food Rev. Int., 6*, 617-646. https://doi.org/10.1080/87559129009540896

AFNOR (1981). *Recueil des Normes Françaises*. Corps Gras, Graines Oléagineuses, Produit Dérivés, 2ème édition, AFNOR, Paris, P438.

Ahmed, E. O., Ali, M. E., Kalid, R. A., Taha, H. M., & Mahammed, A. A. (2010). Investigating the quality changes of raw and hot smoked *Oreochromis niloticus* and *Clarias lazera*. *Pak J Nut., 9*, 481-484. https://doi.org/10.3923/pjn.2010.481.484.

Akande, G. R., Oladosu, O. H., & Tobor, J. G. (1998). A Comparative technical and economic appraisal of fish smoking: Two traditional ovens & a new Improved Magbon-Alade oven. *FAO Fisheries Report, 574*, 70-75.

Akinwumi, F. O. (2014). Effects of Smoking and Freezing on the Nutritive Value of African Mud Catfish, *Clarias gariepinus* (Burchell, 1822). *Journal of Agricultural Science, 6*(11), 143-149.

AOAC (2000). Official Methods of Analysis of AOAC International, 17th Edition. William Horwitz (Ed.). AOAC International, 2200.

AOAC. (1990). *Official methods of analysis*, 15 Edition, Washington DC, 222-245.

Argen, J. J., Al-Amad, H., & Hanninen, O. (1991). Fatty acid content and composition of five fish species in the Persian Gulf. *Comp. Biochem. Physiol. 100B*, 339-341.

Azamand, K. M., & Ali, Y. (2004). Biochemical Assessment on Selected Fresh Fish. *Journal of Biological Sciences, 4*(1), 9-10. https://doi.org/10.3923/jbs.2004.9.10.

Bell, J. G., & Sargent, J. R. (2003). Arachidonic acid in aquaculture feeds: Current status and future opportunities, *Aquaculture, 218*, 491-499. https://doi.org/10.1016/S0044-8486(02)00370-8.

Bligh, E. G., & Dyer, W. J. (1959). A Rapid Method of Total Lipid Extraction and Purification. *Canadian Journal of Biochemistry & Physiology, 37*(8), 911-917. https://doi.org/10.1139/o59-099.

Brody, T. (1994). *Nutritional Biochemistry*. San Diego, CA: Academic Press. 2nd Edn, 761-794.

Christie, W. W. (1993). Preparation of esters derivatives of fatty acids for chromatographic analysis *In Advances in Lipid Methodology*, Two (Christie, W.W. ed.) Oily Press, Dundee, Scotland, 69-111.

Clifford, M. N, Tang, S. L., & Eyo, A. A. (1980). The development of analytical methods for investigating chemical changes during fish smoking, Advances in Fish Science and Technology, Fishing News Books Limited, Farnham, 86-290.

Conner W. E. (1997). The Beneficial Effects of Omega-3 Fatty Acids: Cardio Vascular Diseases and Neurodevelopment, *Current Opinion in Lipidology, 8*(1), 1-3. https://doi.org/10.1097/00041433-199702000-00001

Connor, W. E. (2000). Importance of n-3 fatty acids in health and disease. *Am. J. Clin. Nutr.. 71*, 1715-1755. https://doi.org/10.1093/ajcn/71.1.171S.

Djopnang, D. J., Njinkoue, J. M., Womeni, H. M., Gouado, I., Tomedi-Tabi, E. M., Tiwo, T. C., … Tchoumbougnang, F. (2017). Valeur nutritionnelle et Influence de la période de capture, fumage et cuisson à ébullition sur le potentiel nutritionnel d'Oreochromis niloticus du fleuve Nkam (Yabassi-Cameroun). *Cameroon Journal of Experimental Biology, 11*(01), 34-46. Retrieved from http://www.ajol.info/browse-journals.php/

Dvorak, Z., & Vognarova, I. (1965). Available lysine in meat and meat products. *Journal of Science and Food Agriculture, 16*(6), 305-310. https://doi.org/10.1002/jsfa.2740160604.

Effiong, B. N., & Fakunle, J. O. (2012). Proximate and Mineral Content of Traditional Smoked Fish Species from Lake Kainji, Nigeria. *Bulletin of Environment, Pharmacology and Life Sciences, 1*(4), 43-45.

Eymard, S. (2003). Mise en Evidence et Suivi de l'Oxydation des Lipides au Cours de la Conservation et de la Transformation du Chinchard (*Trachurus trachurus*), Choix des Procédés, Thèse de Doctorat, Université de Nantes, 143P.

Eyo, A. A. (2014). Effects of Smoking and Freezing on the Nutritive Value of African Mud Catfish, *Clarias gariepinus* (Burchell, 1822). *Journal of Agricultural Science, 6*(11), 143-149.

Fallon, S., & Enig, M. G. (2001). *Nourishing Traditions*. The Cook book that Challenges Policitally Correct Nutrition and the Diet Dictocrats. Revised 2nd Edn., 40-45.

FAO & WHO (Food and Agriculture Organization & World Health Organisation) (2007). Protein and amino acid requirements in human nutrition. Report of a joint WHO/FAO/UNU Expert Consultation. WHO Technical Report Series 935. Geneva, Switzerland, Cholé-Doc No 111.

FAO, (2014). Sustainable fisheries and aquaculture for food security and nutrition, Food and Agriculture Organization of the United Nations. 119. Retrieved from http://www.fao.org/documents/card/en/c/63d4de9f-50df-49b1-b6d2-b7d0368744ea.

Franckel, E. N. (1998). *Lipid oxidation*. The Oily press 10, Dundee, Scotland.

Gokoglu, N., Yerlikaya, P., & Cengiz, E. (2004). Effects of cooking methods on the proximate composition and mineral contents of rainbow trout (*Oncorhynchus mykiss*). *Food Chemistry, 84*, 19-22. https://doi.org/10.1016/S0308-8146(03)00161-4.

Hopkins, C., Teugels, G., & Stiassny, M. L. (2007). *Poissons d'eaux douces et eaux saumâtres de basse Guinée, Ouest de l'Afrique Centrale*. Vol1. Paris. France: Institut de Recherche pour le Développement. 805.

Julie, M. J. (2002). Chapter 13: Food safety. *In* Zdzislaw E. Sikorski, Second Edition *Chemical and Functional Properties of Food Components*, ISBN 1-58716-149, pp 291-306.

Kaitaranta, J. K. (1980). Lipids and fatty acids of whitefish (*Coregonus albula*) flesh and roe, *J. Sci. Food Agric., 31*, 1303-1308. https://doi.org/10.1002/jsfa.2740311213.

Krauss, R. M., Eckel, H. R., Howard, B., Appel, L. J., Daniels, S. R., Deckelbaum, R. J., … Bazzarre, T. L. (2000). AHA Dietary Guidelines, Revision 2000: A Statement for Healthcare Professionals from the Nutrition Committee of American Heart Association. *Circulation, 102*, 2284-2299. https://doi.org/10.1161/01.CIR.102.18.2284.

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2003). Fish consumption, fish oil, Omega-3 fatty acids and cardiovascular disease. *Arteriosclerosis Thrombosis Vascular Biology, 23*, 20-31. https://doi.org/10.1161/01.ATV.0000038493.65177.94

Kumolu-Johnson, C. A. A., & Ndimele, P. E. (2010). The effects of smoking on the nutritional qualities and shelf-life of *Clarias gariepinus* (BURCHELL 1822). *African Journal of Biotechnology, 9*(1), 073-076.

Labuza, T. P. (1974). Effect of Dehydration and Storage on Nutrient Retention in Food. *Food Technology, 27*, 20-26.

Liu, Y.C. (1996). *Food Chemistry*. Chinese Light Industry Press, Beijing, 83.

Mouokeu, R. S., Womeni, H. M., Njike, N. F. H., Tonfack, D. F., Djopnang, D. J., Tchoumbougnang F., & Kuiate,

J. R. (2018). Chemical composition and antibacterial activity of oils from *Chrysicthys nigrodigitatus* and *Hepsetus odoe*, two fresh water fishes from Yabassi, Cameroon. *Lipids in Health and Disease, 17*, 45. https://doi.org/10.1186/s12944-018-0690-z.

Mozaffarian, D., Ascherio, A., Hu, F. B., Stampfer, M. J., Willet, W. C., Siscovick, D. S., & Rimm, E. R. (2005). Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. *Circulation, 11*, 157-164. https://doi.org/10.1161/01.CIR.0000152099.87287.83

Olele, N. F. (2012). Nutrient composition of *Gnathonemus tamandua*, *Chrysichtys nigrodigitatus* and *Anchenoglanis bisculatus* caught from river Niger. *Nig. J. Environ., 8*(2), 21-27.

Onyeike, E. N., Ayoologu, E. O., & Ibegbulam, C. O. (2000). Evaluation of the nutritional value of some crude oil in polluted freshwater fishes, *Global Journal of Pure and Applied Sciences, 6*, 227-233. https://doi.org/10.4314/gjpas.v6i2.16112.

Onyeike, N. E., & Oguike, U. J. (2003). Influence of Heat Processing Methods on the Nutrient Composition and Lipid Characterization of Groundnut (*Arachis hypogeaa*) Seed Pastes, *Biokemistri, 15*(1), 34-43.

Onyeike, N. E., & Oguike, U. J. (2003). Influence of Heat Processing Methods on the Nutrient Composition and Lipid Characterization of Groundnut (*Arachis hypogeaa*) Seed Pastes. *Biokemistri, 15*(1), 34-43.

Osborn, H. T., & Akoh, C. C. (2002). Structured lipids-novel fats with medical, nutraceutical and food applications, *Comp. Rev. Food Sci., 3*, 93-103.

Osibona, A. O. (2011). Comparative study of proximate composition, amino and fatty acids of some economically important fish species in Lagos, Nigeria. *Afr. J. Food Sci., 5*, 581-8.

Pauwels, J. M., Van Ranst, E., Verloo, M., & Mvondo, Z. A. (1992). Manuel de laboratoire de pédologie, *Publication Agricole, AGCD, 28*, 263.

Rahman, S. A., Huah, T. S., Nassan, O., & Daud, N. M. (1995). Fatty acid composition of some Malaysian fresh water fish. *J. Food Chem., 54*, 45-49. https://doi.org/10.1016/0308-8146(95)92660-C.

Rao, V. S., Adhikari, H. R., & Nair, P. M. (1995). Fatty acid composition of some Indian fish varieties. *ASEAN Food J., 10*(2), 62-65.

Russel, E.W. (1973). *Soil conditions and plant growth*. Supergene Zone, M. Nedra, 19 (in Russian).

Salán, O. E., Juliana, A. G., & Marilia, O. (2006). Use of smoking to add value to salmoned trout, *Braz. Arch. Biol. Technol., 49*(1), 57-62. https://doi.org/10.1590/S1516-89132006000100007.

Sargent, J. R., Tocher, D. R., & Gordon, B. J. (2002). The lipids, *In* Halver JE, Hardy RW, eds. *Fish nutrition*. Academic Press, Elsevier Science, New York, 181-257.

Sharai, N., Suzuki, H., Tokairin, S., Ehara, H., & Wada, S. (2002). Dietary and Seasonal Effects on the Dorsal Meat Lipid Composition of Japanese (*Silurus asotus*) and Thai Cat- fish (*Clarias macrocephalus* and Hybrid *Clarias macrocephalus* and *Clarias gariepinus*). *Comparative Biochemistry and Physiology Part A, 132*(76), 609-619. https://doi.org/10.1016/S1095-6433(02)00081-8.

Suzuki, H. S., Park, J., Tamura, M., & Ando, S. (1998). Effect of the long-term feeding of dietary lipids on the learning ability, fatty acid composition of brain stem phospholipids and synaptic membrane fluidity in adult mice: a comparison of sardine oil diet with palm oil diet. *Mechanisms of Ageing and Development, 101*, 119-128. https://doi.org/10.1016/S0047-6374(97)00169-3.

Tenyang, N., Ponka, R., Tiencheu, B., Tonfack, D. F., & Womeni, H. M. (2016). Proximate Composition, Fatty Acid and Mineral Contents of Four Freshwater Fish from Maga Lake (Far North Region of Cameroon). *American Journal of Food Science and Technology, 4*(3), 64-69. https://doi.org/10.12691/ajfst-4-3-2.

Tenyang, N. (2015). *Poissons gras consommés au Cameroun: caractérisation chimique, influence des traitements culinaires et de conservations sur la qualité des lipides et les propriétés fonctionnelles des protéines des tourteaux.* (Thèse de Doctorat/Ph.D en Biochimie non publiée) Université de Dschang, Dschang, Cameroun, 186P.

Tenyang, N., Womeni, H. M., Linder, M., Tiencheu, B., Villeneuve, P., & Tchouanguep, M. F. (2014). The chemical composition, fatty acid, amino acid proiles and mineral content of six ish species commercialized on the Wouri river coast in Cameroon. *LA RIVISTA ITALIANA DELLE SOSTANZE GRASSE GRASSE* - VOL. XCI - APRILE/GIUGNO: 129-138.

Tenyang, N., Womeni, H. M., Tiencheu, B., Hrodrik, N., Takugan, F., Tchouanguep, M. F., Villeneuve, P., &

Linder, M. (2013). Lipid Oxidation of Catfish (*Arius maculatus*) after Cooking and Smoking by Different Methods Applied in Cameroon. *Food and Nutrition Sciences, 4*, 176-187. https://doi.org/10.4236/fns.2013.49A1025

Vivien (1991). *Faune du Cameroun : guide des mammifères et poissons*. GICAM et Ministère de la coopération et du développement. Yaoundé, 271.

Watanabe, T., Arakawa, T., Takeuchi, T., & Satoh, S. (1989). Comparison between eico sapentaenoic and docosahexaenoic acids in terms of essential fatty acid efficiency in juvenile striped jack *Pseudocaranx dentex*. *Nippon Suisan Gakkasihi, 55*, 1989-1995. https://doi.org/10.2331/suisan.55.1989.

Weber, J., Bochi, V. C., Ribeiro, C. P., Victorio, A. M., & Emanuelli, T. (2002). Effect of Different Cooking Methods on Oxidation, Proximate and Fatty Acid Composition of Silver Catfish (*Rhamdia quelen*) Fillets. *Food Chemistry, 106*(1), 140-146. https://doi.org/10.1016/j.foodchem.2007.05.052.

Yeannes, M. J., & Almandos, M. E. (2003). Estimation of fish proximate composition starting from water content. *Journal of Food Composition and Analysis, 16*, 81-92. https://doi.org/10.1016/S0889-1575(02)00168-0.

Zenebe, T., Ahlgren, G., & Boberg, M. (1998a). Fatty acid content of some freshwater fish of commercial importance from tropical lakes in the Ethiopian Rift Valley. *J Fish Biol., 53*, 987-1005. https://doi.org/10.1111/j.1095-8649.1998.tb00458.x.

Zenebe, T., Ahlgren, G., Gustafsson, I. B., & Boberg, M. (1998b). Fatty acid and lipid content of *Oreochromis niloticus* L. in Ethiopian lakes dietary effects of phytoplankton. *Ecol. Freshw. Fish, 7*, 146-58. https://doi.org/10.1111/j.1600-0633.1998.tb00181.x.

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Effects of Varying Grilled Sorghum Content on the Quality Parameters of *Urwagwa*, a Traditional Rwandese Banana-based Alcoholic Beverage

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Abstract

An alcoholic beverage called *Urwagwa* is a common and popular traditional Rwandese drink that is produced from banana juice supplemented with coarsely ground grilled sorghum. Due to limited information on how different sorghum concentration affected the physico-chemical characteristics of this traditional beverage, a study was carried out to investigate how varying this ingredient influenced the quality of the final product. Different levels of grilled coarsely ground sorghum (0%, 2%, 5%, 7%, 10% and 15%) were mixed with banana juice and natural fermentation allowed to take place in six separate fermenters for six days at room temperature (25°C). Four parameters; ethanol content, total soluble solids, pH and titratable acidity were monitored after every 24 hours for 144 hours. Results obtained at the end of fermentation indicated that the ethanol content in all fermenters ranged from 10-12% v/v, total soluble solids 8.1-9.7°Brix, pH 4.0-4.7 and titratable acidity 0.6-0.9%. The highest fermentation rate was observed in fermenters containing ground grilled sorghum compared to the control (0% sorghum added).The highest total soluble solid at the completion of fermentation was found in banana beverage with zero percent of sorghum while the highest pH was generally found in fermenters with sorghum combinations. High titratable acidity was observed in fermenter with zero percent of sorghum. The concentration of 5% of ground grilled sorghum was found to result to the most desirable alcoholic banana beverage as it gave the highest yield of ethanol content and other biochemical parameters, as well as a more desirable flavor. Based on these findings, it could be recommended that the traditional *Urwagwa* brewers adopt the 5% of sorghum content in the production process in order to obtain a better quality and more acceptable alcoholic beverage.

Keywords: alcoholic drinks, banana, banana beverage, traditional alcoholic beverage, *urwagwa*

1. Introduction

Alcoholic beverages are among the leading drinks in many African communities in terms of consumption (Shale, Mukamugema, Lues, & Venter, 2014). Due to their high consumption levels, these beverages have vitalized both traditional and industrial production. Almost all African societies have well established domestic production industries for the fermented alcoholic beverages, which to a great extent depends on traditional techniques, processes and resources (Mohapatra, Mishra, & Sutar, 2010). Commercial production is of a larger scale, adopting an industrial outlook that is often oriented towards mass production for consequent economic value. Despite the lower production levels of traditionally produced alcoholic beverages compared to commercially produced ones, their outlook and preference for consumption, especially by low-income earners and rural folks is on an upward trajectory in developing countries, Rwanda included. The reasons for this preference are possibly due to their low prices and the strong connection they have with the traditional cultures.

In Rwanda, *Urwagwa*, a popular traditional banana-based beverage, has a long historical significance to the consuming communities. This alcoholic beverage, which is made from banana juice blended with ground grilled sorghum through spontaneous fermentation, is mainly confined to domestic production with annual production of 700 million litres and an average consumption per capita of about 1.2 litres per day (Immaculate Kanyana, 2013). Although this alcoholic beverage can be made using a wide variety of ripe bananas cultivars (AAA-EAHB), the most commonly used for its production in Rwanda are forty cultivars belonging to Lujugira-Mutika subgroup, with 'Intuntu', 'Intokatoke', 'Injagi, 'Mbwaziruma, Gisubi (ABB), Gros Michel (AAA) and 'Kamaramasenge being the most preferred (Karamura, Karamura, & Tinzaara, 2012). During its production, sorghum which is thought to accelerate the fermentation process and enhance flavour development in the end product is included as part of the composite mixture (Wilson, 2012; Munyanganizi, 1975).

The *Urwagwa* production process is however yet to be determined and documented through scientific studies (Wilson,2012). Although traditional banana-based alcoholic beverage production is an expansive domestic industry and has a long historical existence in Rwandan society, there is little research conducted on its production. The limited studies carried out on the fermentation of banana to produce alcoholic beverages have rarely investigated the aspect of sorghum involved in the fermentation process and the physico-chemical changes that normally take place during production (Wilson, 2012). It is for this reason that this study was carried out to investigate the effects of varying sorghum concentration on selected physico-chemical parameters that can have an influence on the final product quality. The study also served to identify the optimum banana juice-sorghum combination that would result to the best quality alcoholic beverage under the prevailing production conditions.

2. Materials and Methods

2.1 Collection and Ripening of Banana for Urwagwa Production

Four physiologically mature banana cultivars of Kayinja (*Musa* genus), Indaya, Intuntu or Igikashi *musa* groups (AA, AB, AAA, AAB, ABB) and Poyo *musa robusta (AAA group)* in which Intuntu represented 67 percent and 33 percent of rest respectively were collected from a local market around Rugende in Gasabo district of Rwanda and transported in plastic crates under normal conditions to Centre de Formations et des Recherches pour le Développement des Technologies Appropriées (CFRDTA) for experimentation. The bananas were placed in plastic container and covered by spear grass (*Imperata cylindirica*) and green banana leaves in order to ensure the warm conditions and promote ethylene production and accumulation necessary for ripening. The bananas took 6 days to ripen indicated by their change in peel colour to yellow and softening.

2.2 Banana Juice Extraction

Ripe bananas were peeled manually by hand peeling. Juice extraction was mechanically made by mixing 69 kg of peeled banana with 6.9 kg of special grass (*Imperata cylindirica)* which had been previously washed and dried in order to remove dust and other miscellaneous materials that could contaminate the beverage. The banana juice extraction, which was aided by *Imperata cylindirica,* was achieved by use of a fabricated juice extracting machine. The juice was filtered using a plastic sieve and the remaining juice in banana-grass pulp was exhausted by pressing using a fabricated juice extracting machine pressor.

2.3 Banana Juice-Sorghum Combinations Used in Urwagwa Production

The obtained banana juice after pulp squeezing was pasteurized at 90^{o}C for 30 minutes to prevent premature fermentation caused by indigenous yeast and lactic acid bacteria and other contaminating microorganisms. Lightly roasted sorghum, often red sorghum in ground form is commonly used in the production of banana beer in Rwanda as an adjunct where it is thought to contribute to the flavour and colour of the finished product (Munyanganizi,1975;Wilson, 2012). Different proportions of sorghum were mixed with a constant amount of extracted banana juice as shown in Table 1. The red sorghum used in this study which was previously washed, dried, lightly roasted and ground for *urwagwa* production was bought from Kimironko market in Rwanda.

Table1. Banana juice-sorghum combinations used in the production of *Urwagwa* through spontaneous fermentation

Banana juice (l)	Sorghum (% w/v)
3	0
3	2
3	5
3	7
3	10
3	15

2.4 Determination of Initial pH of Banana Juice and Banana Juice-Sorghum Mixture

The pH of banana juice and banana juice-sorghum composite mixture before fermentation was evaluated with pH meter (PB-11Sortorius, Germany) at 25^{o}C. A volume of 20 ml was taken and the measurement of each sample was done after calibration of pH-meter with a buffer (4-7).

2.5 Determination of Protein Content in Banana Juice-Sorghum Mixture before Fermentation

The protein content of banana juice-sorghum mixture was determined on the basis of total nitrogen content by using auto titration Kjeltec models 2300 (Denmark). A representative sample of 0.4g of each of the composite mixture was weighted into 100 ml labelled digestion tubes by use of an electronic balance (Sortorius CPA3245). One Kjeltabs Cu 3.5 tablet of catalyst and 6 ml of concentrated H_2SO_4 were added to each digestion tube. The samples were heated at 420°C for 2 hours and after digestion, they were cooled to room temperature (25°C). After cooling the tubes containing the samples were transferred into distillation unit (Foss 2300 Kjeltec) and protein percentage determined according to the Kjeltec machine manufacturer's instructions where mass of each sample was entered in the Kjeltec system and the protein was reported as percentage and calculated according to the formula by (Muriro,2017). On the basis of early determinations, the average nitrogen (N) content of proteins was found to be about 16 percent, which led to use of the calculation N x 6.25 (1/0.16 = 6.25) to convert nitrogen content into protein content. The following equation was used in the calculation of protein content in the samples.

$$\%\ Nitrogen = \frac{(T - B) * N * 14.007 * 100}{W(mg)}$$

$$\%\ Protein\ = \%\ Nitrogen * F$$

Where;

T = Titration volume for sample (ml)

B = Titration volume for blank (ml)

F = Conversion factor for nitrogen to protein

W = Weight of the sample (mg)

2.6 Determination of Total Soluble Solids (TSS) in Banana Juice-Sorghum Mixture before Fermentation

Total soluble solids of the banana juice and banana juice-sorghum were determined as °Brix according to Serpen, (2012) with some modification by which hand held optical refractometer was replaced by A.Kruss Optronic refractometer GmbDR6100 (Germany) which give a direct reading of ^{o}Brix, or relative sugar concentration. The refractometer was cleaned using distilled water before sample analysis. Distilled water was used as control in this experiment (0°Brix). The amount of sugar in the sample was directly read from the refractometer.

2.7 Fermentation of Banana Juice and Banana Juice-Sorghum Mixtures

The fermentation was carried out in six fermenters of 3 litres of volume. One fermenter, with no added sorghum (banana juice only) served as the control. The experiment was carried out in triplicates. The content in fermenters was left to ferment spontaneously at room temperature (25^{o}C) for 6 days. The whole production process, from ripening of the bananas to final fermented alcoholic product took 13 days.

2.7.1 *Monitoring of the Fermentation Process*

During fermentation 100 ml of samples was collected, in sterile bottles from each fermenter after every 24 hours for 6 days to determine various physico-chemical parameters (pH, titratable acidity, ethanol accumulation and sugar). After collection the samples were pasteurized at 60^{o}C for 15 minutes to end fermentation after which they were stored at 4^{o}C in the refrigerator before analysis.

2.7.2 *Determination Ethanol Content*

Test for ethanol content was conducted using WineScan™ SO2 (Denmark) from the beginning to the end of the fermentation process. A 100 ml of sample to be analysed was filtered by using Whatman filters of 11µm size and 20 ml of filtrate transferred into vials from where they were sucked by the column of WineScan™ SO2 for analysis. Before analysing each sample, there was auto-cleaning of WineScan™ SO2 through zero setting the machine according to the manufacturer's instructions.

2.7.3 *Determination of Total Soluble Solids (TSS)*

Total soluble solids of *urwagwa* were determined as described for banana juice-sorghum composite mixture

above.

2.7.4 *Determination of pH*

The pH of the whole fermentation process was evaluated as aforementioned for banana juice and banana juice-sorghum combination before fermentation.

2.7.5 *Determination of Titratable Acidity*

The titratable acidity, expressed in percent of lactic acid, was determined by the titrimetric method according to (Association of Official Analytical Chemist[AOAC], 2005). A 5ml subsample from the 100 ml sample was used for titration after decarbonating by shaking. The sample was diluted twice with distilled water (10 ml) which was previously brought to boil at 90^{o}C and cooled at room temperature (25°C). Three drops of 1% phenolphthalein indicator was added to the sample in a conical flask and titrated with 0.0909M NaOH to a persistent faint pink colour compared against a white background. The titre volume was noted and used for calculations of the amount of total titratable acidity as percentage of lactic acid using the equation below.

$$\%\ lactic\ acid = \frac{ml\ of\ 0.0909M\ NAOH * Normality\ of\ NAOH * MW\ of\ acid}{Volume\ of\ sample * 10}$$

Where MW = molecular weight

3. Results and Discussion

3.1 Banana Juice and Banana Juice-Sorghum Composition in Regard to Soluble Sugar Concentration, Protein Content and pH before Fermentation

The addition of grilled sorghum to banana juice marginally increased the pH, soluble sugar concentration and protein content of the composite mixtures, and this change tended to increase with the percentage increase in grilled sorghum added (Table 2). Out of the three parameters determined, more change was noticeable with protein concentration than with the changes in pH and soluble sugar concentration on addition of grilled sorghum. The pH ranged from 4.68±0.05 to 4.75±0.03 (0% sorghum added and 15% sorghum added respectively), soluble sugar concentration ranged from 21.00±0.07 to 21.37±0.17 (0% sorghum added and 15% sorghum added respectively) and protein content 1.0±0.08 to 2.7±0.20 (0% sorghum added and 15% sorghum added respectively). Total soluble solids and protein are the main nutrients required for growth of yeast in wort fermentation. The slight increase in total soluble solids as well as protein concentration on addition of varying quantities of grilled sorghum can be attributed to the presence of free sugars and protein found in sorghum.

Table 2. Banana juice and banana juice-sorghum composition in regard to pH, soluble sugar concentration and protein content before fermentation. Values are means of triplicate determinations ±SD

Sorghum concentration	pH	Sugar (°Brix)	Protein (g)
0%	4.68±0.05	21.00±0.07	1.0±0.08
2%	4.69±0.02	21.07±0.06	1.3±0.01
5%	4.70±0.01	21.09±0.02	1.6±0.01
7%	4.71±0.06	21.10±0.10	1.8±0.09
10%	4.73±0.04	21.23±0.12	2.1±0.14
15%	4.75±0.03	21.37±0.17	2.7±0.20

3.2 Effect of Sorghum Content on Ethanol Concentration

Generally, the onset of ethanol production started at the same time in all the six fermenters (including the control), with even quantifiable levels at the start of the experiment (Figure 1). It was observed that the production of ethanol for all the composites tended to increase with time as well as the increase in percentage of sorghum added to the banana juice. The trend for the production of ethanol for all the composites except the one containing no sorghum (control) followed a similar pattern. There was a sharp increase in ethanol production for all the composites after the 48th hour except for the one containing no sorghum where the increase in this parameter seemed to occur rather steadily up to the 72nd hour, after which there was a sharp increase. Except for the control and the composite containing 10% sorghum, in all the other combinations, ethanol production tended to start decreasing before or at the start of the 144th hour of fermentation with the composite containing 15% sorghum showing the earliest onset of ethanol production decline. The final ethanol content in all fermenters ranged from (10.3-12.2%) with the control resulting in the least concentration and the composite containing 10% sorghum resulting into the highest concentration (Figure 1).

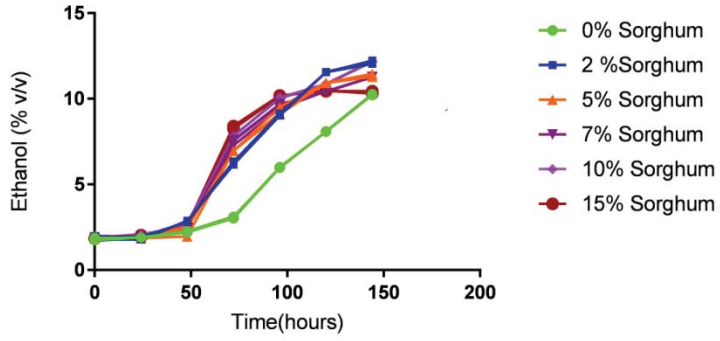

Figure 1. Changes in ethanol concentration during the spontaneous fermentation of *urwagwa* with varying sorghum combinations

These results showed that the alcohol content does not only result from banana juice but also from added roasted sorghum. The initial concentration of soluble sugars in banana juice with no added sorghum was the least at 21°Brix (Table 2). It is therefore not a surprise that the control (banana juice with no added sorghum) yielded the least ethanol than the fermenters containing the composite mixtures. Sugars are normally converted to ethanol by yeasts during fermentation, and the higher the concentration, the higher the expected ethanol yield (Raikar, 2012). From limited data available, previous studies by Davies (1993) and Shale et al.(2013) showed that banana beer with an ethanol content of 11-15% (v/v) and 8.7-18.1% (v/v) was produced from undiluted banana juice and from indigenous banana beer respectively. Similar study done by Byarugaba-Bazirake (2013) and Dieu et al. (2017) reported an alcohol content of 6.4-14.6% (v/v) from banana beverage made from three banana cultivars and 12% (v/v) ethanol in Rwanda banana beverage respectively.

There was early decline of ethanol in fermenter with 15% of sorghum and according to (Ferreira, 2004) the acetic acid bacteria may be responsible for oxidation of ethanol to acetic acid, leading to its reduction. The high trend of ethanol production in all fermenters other than the control could be attributed to the presence of nitrogen and minerals from sorghum that could have favoured the conditions for yeast growth compared to the fermentation of banana juice with no sorghum added (Deesuth, Laopaiboon, Jaisil, & Laopaiboon, 2012). Similar study by Picki-reix et al.(1986) has established the clear relationship between the ethanol production with free amino acids concentration in wort by which high free amino acid corresponded with high ethanol production. According to Carrau et al. (2008), Picki-reix et al. (1986), Barrajón-Simancas et al.(2011), Jiranek et al. (1993) and Sablayrolles et al. (1996) nitrogen deficiency in wort may lead to the sluggish of fermentation, the problem of which could be solved by supplementation with a nitrogen source. The need for a source of nitrogen in fermentation is also highlighted by (Deesuth et al.,2012; Akunna,2015). Sorghum is known to be a good source of nitrogen as well as other essential nutrients including minerals such as potassium that are reported to be necessary for yeast growth according to(Lindsay, 2010). (Kudo, Vagnoli, & Bisson, 1998) have reported the significance played by potassium in early fermentation of wort through acceleration of glucose consumption by yeast. In addition, potassium which is found in high concentration in sorghum plays a key role in pH tolerance of *Saccharomyces* and high yield of ethanol is produced at pH of 4.5 (Asli, 2010). Potassium deficiency may lead to the reduction of fermentation capacity of individual yeast cells or fermentation capacity of culture through the loss of yeast cell viability (Ferreira, 2004).

3.3 Effect of Sorghum Concentration on Soluble Sugar during Fermentation

Sugar is one of the main substrate in alcoholic fermentation of the wort. The initial concentration of total soluble sugars in all the combinations, at time zero, including in the control was on average, 21°Brix. This remained largely unchanged for all the combinations up to the 48th hour of the fermentation except for the sample containing 15% sorghum which showed a slight reduction (20°Brix). There was a sharp decline in soluble sugar concentration after the 48th hour of fermentation for all combinations except with the control where the decrease was observed to be steady (Figure 2).

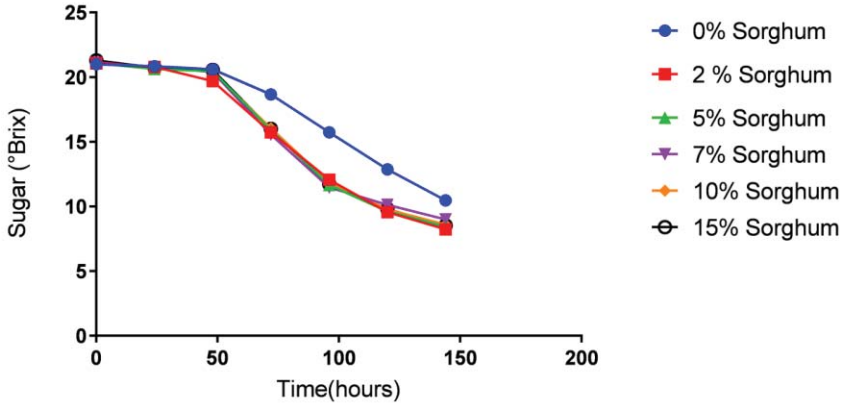

Figure 2. Changes in soluble sugar concentration (°Brix) during fermentation of *urwagwa* with varying sorghum concentration

At the end of the fermentation (144th hour), all the samples seemed to have similar levels of soluble sugars remaining. Previous studies indicates that the main fermentable sugars in ripe bananas are glucose (19-22% of total sugar), fructose (12-17% of total sugar) and sucrose (62-68% of total sugar) (Hammond, Egg, Diggins, & Coble, 1996). According to (Hammond et al.,1996) the ideal sugar content of unfermented wort and which lead to the production of beverage of 8-12% (v/v) ethanol should be around 15-25% (w/w). This observation is in agreement with the findings of this study in terms of soluble sugar concentration and ethanol produced during fermentation as previously discussed. Similar findings have also been reported (Byarugaba-Bazirake, 2013). The sugar level at the end of fermentation was slightly higher in fermenter with zero percent of sorghum (9.7°Brix) which is attributable to high residual sugar.

3.4 Effect of Sorghum Concentration on pH Variation during Fermentation

The pH level is an essential component of the fermentation process as it can have an impact on the quality of the fermenting process as well as outcome of the final product following the process. The initial pH of all the samples, including the control was similar, ranging from 4.0-4.7. From a previous study of Munyanganizi (1974) and (Newilah, Tomekpe, Fokou, & Etoa, 2009), the pH of fully ripened bananas ranges from 4.2 to 4.9, a finding which is in agreement with the results of this study. There was a common trend for all the samples during the fermentation process; the pH decreased as the fermentation progressed (Figure 3).

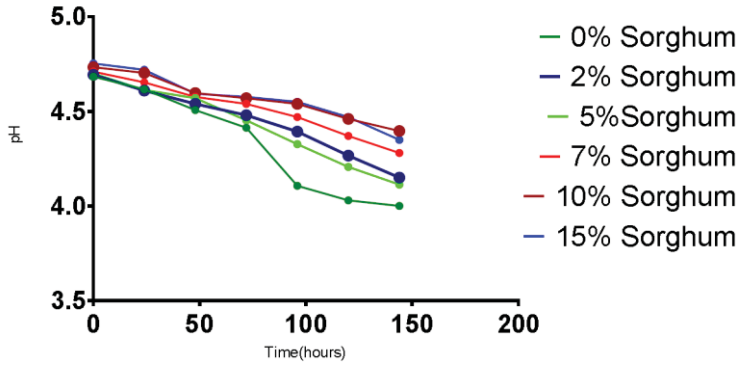

Figure 3. Changes in pH during fermentation of *urwagwa* with varying sorghum concentration

With the exception of the control (no sorghum added), the pH of the rest of the samples were found to decrease steadily up to the end of the fermentation period. There was a sharp decrease of pH in the control sample between the 72nd and 96th hour of fermentation. The less sharp drop in pH in samples containing sorghum compared to the control could be explained by higher content of minerals in sorghum that may have led to high buffering of the wort (Munyanganizi,1976). According to (Rwanda Standard Board,2017), the pH of banana beverages should range from 4.0 to 5.0 which means that the final products of this study complies with the country's requirement in terms of this attribute.

3.5 Effect of Sorghum Concentration on Titratable Acidity

Titratable acidity reflects the number of protons recovered during titration of acid by strong base at specific end point and it is an approximation of total acidity in a given product (Boulton, 1980). All the samples prior to the fermentation process (time zero) had quantifiable acidity which averaged to 0.17. There was a noticeably exponential increase in titratable acidity for all the samples between time zero and 24 hours during fermentation, after which the increase, for all the samples became gradual (Figure 3).

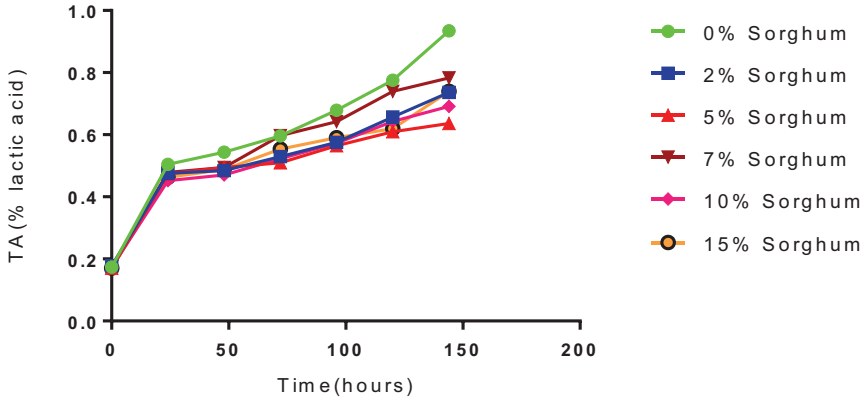

Figure 4. Changes in titratable acidity during fermentation of *Urwagwa* with varying sorghum concentration

Generally, a similar trend for all the samples in the development of the acidity (increase) was observed throughout the fermentation period. The acidity in the final products ranged from 0.64 and 0.93.The highest titratable acidity was found in banana juice fermented without sorghum compared to those with added sorghum. High titratable acidity in sample containing zero percent sorghum may be attributed to the possibility of presence of lactic bacteria involved in early fermentation compared to samples with sorghum added as at this point the fermenters with sorghum added had high ethanol content that could reduce the population of lactic acid bacteria (Sigmon, 1993). According to (Wilson et al.,2012), titratable acidity in traditional alcoholic banana beverage ranges from 0.18 to 0.9% while (Akubor, Obio, Nwadomere, & Obiomah, 2003) found 0.85% in final banana alcoholic beverage. Byarugaba-Bazirake,(2013) also reported titratable acidity expressed as g/L in banana alcoholic beverage produced by conventional and modern techniques from different banana cultivars which ranged from 5.24 to 8.4, which is in agreement with obtained results of this study.

4. Conclusion

Addition of lightly grilled sorghum in banana juice during the production of *Urwagwa* was found to be a good adjunct in fermentation due to its positive influence on the quality parameters assessed. Physico-chemical parameters investigated in this study by using different sorghum concentration were found to display a positive aspect in fermentation which, for example resulted to the sharp ethanol production. It was observed that the production of ethanol for all the composites tended to increase with time as well as the increase in percentage of sorghum added to the banana juice while pH and sugar seemed to decrease steadily up to the end of the fermentation period. Total titratable acidity as one of quality requirements for banana based alcoholic parameter was found generally to be similar in all samples at the completion of fermentation compared to the control. Addition of 5% grilled sorghum was found to be the optimal concentration leading to the best alcoholic beverage quality parameters in terms of ethanol content, pH and total titratable acidity which are in accordance with banana based alcoholic requirements. Economically, there is a big interest to use the minimum possible quantity of sorghum which fits with the highest quality of beverage meeting the standards for *Urwagwa*.

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References

Akubor, P. I., Obio, S. O., Nwadomere, K. A., & Obiomah, E. (2003). Production and Quality Evaluation of Banana Wine, *Plant Foods for Human Nutrition, 58*(3), 1-6

Akunna, J. C. (2015). *Brewing Microbiology*. *Brewing Microbiology*. https://doi.org/10.1016/B978-1-78242-331-7.00019-8

Asli, M. S. (2010). A study on some efficient parameters in batch fermentation of ethanol using Saccharomyces cerevesiae SC1 extracted from fermented siahe sardasht pomace. *African Journal of Biotechnology*, *9*(20), 2906-2912. https://doi.org/10.4314/ajb.v9i20.

Association of Official Analytical Chemist. (2005). *Official Methods of Analysis of AOAC International* (18th ed., Vol. 18th Editi). Gaithersburg, Md. : AOAC International, 2005.

B.Munyanganizi, C. (1976). Etude Comparative de deux procédés d'extraction du jus de banane appliqués ā deux Variétés différentes. *Industries Alimentaires et Agricoles*.

Barrajón-Simancas, N., Giese, E., Arévalo-Villena, M., Úbeda, J., & Briones, A. (2011). Amino acid uptake by wild and commercial yeasts in single fermentations and co-fermentations. *Food Chemistry*, *127*(2), 441-446. https://doi.org/10.1016/j.foodchem.2010.12.151

Boulton, R. (1980). The Relationships between Total Acidity, Titratable Acidity and pH in Wine. *Vitis*, *19*(1), 113-120. Retrieved from http://www.vitis-vea.de/admin/volltext/e017796.pdf

Byarugaba-Bazirake, G. W. P. van R., & W. K. (2013). The influence of commercial enzymes on wine clarification and on the sensory characteristics of wines made from three bana. American Journal of Biotechnology and Molecular Sciences. Institute of Wine Biotechnology, University of Stellenbosch, South Africa. 41-62. https://doi.org/10.5251/ajbms.2013.3.2.41.62

Carrau, F. M., Medina, K., Farina, L., Boido, E., Henschke, P. A., & Dellacassa, E. (2008). Production of fermentation aroma compounds by Saccharomyces cerevisiae wine yeasts : e ¡ ects of yeast assimilable nitrogen on two model strains, *8*, 1196-1207. https://doi.org/10.1111/j.1567-1364.2008.00412.x

Davies, G. (1993). Domestic Banana-Beer Production in Mpigi District, Uganda. *The International Magazine on Banana and Plantain*, *2*(1), 12-15.

Deesuth, O., Laopaiboon, P., Jaisil, P., & Laopaiboon, L. (2012). Optimization of nitrogen and metal ions supplementation for very high gravity bioethanol fermentation from sweet sorghum juice using an orthogonal array design. *Energies*, *5*(9), 3178-3197. https://doi.org/10.3390/en5093178

Dieu, H. J. De, Kubana, I., Claudine, M., Claire, I., Eric, K., Labs, T., … Africa, S. (2017). Comparative Study of Physicochemical and Bacteriological Characteristics of Banana Wines Produced by Conventional and Modern Techniques in Southern province of Rwanda, *5*(5), 192-198. https://doi.org/10.12691/ajfst-5-5-4

Ferreira, J. (2004). *Factors influencing the fermentation performance of commercial wine yeasts by*.

Hammond, J. B., Egg, R., Diggins, D., & Coble, C. G. (1996). Alcohol from bananas. *Bioresource Technology*, *56*(1), 125-130. https://doi.org/10.1016/0960-8524(95)00177-8

Kanyana, I., Ouma, E., & Van Asten, P. (2013). Quality assesment of banana juice and beer in Rwanda. *Journal of Food Technology*.

Shale, K., Mukamugema, J., Lues, R. J., Venter, P., & Mokoena, K. K. (2013). Characterisation of selected volatile organic compounds in Rwandan indigenous beer 'Urwagwa' by dynamic headspace gas chromatography-mass spectrometry. *African Journal of Biotechnology*, *12*(20), 2990-2996. https://doi.org/10.5897/AJB12.1173

Karamura, D. A., Karamura, E., & Tinzaara, W. (2012). Banana cultivar Names,Synonyms and their Usage in East Africa.

Kudo, M., Vagnoli, P., & Bisson, L. F. (1998). Imbalance of pH and potassium concentration as a cause of stuck fermentations. *American Journal of Enology and Viticulture*, *49*(3), 295-301.

Lindsay, J. (2010). Sorghum: An Ancient, Healthy and Nutritious Old World Cereal, 1-28. Retrieved from http://digitalcommons.unl.edu/intsormilpubs%5Cnhttp://digitalcommons.unl.edu/intsormilpubs/7

Mohapatra, D., Mishra, S., & Sutar, N. (2010). Banana and its by-product utilisation: An overview. *Journal of Scientific and Industrial Research*, *69*(5), 323-329.

Munyanganizi, B. (1975). *La technologie de l'extraction du jus de bananes et sa vinification. Thèse de doctorat.* Faculté des Sciences Agronomiques de l'Etat à GEMBLOUX, Belgique.

Muriro, P. A. (2017). Crude Catalyst and Steam Distillation Into Document Control :, 1-19.

Newilah, G. N., Tomekpe, B. K., Fokou, B. E., & Etoa, B. F.-X. (2009). Physicochemical Changes during Ripening of Bananas Grown in Cameroon. *Fresh Produce 3.1*, *3*(1), 64-70. Retrieved from http://www.globalsciencebooks.info/Online/GSBOnline/images/0906/FP_3(1)/FP_3(1)64-70o.pdf

Picki-reix, B. A. T. W., & Ryburgh, G. (1986). e¡ects of yeast assimilable nitrogen ontwo model strains, *92*, 568-571.

Raikar, R. V. (2012). Enhanced production of Ethanol from grape waste. *International Journal of Environmental Sciences*, *3*(2), 776-783. https://doi.org/10.6088/ijes.2012030132004

Rwanda Standard Board. (2017). Uburyo buboneye bwo gukora inzoga z'ibitoki na Divayi zifatiye kubimera, (Ukwakira), 1-99.

Sablayrolles, J. M., Dubois, C., Manginot, C., Roustan, J. L., & Barre, P. (1996). Effectiveness of combined ammoniacal nitrogen and oxygen additions for completion of sluggish and stuck wine fermentations. *Journal of Fermentation and Bioengineering*, *82*(4), 377-381. https://doi.org/10.1016/0922-338X(96)89154-9

Serpen, J. Y. (2012). Comparison of Sugar Content in Bottled 100 % Fruit Juice versus Extracted Juice of Fresh Fruit. *Food and Nutrition Science*, *3*(November), 1509-1513. https://doi.org/10.4236/fns.2012.311196

Shale, K., Mukamugema, J., Lues, R. J., & Venter, P. (2014). Possible microbial and biochemical contaminants of an indigenous banana beer' Urwagwa': A mini review, *8*(July), 376-389. https://doi.org/10.5897/AJFS12.134

Sigmon, K. (1993). *BREWING MICROBIOLOGY Third Edition*. (G.lain Campbell; Priest; Fergus, Ed.) (Third edit). Kluwer Academic/Plenum Publishers New York in 2003. https://doi.org/10.1007/978-1-4419-9250-

Th.Munyanganizi, R. C. (1974). Extraction du Jus de banane. *Industries Alimentaires et Agricoles*.

Jiranek, V., Langridge, P., & Henschke, P. A. (1993). Amino Acid and Ammonium Utilization by Saccharomyces cerevisiae Wine Yeasts From a Chemically Defined Medium. *Copyright © 1995 by the American Society for Enology and Viticulture. All Rights Reserved.*, *46*(1), 79-85.

Wilson, P. (2012). Microbial and Biochemical Changes Occurring During Production of Traditional Rwandese Banana Beer "Urwagwa." *Fermentation Technology*, *01*(03), 1-4. https://doi.org/10.4172/2167-7972.1000104

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Characterisation of Arbequina Extra Virgin Olive Oil from Uruguay

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Abstract

Since the year 2002, the Uruguayan oil-producing sector has been growing at a steady peace, utilizing over 10,000 seeded hectares across the country. The aim of this study was to characterise both the chemical and sensory properties of the extra virgin olive oil variety known as Arbequina through two consecutive harvests. The work was carried out using olives with three different ripening indices; the oil was extracted using an Abencor system. The moisture of the olive, yield extraction, and yield on both a dry and wet basis were determined. The obtained oils were characterized by determining the free acidity, main antioxidant compounds (polyphenols and tocopherols), composition of fatty acids, and sensory profiles. Olive moisture was over 57%, which led to production of pomaces characterized as "difficult pastes", explaining the low yield obtained. The harvest year and ripening index affected different parameters such as Abencor yield and, on a dry basis, the free acidity, polyphenolic content, oleic acid, and linoleic acid contents and fruity, bitter, and pungent intensities. To determine the best time of harvest, it is important to consider the balance between yield (which is significantly greater in olives with a ripening index above 3) and oil quality, as a higher ripening index decreases positive sensory attributes such as pungency and bitterness intensities.

Keywords: Arbequina, chemical characterization, sensory profile, virgin olive oil

1. Introduction

Extra virgin olive oil (EVOO) is a remarkably valuable product that is traditionally produced in Mediterranean countries; 95% of the world's olive oil is produced in Spain (Amelio, 2016; Reboredo-Rodríguez et al., 2016; International Olive Council [IOC], 2017). The olive habitat is concentrated at latitudes between 30° and 45°, both in the southern and northern hemispheres in Mediterranean climatic regions (which is characterized by dry and warm summers and winters with mild temperatures) (Civantos, 2008; Navarro & Parra, 2008; Rapoport, 2008).

Olive oil production has spread in recent years to beyond the Mediterranean region to non-traditional areas (Uruguay XXI, 2013; Villamil & Conde, 2013; Rondanini, Castro, Searles, & Rousseaux, 2014; Katsoyannos et al., 2015; Xiang et al., 2017). This spreading requires the adaptation of olive plants to new climatic conditions (temperature, precipitation, and moisture, for example) associated with latitudes and altitudes differing from those corresponding to the autochthonous regions of olive. Because of this adaptation, oil produced in these regions may differ in quality and composition from those produced in autochthonous regions (Romero, Saavedra, Tapia, Sepúlveda, & Aparicio, 2016).

Uruguay is located between latitudes 30° and 35° which have a mean annual temperature of 17.7°C and mean annual rainfall of 1200–1600 mm (Instituto Nacional de Investigación Agropecuaria [INIA], 2017). In recent years, the production of EVOO has steadily increased from 112,000 kg in 2011 to 900,000 kg in 2017 (Asociación Olivícola Uruguaya [ASOLUR], 2017).

Arbequina is a cultivar typical of the Spanish region of Catalunya (Terragona and Lérida) and of Alto Aragón. It adapts easily to new environmental conditions and, because of its small size, precocity, high oil yield, good oil quality, and other agronomic characteristics, is particularly fit for new high-density olive orchards and mechanised cultivation (Rondanini, Castro, Searles & Rousseaux, 2011; Yousfi, Weiland & García, 2013; Borges et al., 2017). Furthermore, Arbequina olive oil is highly appreciated for its mild taste and is considered ideal for new and emerging markets compared to other oils, which can be more bitter and pungent (B. Jiménez & Carpio,

2008; Borges et al., 2017). Because of these positive factors, countries in South America have begun producing Arbequina, including Chile (García-González, Romero & Aparicio, 2010), Argentina (Torres et al., 2009), and Brazil (Borges et al., 2017). In Uruguay, this cultivar has been cultivated in the past decade and now occupies the largest growing area (51%) and is present in most national plantations (Uruguay XXI, 2013).

The chemical composition of EVOO is influenced by the olive cultivar, pedoclimatic conditions, geographical site, and ripeness stage. Several studies have shown that the choice of the optimal harvest period is essential for obtaining high-quality virgin olive oil (VOO) (Franco, Sánchez, De Miguel, Martínez & Martín-Vertedor, 2015). During ripening, many metabolic processes occur which significantly affect the physical properties, chemical composition, and enzymatic activity of the fruit, which are reflected in the composition and quality of VOO (Lukić et al., 2017). Thus, it is important to determine the best harvest period for each variety to optimize olive productivity and ensure quality (Youssef et al., 2010, M. Beltrán, Sánchez-Astudillo, Aparicio & García-González, 2015). Furthermore, according to B. Jiménez, Sánchez-Ortiz, Lorenzo, & Rivas (2013) and Katsoyannos et al. (2015), as fruit ripening progresses, oxidative stability is reduced because of the decreased total polyphenol content in the ripe fruits.

Previous studies in the Mediterranean region examined modification of the major and minor components in different oil varieties during the ripening process. For example, Franco et al. (2015) evaluated the influence of ripening index (RI) on oil yield, olive moisture, fatty acids, and sensory attributes such as fruity, bitter, and pungent indices of seven varieties from the southwest of Spain, including Arbequina. Currently, Spain shows an increased RI of between 2 and 3 for Arbequina and Picual, indicating very good oil quality and high yield (Franco et al., 2015).

Few studies have examined how geographical and climatic conditions and ripening index affect the properties of Arbequina olive oil produced in Uruguay. Thus, the aim of the present study was to characterise both the chemical and sensory attributes of the EVOO variety Arbequina, considering different RIs, over two years of harvest.

2 Materials and Methods

2.1 Plant Material

2.1.1 Samples

The olives were supplied by five different olive plantations located in the southeast and southwest regions of the country throughout the time of the harvest study in years 2015 and 2016.

The selected olive trees for each producer were approximately the same age of eight years at the beginning of the study. For each sampling, trees were divided into two blocks of six trees each. First, a representative sample of 10 kg was hand-picked from both blocks. The sampling protocol was previously established, which specified that olives were to be picked from all four sides of the tree from different heights, starting from the outside of the tree and ending in the inside. After the olives were harvested, the fruit samples were immediately transported in ventilated storage trays to the Abencor system pilot plant, located in the Chemistry Faculty, to avoid alterations to the fruits.

2.1.2 Determination of Olive RI

RI was determined according to a classification based on fruit colour of both the skin and pulp as described in "Método de Índice de Madurez" (Uceda & Frías, 1975). Olives were classified as green (G) when their RIs were lower than 2.0, medium (M) for values between 2.0 and 3.0, and ripe (R) for values above 3.0, as described by Franco et al. (2015) for qualifying olives.

2.1.3 VOO Extraction Using the Laboratory Plant System Abencor and Yield Determination

VOO extraction from collected olives was conducted at the Facultad de Química laboratory plant within 24 h of harvest as suggested by Aguilera, G. Beltrán, Sánchez-Villasclaras, Uceda, & A. Jiménez (2010), B. Jiménez et al. (2013), and Reboredo-Rodríguez et al. (2015).

The extraction conditions were as follows: the sieve size from the hammer mill was 5 mm and olives were crushed at 3000 rpm. Previous studies (Ellis, 2016) showed that Uruguayan olive pastes from the Arbequina variety have a moisture level higher than 50%, designating them as "difficult pastes" (Aguilera et al., 2010). To ease the separation between the oil and other compounds of olive paste and, as a result, increase extraction yield by depleting the subproducts, micronized natural talc (MNT) was added to the olive paste during the malaxation stage as a technological coadjuvant at 2.3% (Alba, 2008; Aguilera et al., 2010). The MNT was used in all extractions performed during the two years of the study. The paste with the talc was malaxated (using a

thermostatic olive paste mixer) for 50 min at 30°C; in the middle of this process, 200 mL of water was added.

After the malaxation stage, the content of each of the eight vessels from the thermostatic olive paste mixer were centrifuged in an Abencor vertical centrifuge at 3500 rpm for 1 min.

The extracted oil was stored under protection from light in amber-coloured glass bottles at 4°C until analysis.

The yield obtained from the extraction process at the Abencor plant was calculated according to Criado, Motilva, Goñi, & Romero, (2007). Moisture values were expressed as an oil percentage.

2.1.4. Olive Moisture Determination

The moisture content of the olives was determined by gravimetric analysis. Twenty grams of the homogeneous olive paste with the stone, which was freshly milled using the hammer mill of the Abencor system, were weighed in glass Petri dishes and dried in a conventional oven at 105°C for 12 h. The weight decrease was expressed as the moisture percentage according Reboredo-Rodríguez et al. (2015).

2.1.5 Olive Oil Yield Determination by Soxhlet Method

To determine olive oil yield, expressed as the percentage content of oil in olive paste according to UNE 55030, IUPAC Method 1.11, olives were milled with the stone using the Abencor system hammer mill. The fresh olive paste was desiccated in a conventional oven at 105°C for 12 h, and the oil was extracted in a Soxhlet apparatus using petroleum ether (62–68°C boiling point) as a solvent. The system was brought to a boil and extraction was performed or 8 h, after which the solvent was removed and the yield was determined as dry matter weight (% dry weight) and wet matter weight (% wet weight).

2.2 Quality Chemical Parameters

2.2.1 Free Acidity Determination

Free acidity (FA) was determined according to IOC recommendations using ISO 660 "Determination of degree of acidity and free acidity". Free acidity was expressed as the percentage of oleic acid.

2.3 Chemical Characterisation of EVOO

2.3.1 EVOO Total Polyphenol Content Determination

Total polyphenol content in EVOO was determined according to the IOC method/T 20/Doc Nº29 November 2009: "Determination of biophenols in olive oils by HPLC". Syringic acid was used as internal standard.

An HPLC Shimadzu 20 A was used along with a diode array detector (model SPD-M20A) and C18 Phenomenex column (4.6 mm diameter, 25 cm long, 5 µm particle size). Phenolic compounds were quantified at a wavelength of 280 nm using water with 0.2% H_3PO_4 [V/V] /methanol/acetonitrile 96/2/2 [V/V/V] as the mobile phase as recommended by IOC 2009. The external calibration standards were tyrosol and syringic acid.

Before total polyphenol content determination by HPLC, extraction was performed using 2.0 g of EVOO. A methanol/water 80/20 (V/V) solution was used for extraction, along with the addition of syringic acid solution.

2.3.2 EVOO Total Tocopherol Content Determination

For total tocopherol determination in EVOO, the method proposed by Andrikopoulos, Brueschweiler, Felber, & Taeschler, (1991): "HPLC analysis of phenolic antioxidants, tocopherols and triglycerides" was used. Thirty milligrams of EVOO were weighed and dissolved in 1 mL isopropanol. Calibration curves were developed using α-tocopherol, γ-tocopherol, and δ-tocopherol standards (Sigma-Aldrich, St. Louis, MO, USA). An HPLC Shimadzu 20A was used, along with a fluorescence detector (Shimadzu RF 20A XS) set to a 290 nm excitation wavelength and 330 nm emission wavelength, as well as the PDA detector described above. The oven was maintained at 35°C.

2.3.3 EVOO Fatty Acid Composition Determination

The fatty acid composition of EVOO was determined by gas chromatography with previous derivatisation of the oil into fatty acid methyl esters according to IOC/T.20/Doc. Nº24, 2001 "Preparation of fatty acid methyl esters from olive oil and olive pomace oil". Method A: "Cold transesterification with potassium hydroxide methanolic solution" was conducted, followed by IOC, 2015: "Gas chromatography analysis of fatty acid methyl esters" Method AOACS Ch 2-9. IOC/T.20/Doc Nº 33 February 2015 "Determination of fatty acid methyl esters by gas chromatography".

2.4 Sensory Characterisation of EVOO

Samples were evaluated by a panel of 10 trained panellists belonging to the olive oil sensory panel at the Faculty

of Chemistry, which has been approved by the IOC since 2012. The IOC official method IOC/T.20/Doc Nº15/Rev. 8/2015 was applied, along with a descriptive tasting, to evaluate the following descriptors: green (olive leaf, herb, grassy), fig, tomato (plant, leaf, fruit), apple, banana (peel, fruit), nut/almond, sweet, astringent, and other positive attributes.

The intensity of these attributes was evaluated using a linear, non-structured scale ranging from 0 to 10 cm and from "nothing" to "very" as extremes. Furthermore, the "fruity" attribute was described by the evaluator as either "green" or "mature", if perceived.

Samples were served in normalized blue cups (15 mL), codified with a letter and two random numbers, at a temperature of de 28 ± 2°C. Each sample was evaluated in duplicate. Green apple, natural yoghurt, room temperature water, and crackers were used as erasers.

2.5 Statistical Analysis

All chemical analyses were performed in triplicate.

Analysis of variance was performed on chemical and sensory data considering year, RI, and the interaction year × RI as variation factors. Mean ratings and honestly significant differences were determined, based on Tukey's test ($p < 0.05$). All statistical analyses were performed using XL-Stat 2017 software (Addinsoft, New York, NY, USA).

3. Results and Discussion

3.1 Olive Properties

In 2015, olives were harvested during April to obtain 14 samples of EVOO, which presented different RI values: 4 were green, 4 were medium, and 6 were ripe.

In 2016, olives were collected between March and April to obtain 14 samples of EVOO with various RI values: 5 were green, 5 were medium, and 4 were ripe. Thus, during the two years of study, 28 samples of EVOO were obtained.

Producers typically estimate the best harvest time according to the olives' RI, which is calculated based on the colour of the fruit in the olive tree. The aim of this study was to determine the oil content in different RI stages for two years. The results were expressed as yield of dry and wet materials, both as percentages. Additionally, yield was measured using the Abencor System. The results are shown in Table 1.

Olive moisture content was significantly affected by the year ($p = 0.0012$), as was the yield determined with the Abencor ($p < 0.0010$) and yield of dry materials ($p = 0.0297$). In 2015, the moisture content of oil was significantly higher than in 2016, likely because of the high rainfall which occurred during the months before harvest. Rainfall during February and March was 350 mm in 2015, while in the following year during the same months, rainfall was of 200 mm (INIA, 2017). This affected the Abencor yield, which was significantly lower in 2015, as shown in Table 1.

Moisture and yield based on wet weight were not affected by the olive's stage of maturity. However, the Abencor yield and yield based on dry weight were significantly higher for ripe olives than for green olives. Franco et al. (2015) suggested that this increase was caused by water evaporation from ripe olives, while lipid biosynthesis slowly continued. Yield based on dry weight is a more reliable parameter for comparison than yield based on wet weight, as the latter is strongly influenced by the olive's moisture.

Table 1. Results of the olive properties

YEAR	RIPENING INDEX (RI)	Olive fruit properties			
		MOISTURE (%)	ABENCOR SYSTEM YIELD (%)	OIL YIELD (% WET WEIGHT)	OIL YIELD (% DRY WEIGHT)
2015	G	62.6[a]	9.7[a]	15.1[a]	37.0[a]
	M	63.8[a]	10.0[a]	16.1[a]	40.3[a,b]
	R	62.5[a]	11.6[b]	18.0[a]	43.5[b]
	p value	0.7857	**0,0219**	0,3177	**0,0196**
YEAR	RIPENING INDEX (RI)	MOISTURE (%)	ABENCOR SYSTEM YIELD (%)	OIL YIELD (% WET WEIGHT)	OIL YIELD (% DRY WEIGHT)
2016	G	57.8[a]	12.3[a]	15.9[a]	35.1[a]
	M	60.0[a]	12.5[a]	15.1[a]	38.5[a,b]
	R	58.9[a]	13.5[b]	16.0[a]	39.5[b]
	p value	0.3549	**0.0034**	0.7384	**0.0425**

Different letters in the same column denote significant statistical differences al $p<0.05$ (Tukey's test) among ripening index in each year.

While Franco et al. (2015) reported that olive moisture depends on the fruit's ripeness stage, these results do not agree with those found in this study, in which RI did not significantly affect the olive moisture.

Aguilera et al. (2010) characterised olive pastes with moisture higher than 50% as difficult pastes. Particularly, these authors determined the moisture content of paste obtained from Picual olives and characterized it as "very difficult paste", with a moisture value of 56.3%. For difficult pastes, larger and stronger emulsions are formed during the milling phase, which are impossible to break under malaxation conditions. The moisture content of the olives analysed in this work revealed that the resulting pastes were "very difficult pastes", which may explain the low yield obtained from the studied harvests, compared to yields obtained from Arbequina in other countries, as reported by Reboredo-Rodríguez et al. (2015).

3.2 Chemical Quality Parameters

The free acidity percentage is a direct measure of olive oil quality and represents the extent of hydrolytic activities reflecting the care taken from blossoming and fruit set to eventual sale and consumption of the oil (Reboredo-Rodríguez et al*.,* 2016). The results are shown in Table 2. The harvest year significantly influenced free acidity ($p < 0.0001$), with the 2016 oils showing higher values (expressed as percentage oleic acid) than the 2015 oils, as reported by Rodrigues et al. (2018). For the 2015 harvest, free acidity significantly differed between the different RI values ($p < 0.0001$), with an increase from green to ripe RI. Franco et al. (2015) found that the values of free acidity for Arbequina increased as the RI increased. The authors predicted that EVOOs obtained from olives with higher RI had higher percentages of free acidity because of their increased enzymatic activity, particularly lipolytic enzymes, and increased sensitivity to mechanical damage and pathogen infections. This trend was not confirmed for the 2016 harvest, in which no significant differences were found in the free acidity of different RI olives (Table 2).

All analysed oil samples showed free acidity values lower than 0.8%, which is the upper limit established by the IOC (IOC/T.15/NC No 3/Rev.8, 2015, and successive revisions) for an oil to be categorised as EVOO; this demonstrates that the olives used in the present study were in good condition at the time of oil extraction and that the extraction itself and subsequent storage were effectively performed (Rodrigues et al., 2018).

3.3 Chemical Characterisation of EVOO

3.3.1 Polyphenols

Phenolic compounds are considered as natural antioxidants responsible for oil stability through oxidation and contribute to sensory characteristics, such as bitterness and pungent flavours (Reboredo-Rodríguez et al., 2016). According to B. Jiménez et al. (2013), during the fruit ripening process, a series of metabolic processes occurs (chemical and enzymatic reactions), resulting in the production of free phenols and induction of variations in the phenolic profile of several compounds. These changes affect the quality, sensory properties, oxidative stability, and/or nutritional value of the obtained oil.

Table 2 presents the total polyphenol content, expressed in % syringic acid. The harvest year significantly influenced these values ($p = 0.0386$), with the values for 2015 lower than those for 2016. This may be related to the amount of rainfall in 2015, as explained in item 3.1, which is consistent with the moisture values for this

harvest year.

According to Katsoyannos et al. (2015), as the ripeness index increases, the total contents of tocopherols and polyphenols decrease. This was not observed in the present study, as the RI did not significantly affect ($p > 0.05$) the polyphenol content in either harvest year evaluated.

Reboredo-Rodríguez et al. (2015) reported that the total polyphenol content for the Arbequina variety, with an RI of 3.3, was 162 ppm in Galicia, Spain. Values obtained for Uruguayan oils were similar for the 2015 harvest year and higher for the 2016 harvest year.

3.3.2 Tocopherols

According to Reboredo-Rodríguez et al. (2016), tocopherols are the main lipid-soluble antioxidants present in EVOO. Four isomers can be found in EVOO (α, β, γ, and δ), with α-tocopherol (Vitamin E) as the most abundant. Table 2 lists the obtained values for tocopherols in this study. There was no significant difference in total tocopherol content for different RI values and for different harvest years ($p = 0.2465$).

Velasco et al. (2015) found that the total tocopherol normal range in VOO is 100–250%; for the Arbequina variety from the Cabra region in Spain, the authors reported a concentration of 148% as the median RI. Values found in this study were higher than those for both harvest years and for all studied RI values.

3.3.3 Fatty Acid Composition

The main fatty acid composition of EVOO is shown in Table 2; the RI was significantly influenced by linoleic acid in the 2015 harvest and by palmitic, oleic, and linoleic acid in the 2016 harvest. The harvest year significantly influenced the contents of stearic ($p = 0.0192$), linoleic ($p < 0.0001$), and linolenic ($p = 0.0158$) acids, which were higher in 2015, while oleic acid was higher ($p < 0.0001$) in 2016.

The highest fatty acid percentage was obtained for oleic acid, followed by palmitic, linoleic, and stearic acids; this as agrees with the results reported by Reboredo-Rodríguez et al. (2016). The same authors reported that the Spanish Arbequina variety is characterised by its high linolenic acid content (12.57–13.06%) and low oleic content (68.20–65.83%). This agrees with the results of the current study for the 2015 harvest, but not with the results obtained for the 2016 harvest, in which higher values of oleic acid and lower values for linoleic acid were obtained compared to the previous study.

According to studies carried out by Buffa, Tropea, Mattar, Carelli, & Ceci (2013) and Katsoyannos et al. (2015), as the RI of olives increases, the relationship between percentages of oleic/linoleic acid found in the oil decreases. This trend agrees with the results obtained in this study for both harvest years, as shown in Table 2.

Borges et al. (2017) evaluated Arbequina VOO in Australia, Argentina, and Tunisia and found that different cultivation and environmental conditions strongly affect the fatty acid composition of VOO, with oleic and palmitic acids most strongly affected. In addition to the geographical and climate conditions considered in these studies, other factors such as RI, storage conditions, processing conditions, and other environmental factors (light intensity, relative humidity, and evapo-transpiration) may influence the chemical composition of VOO.

All studied fatty acid values were within the limits established by the IOC for EVOOs.

Table 2. Results of the chemical properties of extra virgin olive oils.

Chemical properties of EVOO										
YEAR	RIPENING INDEX (RI)	FREE ACIDITY (%)	POLYPHENOLS (ppm)	TOCOPHEROLS (ppm)	FATTY ACID COMPOSITION (%)					
					16:0	18:0	18:1	18:2	18:3	18:1/18:2
2015	G	0.10[a]	174[a]	171[a]	15.93[a]	1.55[a]	66.83[a]	11.58[a]	0.55[a]	5.77
	M	0.12[b]	164[a]	158[a]	16.45[a]	1.53[a]	65.03[a]	12.65[a,b]	0.55[a]	5.14
	R	0.15[c]	134[a]	149[a]	16.35[a]	1.48[a]	63.83[a]	13.17[b]	0.52[a]	4.84
	p value	**<0.0001**	0.85724	0.4164	0.6043	0.4181	0.2541	**0.0130**	0.6588	
2016	G	0.26[a]	273[a]	191[a]	15.00[a]	1.52[a]	71.20[c]	8.60[a]	0.50[a]	8.28
	M	0.26[a]	251[a]	162[a]	15.84[b]	1.40[a]	69.34[b]	9.24[b]	0.48[a]	7.50
	R	0.24[a]	192[a]	157[a]	16.30[b]	1.38[a]	68.05[a]	9.85[c]	0.48[a]	6.90
	p value	0.8002	0.6198	0.0688	**0.0214**	0.1114	**0.0004**	**0.0001**	0.5746	

Different letters in the same column denote significant statistical differences al p<0.05 (Tukey's test) among ripening index in each year.

3.4 Sensory Characterisation of EVOO

For the data obtained from the sensory panel for the 28 samples of VOO, the median and robust coefficient of variation were calculated for each attribute (positive and negative) according to IOC regulations. According to the results, all VOOs were classified as EVOOs because the defect median was zero and fruity attribute median was higher than zero in all samples.

In the evaluated samples, the sensory panel perceived the attributes fruity, bitter, pungent, green, banana, nut, sweet, and astringent, as is shown in Table 3. According to the analysis of variance results, the interaction year × RI studied were not significant ($p > 0.05$), making it possible to study the effect of each factor independently.

The harvest year significantly affected the intensities of the fruity ($p < 0.0010$), bitter ($p < 0.0010$), pungent ($p < 0.0001$), green ($p < 0.0010$), banana ($p = 0.0108$), sweet ($p = 0.0001$), and astringent ($p = 0.0001$) attributes. As shown in Table 3, 2016 oils had higher intensities of fruity, bitter, pungent, green, banana, and astringent attributes and lower intensity of the sweet attribute. The sensory results were correlated with higher moisture content and a lower polyphenol content in the oils was obtained for the 2015 harvest.

Sensory qualities of VOO are affected by the presence of specific minor compounds, among which phenolic and volatile compounds are the most important. Phenolic compounds are responsible for flavour characteristics such as bitterness, kinesthetic sensations such as pungency, and exhibit antioxidant activity, which is widely responsible for the oxidative stability, shelf life, and nutritional value of VOO (Lukić et al., 2017; Xiang et al., 2017). In the present study, the relationship between sensory characteristics and polyphenol content was confirmed in the studied samples, as the presence of phenolic compounds stimulates taste receptors and the trigeminal nerve, evoking the sensations of bitterness in the first case and pungency and astringency in the second case. Low-weight volatile compounds easily evaporate at room temperature and, following stimulation of nasal receptors, are responsible for the VOO characteristic aroma (De Santis & Frangipane, 2015).

For the 2015 harvest oils, for higher RI values, a significant decrease ($p < 0.05$) was observed in the bitter, pungent, and green attributes, while an increase was observed for the sweet attribute ($p < 0.05$). For the 2016 harvest oils, an increase in RI resulted in a significant decrease ($p < 0.05$) in the pungent, green, and astringent attributes, as shown in Table 3. These results agree with the information reported by B. Jiménez et al. (2013) and Franco et al. (2015), who determined that the bitter, pungent, and green attributes decreased in intensity as the sweet attribute and the olives' RI increased.

The sensory profile of Uruguayan olive oils of the Arbequina variety coincided with reports by other authors for the same olive variety in other regions of the world, who found that these oils have a medium fruity attribute and mild bitter and pungent attributes. Franco et al. (2015) characterised oils obtained from seven different varieties from plantations in southwestern Spain and concluded that oils from the Arbequina variety presented the lowest values for the fruity and pungent attributes. Aparicio & Harwood (2003) used the sensory descriptors soft, slightly pungent, and medium green aroma to characterise this variety. B. Jiménez & Caprio (2008) reported that this oil presents a fruity aroma reminiscent of olives and other fruits, with fluid, sweet, and nearly imperceptible bitterness and pungency; these attributes make the variety particularly suitable for consumers not accustomed to the taste and aroma of virgin olive oils.

Table 3. Results of the sensory analysis of the extra virgin olive oils.

YEAR	RIPENING INDEX (RI)	Sensory characterisation of EVOO							
		FRUITY	BITTER	PUNGENT	GREEN	SWEET	ASTRINGENCY	BANANA	NUT
2015	G	3.5[a]	1.5[b]	1.8[b]	1.7[b]	2.0[a]	0.2[a]	1.5[a]	1.9[a]
	M	3.5[a]	1.6[b]	1.9[b]	1.8[b]	2.2[a,b]	0.2[a]	1.5[a]	1.9[a]
	R	3.6[a]	1.1[a]	1.4[a]	1.1[a]	2.6[b]	0.2[a]	1.8[a]	1.7[a]
	p value	0.9577	**0.0244**	**0.0073**	**0,0005**	**0.0355**	0.9725	0.8054	0.4217

YEAR	RIPENING INDEX (RI)	FRUITY	BITTER	PUNGENT	GREEN	SWEET	ASTRINGENCY	BANANA	NUT
2016	G	4.4[a]	2.6[a]	3.2[b]	2.2[b]	1.3[a]	0.8[b]	2.1[a]	2.1[a]
	M	4.1[a]	2.3[a]	3.0[a,b]	1.9[a,b]	1.7[a]	0.5[a,b]	2.0[a]	1.7[a]
	R	4.4[a]	1.9[a]	2.3[a]	1.6[a]	1.8[a]	0.2[a]	1.9[a]	1.7[a]
	p value	0.3930	0.2236	**0.0388**	**0.0407**	0.2381	**0.0076**	0..8752	0.2698

Main positive mean attributes found in the Arbequina variety throughout two years.

Different letters in the same column denote significant statistical differences al p<0.05 (Tukey's test) among ripening index in each year.

4. Conclusions

The chemical analysis results and sensory profiles of the 28 samples revealed that all oils examined belonged to the EVOO category according to IOC regulations.

Fruit moisture is an important parameter that must be considered, as olives with higher water content typically have a lower oil yield, which also negatively affects the quality of the oil obtained.

For olive physical and chemical properties, the RI significantly affected different parameters depending on the harvest year.

All pastes studied were considered "difficult pastes" because of the high moisture content in the olives; this should be considered by producers when determining the optimal harvest time, to achieve a balance between yield (which is significantly greater in olives with an RI above 3) and oil quality. At higher RI, sensory aspects, such as bitter and pungency decrease along with the content of antioxidants, such as polyphenols.

References

Alba, J. (2008). Elaboración del aceite de oliva virgen. In: El cultivo del olivo. (Barranco, D., Fernández-Escobar, R., Rallo, L.) 6ta Ed. Ediciones Mundi-Prensa, Madrid, España. pg. 657-697.

Aguilera, M. P., Beltrán, G., Sánchez-Villasclaras, S., Uceda, M., & Jiménez, A. (2010). Kneading olive paste from unripe "Picual" fruits: I. Effect on oil process yield. *Journal of Food Engineering, 97*, 533-538. http://dx.doi.org/ 10.1016/j.jfoodeng.2009.11.013

Amelio, M. (2016). The official method for olive oil sensory evaluation: An expository revision of certain sections of the method and a viable means for confirming the attribute intensities. *Trends in Food Science & Technology, 47,* 64-68. http://dx.doi.org/10.101016/j.tifs.2015.11.001

Andrikopoulos, N. K., Brueschweiler, H., Felber, H., & Taeschler, Ch. (1991). HPLC analysis of phenolic antioxidants, tocopherols and triglycerides. *Journal of the American Oil Chemists' Society, 68*, 359-364. https://doi.org/10.1007/BF02663750

Aparicio, R., & Harwood, J. (2003). Manual del aceite de oliva. AMV Ediciones, Madrid, España.

ASOLUR (Asociación Olivícola Uruguaya), 2017. Retrieved from http://www.asolur.org.uy/s/es/search

Beltrán, M., Sánchez-Astudillo, M., Aparicio, R., & García, G. D. L. (2015). Geographical traceability of virgin olive oils from south-western Spain by their multi-elemental composition. *Food Chemistry, 169,* 350-357. http://dx.doi.org/10.1016/j.foodchem.2014.07.104

Borges, T. H., Pereira, J. A., Cabrera-Vique, C., Lara, L., Oliveira, A. F., & Seiquer, I. (2017). Characterization of Arbequina virgin olive oils produced in different regions of Brazil and Spain: Physicochemical properties, oxidative stability and fatty acid profile. *Food Chemistry, 215*, 454-462. https://doi.org/10.1016/j.foodchem.2016.07.162

Buffa, M. A., Tropea, G., Mattar, S., Carelli, A., & Ceci, L. (2013). Análisis químico y sensorial de aceites de oliva de la provincia de San Juan (Argentina). XVI simposio científico-técnico del aceite de oliva, Expoliva.- Jaén, España.

Civantos, L. (2008). La olivicultura en el mundo y en España. In*: El cultivo del olivo*. (Barranco, D., Fernández-Escobar, R. Rallo, L.) 6ta Ed. Ediciones Mundi-Prensa, Madrid, España. pg. 17-35.

Criado, M. N., Motilva, M. J., Goñi, M., & Romero, M. P. (2007). Comparative study of the effect of the maturation process of the olive fruit on the chlorophyll and carotenoid fractions of drupes and virgin oils from Arbequina and Farga cultivars. *Food Chemistry, 100*, 748-755. http://dx.doi.org/10.1016/j.foodchem.2005.10.035.

De Santis, D., & Frangipane, M.T. (2015). Sensory Perceptions of Virgin Olive Oil: New Panel Evaluation Method and the Chemical Compounds Responsible. *Natural Science, 3*, 132-142. http://dx.doi.org/10.4236/ns.2015.73015

Ellis, A. C. (2016). *Calidad sensorial y fisicoquímica de aceite de oliva nacional variedad Arbequina y Picual* (Doctoral dissertation). University of the Republic, Scholl of Chemistry, Montevideo, Uruguay.

Franco, M. N., Sánchez, J., De Miguel, C., Martínez, M., & Martín-Vertedor, D. (2015). Influence of the Fruit's Ripeness on Virgin Olive Oil Quality. *Journal of Oleo Science, 64*(3), 263-273. http://dx.doi.org/10.5650/jos.ess14148

García-González, D. L., Romero, N., & Aparicio, R. (2010). Comparative study of virgin olive oil quality from

single varieties cultivated in Chile and Spain. *Journal of Agricultural and Food Chemistry*, *58*(24), 12899-12905. https://doi.org/10.1021/jf1031313

INIA (Instituto Nacional de Investigación Agropecuaria), 2017. Retrieved from http://www.inia.uy

IOC (International Olive Council), 2017. Retrieved from http://www.internationaloliveoil.org

IOC (2015). Chemistry/TestingMethods/Chemical testing methods.

Jiménez, B., & Carpio, A. (2008). La cata de aceites: aceite de oliva virgen. Características organolépticas y análisis sensorial. Edita: Junta de Andalucía. Instituto de Investigación y Formación Agraria y Pesquera, Consejería de Agricultura y Pesca, Spain

Jiménez, B., Sánchez-Ortiz, A., Lorenzo, M. L., & Rivas, A. (2013). Influence of fruit rippening on agronomic parameters, quality indices, sensory attributes and phenolic compounds of Picudo olive oils. *Food Research International, 54*, 1860-1867. http://dx.doi.org/10.1016/j.foodres.2013.08.016

Katsoyannos, E., Batrinou, A., Chatzilazarou, A., Bratakos, S. M., Stamatopoulos, K., & Sinanoglou, V. J. (2015). Quality parameters of olive oil from stoned and nonstoned Koroneiki and Megaritiki Greek olive varieties at different maturity levels. *Grasas Aceites, 66*(1), e067. http://dx.doi.org/10.3989/gya.0711142.

Lukić, I., Žanetić, M., Špika, M. J., Lukić, M., Koprivnjak, O., & Bubola, K. B. (2017). Complex interactive effects of ripening degree, malaxation duration and temperatura on Oblica cv. Virgin olive oli phenols, volatiles and sensory quality. *Food Chemistry, 232*, 610-620. http://dx.doi.org/10.1016/j.food.chem.2017.04.047

Navarro, C., & Parra, M. A. (2008). Plantación. In: El cultivo del olivo. (Barranco, D., Fernández-Escobar, R., Rallo, L.) 6ta Ed. Ediciones Mundi-Prensa, Madrid, España. pg. 189-238.

Rapoport, H. F. (2008). Botánica y morfología. In: El cultivo del olivo. (Barranco, D., Fernández-Escobar, R., Rallo, L.) 6ta Ed. Ediciones Mundi-Prensa, Madrid, España. pg. 39-61

Reboredo-Rodríguez, P., González Barreiro, C., Cancho-Grande, B., Valli, E., Bendini, A., Gallina, T., T., & Simal-Gandara, J. (2016). Characterization of virgin olive oils produced with autochthonous Galician varieties. *Food Chemistry, 212,* 162-171. http://dx.doi.org/10.1016/j.foodchem.2016.05.135.

Reboredo-Rodríguez, P., Gonzalez-Barreiro, C., Cancho-Grande, B., Fregapane, G., & Salvador, M. (2015). Characterisation of extra virgin olive oils fron Galician autochthonous varieties and their co-crushings with Arbequina and Picual cv. *Food Chemistry, 176*, 493-503. http://dx.doi.org/10.1016/jfoodchem.2014.12.078

Rodrigues, N., Casal, S., Peres, A. M., Baptista, P., Bento, A., Martín, H., Asensio-S-Manzanera, M. C., & Pereira, J. A. (2018). Effect of olive tres density on the quality and composition of olive oil from cv. Arbequina. *Scientia Horticulturae, 238*, 222-233. http://dx.doi.org/10.1016/j.scienta.2018.04.059

Romero, N., Saavedra, J., Tapia, F., Sepúlveda, B., & Aparicio, R. (2016). Influence of agroclimatic parameters on phenolic and volatile compounds of Chilean virgin olive oils and characterization based on geographical origin, cultivar and ripening stage. *Journal of the Science of Food and Agriculture, 96*(2), 583-592. https://doi.org/10.1002/jsfa.7127

Rondanini, D. P., Castro, D. N., Searles, P. S., & Rousseaux, M. C. (2014). Contrasting patterns of fatty acid composition and oil accumulation during fruit growth in several olive varieties and locations in a non-Mediterranean region. *European Journal of Agronomy, 52*, 237-246. https://doi.org/10.1016/j.eja.2013.09.002

Rondanini, D., Castro, D., Searles, P., & Rousseaux, M. (2011). Fatty acid profile or varietal virgin olive oils (Olea europaea L.) from mature orchards in warm arid valleys of Northwestern Argentina (La Rioja). *Grasas Aceites, 62*(4), 399-409. http://dx.doi.org/10.3989/gya.125110

Torres, M. M., Pierantozzi, P., Cáceres, M. E., Labombarda, P., Fontanazza, G., & Maestri, D. M. (2009). Genetic and chemical assessment of Arbequina olive cultivar grown in Córdoba province, Argentina. *Journal of the Science of Food and Agriculture, 89*(3), 523-530. https://doi.org/10.1002/jsfa.3483

Uceda, M., & Frías, L. (1975). Trend of the quality and quantitive composition of olive fruit oil during ripening. *Proceedings of the International Meeting on Olive Oil.* 25-46. Córdoba, Spain.

URUGUAY XXI. (2013) Sector Olivícola. Promoción de Inversiones y Exportaciones. Presidencia de la República Oriental del Uruguay. Retrieved from http://www.uruguayxxi.gub.uy/exportaciones/wp-content/uploads/sites/2/2014/09/Sector-Olivicola-Dec-201

3-Uruguay-XXI.pdf.

Velasco, L., De la Rosa, R., & León, L. (2015). Advanced olive selections with enhanced quality for minor constituents. *Grasas Aceites, 66*(4), 1-7. http://dx.doi.org/10.3989/gya.0227151.

Villamil, J., & Conde, P. (2013). Variedades. In: Aceites de oliva: de la planta al consumidor. Vol. 1 (Grompone, M. A.; Villamil, J.) Ed. Hemisferio Sur. Montevideo, Uruguay.

Xiang, C., Xu, Z., Liu, J., Li, T., Yang, Z., & Ding, C. (2017). Quality, composition, and antioxidant activity of virgin olive oil from introduced varieties al Liangshan. *Food Science and Technology, 78*, 226-234. http://dx.doi.org/10.1016/j.lwt.2016.12.029

Yousfi, K., Weiland, C. M., & García, J. M. (2013). Responses of fruit physiology and virgin oil quality to cold storage of mechanically harverest "Arbequina" olives cultivated in hedgerow. *Grasas Aceites, 64*(5), 572-582. http://dx.doi.org/10.3989/gya.063013

Youssef, N. B., Zarrou, K. W., Carrasco-Pamcorbo, A., Ouni, Y., Segura-Carretero, A., ... Fernández-Gutiérrez, A. (2010). Effect of olive rippeness on Chemicals propierties and phenolic composition of Chetoui virgin olive oil. *Journal of the Science of Food and Agriculture, 90*, 199-204. http://dx.doi.org/10.1002/jsfa.3784

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Antiviral Activity of Geopropolis Extract from Scaptotrigona Aff. Postica against Rubella Virus

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Abstract

The search for functional foods, which possess bioactive substances, is a new trend for the obtention of alternative and more effective treatments of many diseases with fewer side effects. Geopropolis, elaborated by stingless bees, is a mixture of plant resin sources, wax and soil. In the geopropolis from *Scaptotrigona affinis postica* (Latreille, 1807), (Hymenoptera, Apidae, Meliponini) was not observed the presence of soil. In a previous study, the extract of geopropolis provided by the beekeeper, from *S. postica* of Barra do Corda, Maranhão State, exhibited potent antiviral activity against herpes simplex virus. In this study, the propolis extract was prepared experimentally and characterized by RP-HPLC-DAD-ESI-MS/MS. The objective of this study was to evaluate the antiviral activity of an experimentally prepared geopropolis extract from *S. postica* against Rubella Virus infected Statens Serum Institute Rabbit Cornea (SIRC) cells. Rubella virus infection of susceptible women during the first trimester of pregnancy, often results in a combination of birth defects in newborns. There is not an effective treatment for rubella virus infection. Different protocols were carried out to evaluate, the antiviral effect of geopropolis extract on the viral replication of infectious RV. Cell viability and cell proliferation assays indicated that this geopropolis was not toxic to cultured SIRC cells. In the viral binding assay, antiviral assay, real-time PCR, and transmission electron microscopy, was observed that different concentrations of geopropolis (17, 34 and 68 μg/mL) was able to inhibit the binding of virions to the cell receptor and the production of infectious RV particles in post treated and pre treated infected SIRC cells. The antiviral activity could to be attributed to the high contents of the apigenin derivatives, vicenin-2 and schaftoside. As far as we know, this is the first report about the antiviral activity of geopropolis from *Scaptotrigona postica* against a *Togaviridae* virus.

Keywords: Rubella virus, antiviral activity, stingless bee, geopropolis, flavones-C-glycosides

1. Introduction

Rubella virus is classified as the only member of the genus Rubivirus belonging to the family Togaviridae. Rubella virus (RV) is a positive-sense, single-stranded RNA virus, hemagglutinin-containing surface projections. Chikungunya and Mayaro virus belong to the same family. Rubella, known more popularly as German measles, is a childhood disease, possessing a worldwide distribution (Parkman, 1996). Rubella virus is formed by the structural polypeptides, the membrane glycoproteins E1 and E2 and a single nonglycosylated RNA-associated capsid protein C (Lee & Bowden, 2000). In a recent research, five genotypes of RV, 1E, 2B, 1J, 1I, and 1a were identified (Martínez-Torres et al., 2016). The vaccine is a live attenuated preparation of the virus (RA 27/3), which induces immunity by producing a modified rubella infection (Parkman, 1996). The vaccines produced by attenuated rubella virus are effective, however possess some side effects and are uneffective for pregnant women

and immunodeficiency people (Petrova et al., 2016).

Postnatal rubella infection causes mild febrile illness accompanied by maculopapular rash and lymphadenopathy, while maternal infections during the first trimester of pregnancy result in a combination of birth defects in newborns, known as congenital rubella syndrome (Plotkin, 2011). RV can establish persistent infection in the developing fetus. Beside this, its replication can induce multiple pathological changes (Curti et al., 2013). It is estimated that more than 100,000 cases of congenital rubella syndrome occur in developing countries every year, representing a considerable social and economic burden ("WHO | World Health Organization," 2017). In a study carried out among 2012 and 2013, 68,968 rubella cases were registered in 28 countries of the WHO European Region (Muscat et al., 2014). The treatment for this virus infection is limited, since the commonly used antiviral drugs, acyclovir or immunoglobulin, are inefficient in the elimination of RV from chronically infected hosts (Gualberto et al., 2013).

Propolis is produced by *Apis mellifera* (Apidae) from resin of the leaf buds of numerous tree species, like *birch*, *poplar*, *conifers*, *pine*, *alder*, *willow*, *palm*, *Baccharis dracunculifolia* and *Dalbergia ecastaphyllum* (Huang et al., 2014; Li et al., 2016). Meliponinae is genera of Hymenoptera, known as stingless bees, which is highly social organisms that occur in tropical and subtropical areas throughout the world, including Brazil. Propolis produced by stingless bees, is a mixture containing plant resin source, wax and clay or soil particles (Massaro et al., 2014, Carneiro, et al., 2016). A study demonstrated that *Corymbia torelliana* is the resin source for the elaboration of geopropolis from Australian *Tetragonula carbonaria*, as was evidenced by the great similarity in their methylated flavanone profiles by HPLC analyses of their respective extracts (Massaro et al., 2014). Geopropolis from stingless bee *Scaptotrigona affinis postica* (Latreille, 1807), (Hymenoptera, Apidae, Meliponini), analysed in this study, contains no soil. Propolis and geopropolis possess the same chemical class of compounds, which are extracted from its respective resin source. Flavonoids, terpenes, phenylpropanoids, triterpenoids, catechins and caffeoylquinic acid derivatives were detected in geopropolis (Silva et al., 2014; Dutra et al., 2014; Batista et al., 2016; Sawaya et al. 2009; Ferreira et al., 2017). A pyrrolizidine alkaloid derived from retronecine was detected in geopropolis from stingless bee *S. postica* (Coelho et al., 2015). However, the chemical composition of propolis from *Apis mellifera* is qualitatively the same in the geographic region where it was produced. As for example, the resin source for European propolis is *poplar* species, for Brazilian green propolis is *Baccharis dracunculifolia* and for red South American propolis is *Dalbergia ecastophyllum* (Osés et al., 2015; Huang et al., 2014; Valenzuela-Barra et al., 2015).

On the other hand, in general, geopropolis show a wide variation even among samples from the same region, since stingless bee collect material from plants near their hives. Different chemical profile was observed among geopropolis samples from *Melipona fasciculata* Smith harvested in municipalities of Maranhão State, northeastern Brazil. Cycloartane, ursane and oleanane derivatives and phenolic acids (protocatechuic acid and gallic acid) were detected in geopropolis harvested in Palmeirândia, while gallic and ellagic acid were the main constituents detected in geopropolis harvested in Fernando Falcão (Batista et al., 2016). Phenolic acids and hydrolyzable tannins (gallotannins and ellagitannins) were detected in geopropolis from *Melipona fasciculata* harvested in Baixada Maranhense, also in Maranhão State (Dutra et al., 2014). However, samples of geopropolis from stingless bee *Tetragonisca angustula*, independently of their geographic origin, presented a similar composition to the flowers extracts of *Schinus terebinthifolius* Raddi (Anacardiaceae), their possible resins source (Carneiro et al, 2016).

There are more studies for *Melipona* than *Scaptotrigona* stinglees bee species (Santos et al., 2017). Sawaya et al. (2009) analysed geopropolis extract from three species of *Scaptotrigona* harvested monthly from two distinct regions in Brazil. Geopropolis from *Scaptotrigona* ssp. was harvested in the state of Maranhão, Northeastern region of Brazil, while geopropolis from *Scaptotrigona aff. depillis* and *Scaptotrigona bipunctata* was harvested in the state of São Paulo, South eastern region of Brazil. Diterpenes acid derivatives were found as the main constituents. However, was observed that, the chemical profile obtained for geopropolis from *Scaptotrigona* ssp., harvested in Maranhão State, was different for that obtained for the *Scaptotrigona* species harvested in São Paulo State (Sawaya et al. 2009). Flavonols, such as quercetin methyl ethers, and methoxy chalcones were detected in geopropolis from *Scaptotrigona aff. depillis*, harvested in the state of Rio Grande do Norte, Northeast region of Brazil (Ferreira et al., 2017).

In this study was carried out the chemical analysis and antiviral activity of an experimentally prepared extract of geopropolis (HMEG) from *Scaptotrigona affinis postica* harvested in Barra do Corda, Maranhão State, Brazil. HMEG were characterized by RP-HPLC-DAD-ESI-MS/MS. In the previous study was reported the chemical composition of an extract from *S. postica* of Barra do Corda, provided by the beekeeper, which exhibited the flavones-6,8-di-C-glycosides (vicenin-2 and schaftoside), a pyrrolizidine alkaloid derived from retronecine,

catechin-3-O-gallate, 3,5-dicaffeoyl quinic acid and caffeoylquinic acid-O-arabinoside as the main constituents (Coelho et al., 2015). The ecosystems from Barra do Corda, Maranhão State, included mangrove swamps, floodplains, lakes, babassu palm and forests. In geopropolis from *S. postica* were identified 94 pollen types, which belonging to 35 plants families. *Borreria verticillata* (34.17%) was the most frequent pollen type, followed by *Anadenanthera* sp. (13.65%) and *Mimosa caesalpiniifolia* (10.5%) (Souza et al., 2015).

The search for functional foods, that possess bioactive substances, is a new trend, which can provide more effective treatments of diseases with fewer side effects. The numerous bioactive compounds collected by honeybees from exudates and buds of plants, are utilized in the elaboration of propolis that exert a defensive barrier against microorganism (Saeed et al., 2016; Salas et al., 2016). It is extensively used for centuries, in foodstuffs and beverages to improve health related disorders. Propolis and geopropolis exhibited a wide variety of pharmacological properties, such as, anti-inflammatory, antioxidant, antitumor, antiulcer and for treatment of respiratory diseases (Berretta et al., 2017; Montenegro & Mejías, 2013; Pippi et al., 2015; Nina et al., 2015). The antiviral activity of propolis from different geographic regions is known, since ancient times. Propolis has been pointed out as an alternative for the treatment of disease caused by virus, since its antiviral properties has been evidenced in different steps of viral replication (Silva-Carvalho et al., 2015; Saeed et al., 2016; Salas et al., 2016). Propolis exerted antiviral activity against influenza virus A and B, herpes, Vaccinia Virus, Hepatitis B Virus, Calicivirus, Newcastle disease virus, Avian reo virus, Bursal disease virus and human immunodeficiency virus (HIV) (Silva-Carvalho et al., 2015; Oldoni et al., 2015). The green propolis and its resin source *Baccharis drancunculifolia* exhibited antiviral activity on poliovirus type 2 (Búfalo et al., 2009). Propolis extracts exhibited high anti-herpetic activity against Herpes virus type I and II, by different mechanism of action (Nolkemper et al., 2010; Schnitzler et al., 2010), and anti-influenza virus activity against influenza infection in mice (Shimizu et al., 2008). The hydroalcoholic extract from Brazilian brown propolis promoted protective effect on herpes infected mice, acting on inflammatory and oxidative processes (Sartori et al. 2012). Hatay propolis samples exhibited antiviral effects against Herpes virus type I and II (Yildirim et al., 2016). Propolis extract collected in a Canadian region, rich in poplar trees, exhibited high virucidal effect against herpes simplex viruses type 1 and type 2, due to its interference in virus adsorption (Bankova et al., 2014). The geopropolis from *S. postica*, that contain high contents of vicenin-2 and schaftoside, exhibited high antiviral activity against herpes virus (Coelho et al. 2015). The results obtained in different studies had shown that propolis with different chemical profile, harvested in different geographic region exhibited antiviral activity against herpes simplex viruses and other types of virus (Coelho et al. 2015, Bankova et al., 2014, Yildirim et al., 2016). Attachment to cellular receptors and entry into the host cell are the first steps in viral infection (Rasbach et al., 2013). It is known that flavonoids can prevent the virus binding to host cell receptor and penetration within cells, exerting an inhibitory effect on the early stage of virus infection (Ahmad et al., 2015; Kai et al., 2014).

The aim of this study was to evidence the effectiveness of an experimentally prepared extract of geopropolis (HMEG) from *S. postica*, harvested in Barra do Corda, Maranhão State, against Rubella virus infected Statens Serum Institut Rabbit Cornea (SIRC) cells. In the present study, viral binding and penetration assays were included, to determine if treatment of RV with an extract rich in flavones-6,8-di-*C*-glycosides could disrupt virions from binding to the SIRC receptor of the cell membrane and its penetration into the cell.

2. Material and Methods

2.1 Cells

The SIRC cells (rabbit cornea — ATCC CCL-60) were grown in 75 cm^2 plastic cell culture flasks, in DMEM medium (Dulbecco's minimum Eagle essential medium) supplemented with 10% inactive fetal bovine serum (FBS) and 20 mM L-glutamine (Invitrogen, EUA).

2.2 Preparation and Phytochemical Analysis of Experimentally Prepared Extract of Geopropolis (HMEG) from S. Postica using Reversed Phase HPLC-DAD-ESI-MS/MS.

Geopropolis sample (15 g) from *S. postica* harvested in the region of Barra do Corda, Maranhão state, Brazil, (5º 30'S, 45º 14'O) was treated with solvents of increasing polarity (hexane, chloroform, ethyl acetate and methanol) in Sohxlet apparatus. The obtained fractions were concentrated and stored at freezer until sample workup. The yield of methanolic extract was 15.08 % by dry weight. For antiviral tests, the dry methanolic fraction, was dissolved in water and denominated HMEG. This extract rich in hydrosoluble compounds was analyzed by HPLC-DAD-ESI-MS/MS. The analysis was conducted on DADSPD-M10AVP Shimadzu system equipped with a photodiode array detector coupled to Amazon speed ETD, Bruker Daltonics, as previously described by Coelho et al. (2015). The identification of constituents was established on the basis of their UV and mass spectral (MS) data, which were compared with MS data reported by Coelho et al. (2015), Negri et al. (2018), Mihajlovic et al.

(2015) and the chemical databases Phenol-Explorer (www.phenol-explorer.eu), Chem. Spider (http://www.chemspider.com), METLIN (http://metlin.scripps.edu) and HMDB (www.hmdb.ca).

2.3 Cell Viability - MTT Assay

Cell viability were determined using MTT (3-[4,5-dimethylthiazol 2-yl]-2,5 diphenyl tetrazolium bromide). The MTT assay was carried out using the methodology reported by Coelho et al., (2015) with modifications. SIRC cells were seeded at concentration of 10^4 cells/well in 96-well plates, which was grown at 37 °C for 1 day. After 48 hs, cells were treated with different concentrations of HMEG (0, 0.3, 0.6, 1.2, 2.4, 8.6, 17.2, 34, 68, and 150 ug/mL) and phosphate buffered saline (PBS) (control negative).

2.4 Determination of the Virus Infectious Dose

The determination of the virus infectious dose was carried out using the methodology reported by Coelho et al. (2015) with modifications. The confluent monolayers were dispersed with 0.2% trypsin and 0.02% versene, resuspended in Dulbecco's minimum Eagle essential growth medium (DMEM) with 100 IU/ml penicillin G and 100 mg/ml streptomycin. The SIRC cell suspension was diluted to $2.0x10^4$ cells/ml and placed into 96-well plate. Plates were seeded with 200 µL of cell suspension and incubated at 37ºC in a humidified 5% CO_2 atmosphere. RA 27/3 strain (Meruvax II, Merck, Sharp and Dohme) stock virus was quantified by medium tissue, using cell culture infections with 0.01 MOI (multiplicity of infection). HMEG was added to the cells at 3 h prior of the virus infections (pre treatment) and 1 h after virus infection (post treatment). The antiviral screenings were repeated three times with different concentrations of HMEG (0.6, 2.4, 8.6, 17, 34 and 68 µg/mL).

2.5 Antiviral Effect of Geopropolis on Infected SIRC Cells

The antiviral effect was evaluated according to the method described by Carvalho et al. (2017) and Coelho et al. (2015) with modifications. SIRC cells were grown to approximately 90% confluence in 96 well plates in DMEM, supplemented with 2 mM of L-glutamine and 10% phosphate buffered saline (PBS). Plates were incubated at 37°C in a humidified 5% CO_2 atmosphere. The confluent cells were infected with RA 27/3 (MOI=0.1) and monitored for cytopathic effects, for 3 days. The extract was added to the cells at 3 h prior of the virus infections (pre treatment) and 1 h after virus infection (post treatment). The antiviral screenings were independently repeated, three times with different concentrations of HMEG (0.6, 2.4, 8.6, 17, 34 and 68 µg/mL). After this, the determination of the HMEG effect on the infected cells was carried out using Real-Time quantitative polymerase chain reaction (qPCR).

2.6 Binding-penetration Assays

The aim of binding-penetration assays is to measure interactions between virus and cells. These assays were carried out according to the method described by Carvalho et al. (2017) with modifications. The binding assay was carried out at 4°C, a temperature that allowed the binding of RV to cell receptors. In this temperature RV cannot penetrate within cells. The penetration occurs most efficiently at 37°C. SIRC cells were placed in 24-well plate and allowed to reach confluency. The cells were infected with RV (MOI=0.1) dilutions and treated with different concentrations of HMEG (17, 34 and 68µg/mL). The infected SIRC cells treated or untreated were allowed to adsorb for 1 h at 4 °C. Unabsorbed virions were then aspirated, and the cells were washed twice with PBS and were again treated with different concentrations of HMEG (17, 34 and 68µg/mL) for 1h at 37°C and 5% CO_2. RV virions penetrated within cells at 37°C to complete its life cycle. Unabsorbed virions were then aspirated, and the cells washed with PBS twice. After this, cells were incubated for 72 h at 37°C and 5% CO_2. The results were analysed using qPCR.

2.7 Quantitative Real-time PCR Assay - qPCR

Quantitative real-time PCR (qPCR) is used for the quantification of viral nucleic acids, being a reliable method for measure gene expression (Carvalho et al. 2017). The total RNA for evaluation of antiviral activity of HMEG in pre treatment, post treatment, binding and penetration assay was measured by qPCR. The quantification was carried out according to the method described by Coelho et al., (2015) and Carvalho et al. (2017) with slight modification. The extraction of total RNA from homogeneous cell group, were carried out using the MagNA Pure extractor (Roche, Basel, Switzerland). To amplify the RV genomic sequence, Real-Time quantitative polimerase chain reaction (RT-PCR) was performed using the Superscript III Step RT-PCR kit (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's recommendations. The set of primers used was described by CDC/USA (Abernathy et al. 2009). The assay was performed in triplicate with 25µl reactions mixtures containing reaction buffer (Invitrogen, Carlsbad, CA, USA), 0.5 U of a Superscript-Taq enzyme mixture, 0.2µM of each primer, 0.1µM of the labeled probe (Invitrogen, Carlsbad, CA, USA) and 5µl of RNA. The assay was carried out also including a no-template control. The thermal cycling was carried out with an Applied

Biosystems 7500 thermal cycle with the following procedure: 50 C for 10 min; 95 C for 2 min; and 40 cycles of 95 C for 15 s and 60 C for 1 min. The presence of intact RNA in the samples was confirmed with primers specific for RNase P RNA. Standard curves were prepared by qPCR using serial dilutions of known copy numbers of the purified amplification product for RV. A reaction mixture containing water as the template was run on each plate as negative control. The percentage of reduction was defined as follows: [copy no. of infected cells - copy no. of treated cells/copy no. of infected cells X 100]. The data were analysed with SDS software (version 2.1; Applied Biosystems, Grand Island, NY, USA).

2.8 Transmission Electron Microscopy (TEM)

The transmission electron microscopy was carried out according to the methodology reported by Coelho et al., (2015) and Carvalho et al. (2017) with slight modification. SIRC cells were cultivated on Aclar film seeded in 24-well plates and incubated for 48 h at 37 ^{o}C in a humidified atmosphere with 5% CO_2. RVs (MOI = 0.1) were treated with 68 µg/mL of HMEG for 1 h at 37 ^{o}C prior to cell infection. DMSO used as the negative control, did not exhibited any noticeable effects on the cell lines. The cells inoculated with RV, treated and untreated with HMEG, and allowed to adsorb RV for 1 h at 37 ^{o}C in 5% CO_2. After 48 h, the cells were fixed with 2.5% glutaraldehyde (Sigma, St. Louis, MO, USA) in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 hours at 4°C. After rinsing with cacodylate buffer, the cultures were post-fixed in a solution containing 1% osmium tetroxide, washed in 0.1 M sodium-cacodylate buffer, dehydrated in graded acetone, and embedded in epoxy resin. Ultrathin sections stained with uranyl acetate and lead citrate were examined under a Jeol Transmission Electron Microscope at 80 kV. Images were recorded under a JEM-1011 transmission electron microscope (JEOL, Tokyo, Japan).

2.9 Statistical Analysis

Statistical analysis was performed using Exstat software. For analyses of the cell viability data and the antiviral activity by mRNA quantification, the Student's T test was used with the *p* value corrected by the Bonferroni–Sidak method.

3. Results

3.1 Antiviral Effect of HMEG on SIRC Infected Cells

Results of cell viability and cell proliferation assays indicated that HMEG was not toxic to cultured SIRC cells. SIRC infected cells were treated with different concentrations of HMEG (0.6, 2.4, 8.6, 17, 34 and 68 µg/mL) prior to RV infection (pre treated cells) and after infection (post treated cells). In the MTT assay was not observed cytotoxic effect on SIRC cells treated with HMEG at concentration of 68 ug/mL, as can be seen in Figure 1. The 50% cytotoxic concentration (CC50) of HMEG was 150 ug/mL. As can be seen in Figure 2, the post treatment and pre treatment with HMEG reduced the number of copies of RV in the cell lysates, reducing the viral load, which was dose dependent. In the pre treatment with HMEG, the reduction of viral load ranged from 20% to 90%. On the other hand, was observed a reduction of 80% of viral load, after post treatment with 0.6 µg of HMEG. The post treatment carried out with 68 µg/mL of HMEG resulted in 98% of inhibition of the viral replication (see Figure 2).

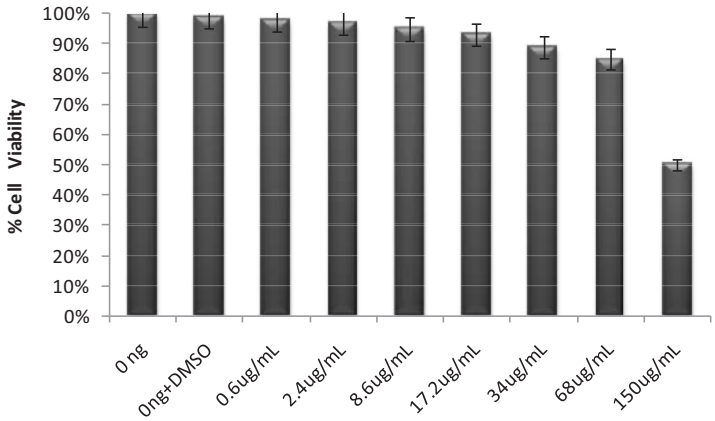

Figure 1. Cell viability of SIRC cells treated with different concentrations of HMEG. The number represents the mean of three replicates

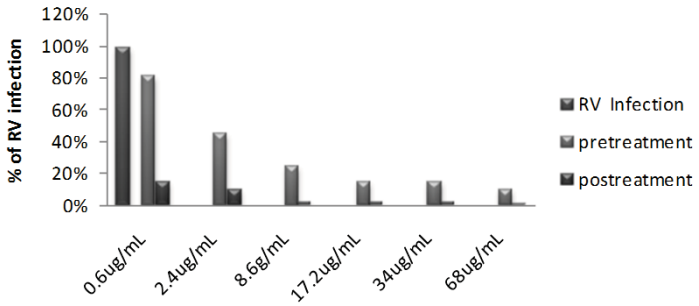

Figure 2. RV treated with geopropolis (pre treatment and post treatment). The infectivity of RV decreased after pre treatment and post treatment, with HMEG. Infectivity was determined by qPCR. The errors bars represent the SD from three replicates for each set of values. It is important to observe that the inhibitory infection was more than 90% with concentrations of HMEG 2.4 ug/mL.

The viral binding assay was performed at 4°C, to determine if treatment of RV with HMEG could disrupt virions from binding to the SIRC cellular receptor. In this temperature did not occur the penetration of RV within SIRC cells, which occur after the increase of temperature at 37° C, completing its life cycle. The cytopathic effect was observed in the cells infected and untreated and in cells DMSO-treated RVs, however was not observed with RV infected SIRC cells treated with HMEG. In the viral binding assay was observed that the treatment of infected SIRC cells with different concentrations of HMEG (17, 34 and 68µg/mL), was efficient to block the binding the virus on cell receptor, inhibiting the infection of SIRC cells, as can be seen in Figure 3. Thus, the results (repeated in triplicates) indicate that HMEG was able to inhibit the binding of virions to the SIRC cells receptor. Virus binding to cellular receptors leads to the direct penetration into cells. Beside this, the results obtained by penetration assays (Figure 4), indicated that HMEG efficiently prevented viral penetration and replication.

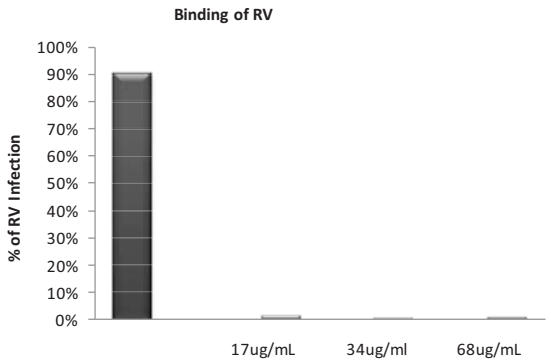

Figure 3. Binding assay of untreated virus and RV treated with HMEG (17, 34 and 68ug/mL) on receptor of SIRC cells. Infectivity was determined by qPCR. The numbers represent the mean triplicate trials

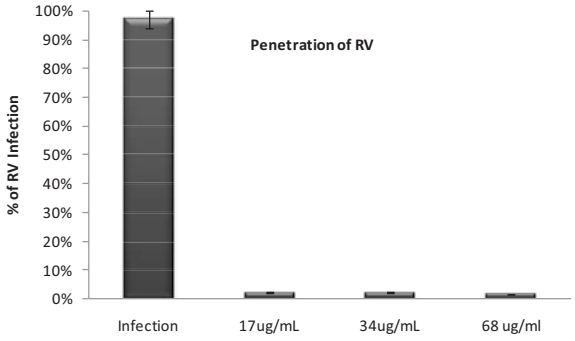

Figure 4. Penetration assay of untreated or RV treated with HMEG (17, 34 and 68ug/mL) within SIRC cells. Infectivity was determined by qPCR. The numbers represent the mean of triplicate trials

The reduction of viral load was observed by qPCR, which indicated a decrease of the RNA copy number of RV. These results were corroborated by TEM assay. As can be seen in Figure 5, in the electron micrographs, in the cytoplasm of infected SIRC cells treated with HMEG, were not observed the rearrangement of organelles and the presence of RV-like particles.

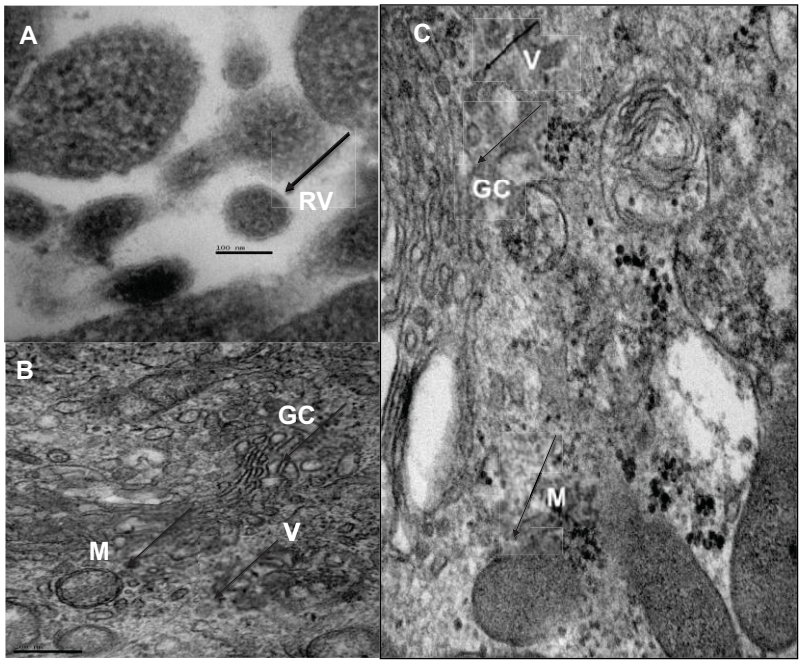

Figure 5. The SIRC cells were cultivated on Aclar film and after 48 hours were inoculated with binding sample and processed by TEM. A- SIRC cells inoculated with RV. Note the presence of a typical particle viral. B- SIRC cells inoculated with binding sample. Note Golgi complex (GC), Vesicles (V) and Mitochondria (M). C- SIRC cells inoculated with virucida sample. It is important to note that RV like particles is not found. The Golgi complex (GC), Vesicles (V) and Mitochondria (M) are marked in the cells.

3.2 Phytochemical Analysis of Experimentally Prepared Extract of Geopropolis (HMEG) using Reversed Phase HPLC-DAD-ESI-MS/MS

The chemical profile of experimentally prepared extract of geopropolis (HMEG) from *S. postica* was similar that obtained for extract provided by beekeeper (Coelho et al., 2015). The only difference observed was the presence of low contents of hydroxycinnamic acid amide derivatives in HMEG. The presence of hydroxycinnamic acid amide derivatives were reported in *Apis mellifera* pollen (Negri et al. 2011, 2018). Table 1 summarises the MS data of compounds **1 – 22** detected in HMEG, through reversed phase HPLC-DAD-ESI-MS/MS. The method used for the identification of vicenin-2 (**7**); schaftoside (**10**); 5-O-caffeoylquinic acid arabinoside (**23**) and the pyrrolizidine alkaloid 7-(3-methoxy-2-methylbutyryl)-9-echimidinyl retronecine (**2**) was reported by Coelho et al., (2015). The flavones-6,8-di-C-glycosides, vicenin-2 (**7**) and schaftoside (**10**) was found as the main constituents in both extracts. Catechin-C-arabinoside (**8**) and catechin-C-rhamnoside (**9**) were identified based on mass spectral data reported in literature (Karar & Kuhnert, 2015). The identification of hydroxycinnamic acid amide derivatives were performed according to the method reported by Negri et al. (2011, 2018) and Mihajlovic et al. (2015). As can be seen in Table 1, the MS/MS experiments in protonated hydroxycinnamic acid amide (HAA) derivatives produced abundant fragment ions attributed to the acyl neutral losses, as for example 176 Da for feruloyl, 162 Da for caffeoyl, and 146 Da for coumaroyl moieties, which was followed by neutral water loss (18 Da) (Negri et al., 2011). While the MS/MS experiments in deprotonated hydroxycinnamic acid amide derivatives produced abundant fragment ions, attributed to the loss of 120 Da for HAA containing coumaric acid moiety; the loss of 136 Da for HAA containing caffeic acid moiety; and the loss of 150 Da for HAA containing ferulic acid moiety (Mihajlovic et al., 2015, Negri et al., 2018).

Compound **20** was tentatively identified as 6-C-fucosyl luteolin, since in its MS/MS spectrum was observed the loss of water (Table 1), which is representative of C-6-isomers (Elliger et al., 1980). The presence of

pyrrolizidine alkaloids was reported in bee products. When bees collect resins of plants that contain pyrrolyzidine alkaloids, these compounds can be transferred into geopropolis, propolis or honey (Dübecke, Beckh, & Lüllmann, 2011). Pyrrolizidine alkaloids possess 1-hydroxymethyl pyrrolizidine necine base. They rarely occur in the free form, generally occurring as esters (mono-, di- or macrocyclic diesters) (Moreira et al., 2018). The pyrrolizidine alkaloids 7-(3-methoxy-2-methylbutyryl)-9-echimidinyl retronecine (**2**) and 7-(3-dihydroxy-propoxy-2-methylbutyryl)-9-echimidinyl retronecine (**6**) (Table 1) occur as necine base (retronecine) and contain esters groups at C-9 and C-7.

Table 1. Compounds **1-22** detected in experimentally prepared extract of geopropolis from *S. postica* (HMEG) of Barra do Corda, Maranhão State, by HPLC-DAD-ESI-MS/MS analyses

N°	Tr min	HPLC/DAD Max (nm)	HPLC/(+)ESI-MS/MS *m/z* (% base peak)	HPLC/(-)ESI-MS/MS *m/z* (% base peak)	Identification
1	2.8	320		$[M - H]^-$ - 341 MS/MS - 179	6-*O*-caffeoyl glucoside[a]
2	3.5	-	$[M + H]^+$ - 430 MS/MS - 412 (100), 385 (70), 315 (20)	$[M - H]^-$ - 428 MS/MS - 398	7-(3-methoxy-2-methylbutyryl)-9- echimidinyl retronecine[a]
3	4.1	-	$[M + H]^+$ - 541 MS/MS - 523 (100), 472 (20), 444 (40)		methoxy-heptahydroxy-flavone-3-O-glucuronide[c]
4	5.5	260 - 355	$[M + H]^+$ - 555 MS/MS - 537 (100), 486 (20), 454 (30)		dimethoxy- hexahydroxy-flavone-3-O-glucuronide[c]
5	6.0	260 - 355	$[M + H]^+$ - 641 MS/MS - 479 (90), 317 (100)		Isorhamnetin-7,3-O-diglucoside[c]
6	6.5	-	$[M + H]^+$ - 490 MS/MS - 473 (40), 445 (100), 315 (60)		7-(3-dihydroxy-propoxy-2-methylbutyryl)-9-echimidinyl retronecine[c]
7	10.3	270 - 335	$[M + H]^+$ - 595 MS/MS - 577 (100), 559 (30), 529 (40), 511 (50), 499 (30), 475 (30), 457 (80)	$[M - H]^-$ - 593 MS/MS - 575 (20), 503 (40) 473 (100), 383 (40), 353 (50)	vicenin-2[a]
8	11.2	280	[M + Na]+ - 445 MS/MS - 427 (100) [M + H]+- 423 MS/MS - 405 (100), 387 (30), 357 (80), 327 (50)	$[M - H]^-$ - 421 MS/MS - 403 (20), 331 (80), 301 (100)	catechin-C-arabinoside[a]
9	12.6	280	[M + H]+ - 437 MS/MS - 419 (100), 371 (60), 341 (40)		Catechin-C-rhamnoside[a]
10	13.0	270 - 335	[M + H]+ 565 MS/MS - 547 (100), 529 (70), 511 (80), 427 (85)	[M - H] - 563 MS/MS - 545 (40), 503 (50), 473 (80), 443 (100), 383 (60), 353 (60)	schaftoside[a]
11	14.0	-	[M + H]+ - 333 MS/MS - 315 (20), 206 (80), 179 (100)		2-galloyl glucose[c]
12	15.1	-	[M + Na]+ - 587 MS/MS - 569 (100), 551 (40) [M + H]+ - 565 MS/MS - 547 (100),	[M - H] - 563	isoschaftoside[a]

			529 (30), 499 (60), 457 (50)		
13	19.4	300 - 330	[M + H]+ - 632 MS/MS - 470 (100), 452 (20)	[M - H] - 630 MS/MS - 494 (80), 468 (100), 358 (40)	N',N",N'"-tris-caffeoyl spermidine[b]
14	19.6	300 - 330	[M + H]+ - 616 MS/MS - 454 (100), 436 (18)	[M - H] - 614 MS/MS - 478 (100), 452 (80), 358 (70)	N',N"-dicaffeoyl,N'"-couma royl spermidine[b]
15	19.8	300 - 330	[M + H]+ - 600 MS/MS - 454 (80), 438 (100),	[M - H] - 598 MS/MS - 478 (100), 358 (60)	N'-caffeoyl-N",N'"-dicoumaroyl spermidine[b]
16	20.5	-	[M + H]+ - 770 MS/MS - 562 (30), 544 (100)		N'-hydroxyferuloyl-N"-dihy drohydroxy sinapoyl-N'"-dihydrosinapoyl spermidine[b]
17	22.2	300 - 330	[M + Na]+ - 606 MS/MS - 460 (100)	[M - H] - 582 MS/MS - 462 (100), 342 (40)	N',N",N'"-tris-p-coumaroyl spermidine[b]
			[M + H]+- 584 MS/MS - 438 (80), 420 (100)		
18	23.2	300 - 330	[M + H]+ - 764 MS/MS - 558 (100), 540 (80)	[M - H] - 762 MS/MS - 596 (40), 582 (100)	N',N",N'"-tris-p-sinapoyl spermidine[b]
19	23.6	300 - 330	[M + Na]+ - 696 MS/MS - 520 (100), 344 (40)	[M - H] - 672 MS/MS - 522 (100)	N',N",N'"-tris-p-feruloyl spermidine[b]
			[M + H]+ -674 MS/MS - 498 (60), 480 (100)		
20	25.0		[M + Na]+ - 455 [M + H]+ - 433 MS/MS - 415 (80), 311 (50), 293 (100)	[M - H] - 431 MS/MS - 413 (10), 309 (80), 265 (100)	6-C-fucosyl luteolin[c]
21	25.4	-	[M + Na]+ - 929 MS/MS - 753, 577	[M - H] - 905 MS/MS - 755 (100), 605 (40)	catechin diferuloyl diarabinoside[c]
			[M + H]+ -907 MS/MS - 731 (100), 713 (50)		
22	35.4		[M + H]+ - 487 MS/MS - 469 (100), 451 (80), 433 (40)		5-O-caffeoylquinic acid arabinoside[a]

[a]Constituents previously reported for geopropolis from S. postica of Barra do Corda, Maranhão State (Coelho et al. 2015). [b]Constituents identified based on MS spectral data reported by Negri et al. (2011, 2018), and Mihajlovic et al., (2015). [c]Constituents tentatively identified based on MS data.

4. Discussion

The potent antiviral activity of the extract of geopropolis from *S. postica* provided by beekeeper, against herpes simplex virus was reported previously (Coelho et al., 2015; Silva-Carvalho et al., 2015). The present study evaluated the effect of an experimentally prepared extract of geopropolis from *S. postica* (HMEG) on RV infected SIRC cells. Rubella was described in 1866, by Henry Veale, a British Army surgeon (Muscat et al., 2014). Generally, cells cultivated with RV strains cause cytopathic effects or morphological changes in the host cell (Carvalho et al. 2017). In this study, SIRC cells cultivated with RA 27/3 exhibited clear growth of the RV and readily detectable cytopathic effects. The replication of RV was observed on untreated SIRC cells cultivated with RA 27/3 after 48 h, as described by Figueiredo et al., (2000). The growth of the RV, with the arrangement of

organelles was not observed in cells infected and treated with HMEG. The binding-penetration assays indicated that HMEG inhibited RV entry into SIRC cells by interfering with the binding/ adsorption of the virions to the cellular receptor, and consequently caused not only a reduction of viral load but also a decrease of cytopathic effects and viral protein synthesis. Thus, vicenin-2 and schaftoside was been able to block the binding of RV with receptors on SIRC plasma membrane and prevent the penetration within cells, affecting the steps of viral cycle replication into SIRC cells or lead to the DNA degradation, before the virus entry into cells.

Endoplasmic reticulum, membranous networks of the cell, is a crucial organelle used for viral entry and viral replication. Rubella virus possesses the ability to rearrange cellular membranes to facilitate its viral replication (Lee & Bowden, 2000, Petrova et al., 2016). The endoplasmic reticulum, Golgi complex, and mitochondria are often closely arranged around the virus replication complex, in RV infected SIRC cells (Lee & Bowden, 2000). The results measured by qPCR and visualized by transmission electron microscopy (TEM) demonstrated a reduction in infectivity on the RV infected SIRC cells treated with HMEG. In TEM assay, was not observed the rearrangement of organelles, the typical replication complex, rubella virions and RV-like particles on infected SIRC cells treated with HMEG in concentrations of 0.6-68 ug/mL. In qPCR assays was observed that the inhibition of the cytopathic effect and viral replication on infected and treated SIRC cells, was dose dependent. Thus, was observed that pre treatment of SIRC cells with HMEG, carried out 3 h before of the virus infection and post treatment 1 h after of virus infection, inhibited the viral replication. The post treatment exhibited the best antiviral activity. The results indicated that HMEG inhibited RV entry into target cells interfering with the binding/adsorption of the virions to the cellular receptor.

The pharmacological activities of propolis had been attributed to flavonoids, generally, its main constituents. The antiviral property of flavonoids is known, since 1940 (Kaul, Middleton, & Ogra, 1985). Many flavonoids are used extensively in the fields of nutrition, food safety and health (Ahmad etal., 2015; Panche, Diwan & Chandra, 2016; Kumar & Pandey, 2013). Quercetin, naringin, hesperetin and catechin affected the replication and infectivity of some RNA and DNA viruses (Panche, Diwan & Chandra, 2016; Kumar & Pandey, 2013). The antioxidant activity of flavonoids can to inhibit essential enzymes associated with the life cycle of viruses (Kumar & Pandey, 2013), disrupt cell membranes, to prevent viral binding and penetration into cells and increase the host cell self-defense mechanism (Friedman, 2014). Moreover, can inhibit the enzyme viral polymerase and the bind of viral nucleic acid or viral capsid proteins on host cell receptor (Hossain et al., 2014; Kumar & Pandey, 2013; Song et al., 2015). Several flavone 6-C-monoglycosides exhibited potent, *in vitro*, antiviral effect (Wang et al., 2012). The flavonoids baicalein, fisetin, and quercetagetin exhibited high antiviral activity against Chikungunya virus and extracellular Chikungunya vírus particles (Lani et al., 2016) and demonstrated anti-noroviral activity against murine norovirus and feline calicivirus (Seo et al., 2016).

Vicenin-2 and schaftoside are apigenin derivatives. The antiviral activity of apigenin derivatives and other flavones are known, since 1994 (Panche, Diwan & Chandra, 2016). Apigenin exhibited antiviral activity against eleven different types of viruses (Ahmad et al., 2015), among them, herpes simplex virus, aujeszky virus (Kumar & Pandey, 2013), poliovirus type 2 (Visintini Jaime et al., 2013), enterovirus 71 (Ji et al., 2015; Lv et al., 2014; Wang et al., 2014) and hepatitis C virus (Shibata et al., 2014). Beside this, apigenin and luteolin inhibited the neuraminidase of influenza virus (Liu et al., 2008) and exhibited high antiviral activity against oseltamivir- and peramivir-sensitive and oseltamivir- and peramivir-resistant influenza viruses (Kai et al., 2014). 3,2'-Dihydroxyflavone and 3,4'dihydroxyflavone, exhibited potent anti-influenza activity, attributed to the inhibition of the viral neuraminidase activity and viral penetration into cells (Hossain et al., 2014). Luteolin 7-O-methylether-3′-O-beta-D-glucoside exerted an inhibitory effect on the first stage of herpes virus-2 infection, attributed to the inhibition of herpes virus-2 binding to receptor of the cell membrane and its penetration into the cell (Behbahani, Zadeh, & Mohabatkar, 2013). An extract of Mexican propolis, possessing high contents of quercetin, pinocembrin and naringenin exhibited antiviral activity against Canine Distemper virus (González-Búrquez et al., 2018). Low contents of flavonoids inhibited the replication of Hand Foot Mouth Disease, caused from human enterovirus A71 infection, which can produce severe neurological complications, mainly in young children (Min et al., 2018). Oroxylin A (bacalein-6-methyl ether, an O-methylated flavone), exhibited antiviral activity against Coxsackievirus B3 (Kwon et al., 2016).

The antiviral activity of caffeolyquinic acids, catechins and hydroxycinnamic acid amide derivatives was also known. An aqueous extract of Brazilian green propolis, rich in caffeoylquinic acids derivatives, exhibited anti-influenza activity (Takemura et al., 2012; Urushisaki et al., 2011). Catechins inhibited RNA replication of influenza virus (Song, Lee, & Seong, 2005), and the process of fusion of HIV virus with the cell receptor (Liu et al., 2005). Hydroxycinnamic acid amide derivatives or triacylated spermidines derivatives exhibited antimicrobial activity against viruses, bacteria and fungi (Mihajlovic et al., 2015). The geopropolis from *S.*

Postica is used by the population of Maranhão State, in the treatment of wounds (Coelho et al., 2015; Souza et al., 2015). Pyrrolizidine alkaloids exhibited antimicrobial activity and are promising prototypes for new drugs, especially for topical use (Silva Negreiros Neto et al., 2016).

5. Conclusion

There is not an effective treatment for rubella virus infection. This study indicated that geopropolis from *Scaptotrigona postica* of Barra do Corda, Maranhão State, possess potent antiviral activity against Rubella, a *Togaviridae* virus. HMEG at low concentrations, was able to inhibit the replication of Rubella virus. The best antiviral activity was observed in the post treatment with HMEG. Results of cell viability and cell proliferation assays indicated that HMEG was not toxic to cultured SIRC cells. The results obtained by viral binding assay, antiviral assay, PCR, real-time PCR, and transmission electron microscopy demonstrate that HMEG can be able to inhibit the production of infectious RV particles. This activity could be attributed to the high content of vicenin-2 and schaftoside, which probably acted blocking the RV binding to the receptor of SIRC cell membrane, and the penetration within the cell, preventing the viral replication.

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Conflict of interests

We confirm that there are not conflict of interest associated with this work.

List of Abbreviations

CRS - congenital rubella syndrome, EPEG – experimentally prepared hydroethanolic extract from geopropolis, DEM – Direct electron microscopy, DMEM - Dulbecco's minimum Eagle essential medium, PBS - phosphate buffered saline, FBS - fetal bovine serum, mRNA – Messeger ribonucleic acid, MTT - 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, PCR – Polimerase chain reaction, qPCR – quantitative polimerase chain reaction, RV – Rubella Virus, SIRC - Serum institut Rabbit Cornea (SIRC) cells, TEM - Transmission electron microscopy, WHO – World Health Organization

References

Abernathy, E., Cabezas, C., Sun, H., Zheng, Q., Chen, M., Castillo-Solorzano, C., … Icenogle, J. (2009). Confirmation of rubella within 4 days of rash onset: comparison of rubella virus RNA detection in oral fluid with immunoglobulin M detection in serum or oral fluid. *Journal of Clinical Microbiology, 47*, 182-188. https://doi.org/10.1128/JCM.01231-08

Ahmad, A., Kaleem, M., Ahmed, Z., & Shafiq, H. (2015). Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections-A review. *Food Research International, 77*, 221-235. https://doi.org/10.1016/j.foodres.2015.06.021

Bankova, V., Galabov, A. S., Antonova, D., Vilhelmova, N., & Di Perri, B. (2014). Chemical composition of Propolis Extract ACF® and activity against herpes simplex virus. *Phytomedicine, 21*, 1432-1438. https://doi.org/10.1016/j.phymed.2014.04.026.

Batista, M. C. A., Abreu, B. V. B., Dutra, R. P., Cunha, M. S., Mendonça do Amaral, F. M., Torres, L. M. B., Ribeiro, M. N. S. (2016). Chemical composition and antioxidant activity of geopropolis produced by Melipona fasciculata (Meliponinae) in flooded fields and cerrado areas of Maranhão State, northeastern Brazil. *Acta Amazonica, 46*, 315-322. http://dx.doi.org/10.1590/1809-4392201600034

Behbahani, M., Zadeh, M. S., & Mohabatkar, H. (2013). Evaluation of antiherpetic activity of crude extract and fractions of Avicenna marina, in vitro. *Antiviral Research, 97,* 376-80. https://doi.org/10.1016/j.antiviral.2013.01.001.

Berretta, A. A., Arruda, C., Miguel, F. G., Baptista, N., Nascimento, A. P., Marquele Oliveira, F., … Bastos, J. K. (2017). Functional Properties of Brazilian Propolis: From Chemical Composition Until the Market. Agricultural and Biological Sciences » "Superfood and Functional Food - An Overview of Their Processing and Utilization", book edited by Viduranga Waisundara and Naofumi Shiomi, ISBN 978-953-51-2920-2.

Búfalo, M. C., Figueiredo, A. S., De Sousa, J. P. B., Candeias, J. M. G., Bastos, J. K., & Sforcin J. M. (2009). Anti-poliovirus activity of Baccharis dracunculifolia and propolis by cell viability determination and

real-time PCR. *Journal of Applied Microbiology, 107*, 1669-1680. https://doi.org/10.1111/j.1365-2672.2009.04354.x

Carneiro, M. J., López, B. G-C., Lancellotti, M., Franchi G. C., Nowill, A. E., & Sawaya, A. C. H. F. (2016). Evaluation of the chemical composition and biological activity of extracts of Tetragonisca angustula propolis and Schinus terebinthifolius Raddi (Anacardiaceae). *Journal of Apicultural Research, 55,* 315-323. https://doi.org/10.1080/00218839.2016.1243295

Carvalho, N. D., Mendonça, R. Z., Oliveira, M. I., Curti, S. P., Barbosa, T. F., Silva, P. E., … Figueiredo, C. A. (2017). Antiviral activity of hemolymph of Podalia against rubella virus. *Cytotechnology, 69*, 31-37. https://doi.org/10.1007/s10616-016-0035-6

Coelho, G. R., Mendonça, R. Z., De Senna, V. K., Figueiredo, C. A., Badari, J. C., Taniwaki, N., … Negri, G. (2015). Antiviral action of hydromethanolic extract of geopropolis from scaptotrigona postica against antiherpes simplex virus (HSV-1). *Evidence-Based Complementary and Alternative Medicine.* http://dx.doi.org/10.1155/2015/296086

Curti, S. P., Figueiredo, C. A., de Oliveira, M. I., Andrade, J. Q., Zugaib, M., Frugis Yu, A. L., Oliveira, D.B., & Durigon, E. L. (2013). Molecular epidemiology of rubella viruses involved in congenital rubella infections in São Paulo, Brazil, between 1996 and 2009. *Journal of Medical Virology, 85*, 2034-2041. https://doi.org/10.1002/jmv.23675.

Dübecke, A., Beckh, G., & Lüllmann, C. (2011). Pyrrolizidine alkaloids in honey and bee pollen. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment*, *28*, 348-58. https://doi.org/10.1080/19440049.2010.541594.

Dutra, R. P., De Barros, A. B. V., Cunha, M. S., Batista, M. C. A., Torres, L. M., Nascimento, F. R., Ribeiro, M. N., & Guerra, R. N. (2014). Phenolic acids, hydrolyzable tannins, and antioxidant activity of geopropolis from the stingless bee melipona fasciculata smith. *Journal of Agricultural and Food Chemistry, 62,* 2549-2557. https://doi.org/10.1021/jf404875v.

Elliger, C. A., Chan, B. G., Waiss, A. C., Lundin, R. E., & Haddon, W. F. (1980). *C*-Glycosylflavones from *Zea mays* that inhibit insect development. *Phytochemistry*, 293-297. https://doi.org/10.1016/S0031-9422(00)81977-9.

Ferreira, J. M., Fernandes-Silva, C. C., Salatino, A., Message, D., & Negri, G. (2017). Antioxidant Activity of a Geopropolis from Northeast Brazil: Chemical Characterization and Likely Botanical Origin. *Evidence-Based Complementary and Alternative Medicine*. https://doi.org/10.1155/2017/4024721

Figueiredo, C. A., Oliveira, M. I., Curti, S. P., Cruz, A. S., Moreira, E., Afonso A. M. S., & Salles-Gomes, L. F. (2000). RC-IAL cell line: sensitivity of rubella virus grow. *Revista de Saúde Pública, 34*, 353-357.

Friedman, M. (2014). Antibacterial, antiviral, and antifungal properties of wines and winery byproducts in relation to their flavonoid content. *Journal of Agricultural and Food Chemistry, 62*, 6025-6042. https://doi.org/10.1021/jf501266s.

González-Búrquez, M. J., González-Díaz, F. R., García-Tovar, C. G., Carrillo-Miranda, L., Soto-Zárate, C. I., Canales-Martínez, M. M., … Fonseca-Coronado, S. (2018). Comparison between In Vitro Antiviral Effect of Mexican Propolis and Three Commercial Flavonoids against Canine Distemper Virus. *Evidence-Based Complementary and Alternative Medicine*. https://doi.org/10.1155/2018/7092416

Gualberto, F. A. S., Curti, S. P., de Oliveira, M. I., Moraes-Vasconcelos, D., & Figueiredo, C. A., (2013). Intermittent rash, lymph node swelling, arthralgia and vaccinal viral detection after rubella immunization. *Journal of Clinical Virology, 56*, 93-95. https://doi.org/10.1016/j.jcv.2012.07.017.

Hossain, M. K., Choi, H. Y., Hwang, J.-S., Dayem, A. A., Kim, J.-H., Kim, Y. B., Poo, H., & Cho, S. G. (2014). Antiviral activity of 3,4'-dihydroxyflavone on influenza a virus. *Journal of Microbiology*, *52*, 521-526. https://doi.org/10.1007/s12275-014-4212-z.

Huang, S., Zhang, C.-P., Wang, K., Li, G., & Hu, F. L. (2014). Recent advances in the chemical composition of propolis. *Molecules*, *19*, 19610-19632. https://doi.org/10.3390/molecules191219610.

Ji, P., Chen, C., Hu, Y., Zhan, Z., Pan, W., Li, R., Li, E., Ge, H. M., & Yang, G. (2015). Antiviral activity of Paulownia tomentosa against Enterovirus 71 of hand, foot, and mouth disease. *Biological Pharmaceutical Bulletin*, *38*, 1-6. https://doi.org/10.1248/bpb.b14-00357.

Kai, H., Obuchi, M., Yoshida, H., Watanabe, W., Tsutsumi, S., Park, Y. K., Matsuno, K., Yasukawa, K., &

Kurokawa, M. (2014). In vitro and in vivo anti-influenza virus activities of flavonoids and related compounds as components of Brazilian propolis (AF-08). *Journal of Functional Foods, 8*, 214-223. https://doi.org/10.1016/j.jff.2014.03.019

Karar, M. G. E., & Kuhnert, N. (2015). UPLC-ESI-Q-TOF-MS/MS Characterization of Phenolics from Crataegus monogyna and Crataegus laevigata (Hawthorn) Leaves, Fruits and their Herbal Derived Drops (Crataegutt Tropfen). *Journal of Chemical Biology & Therapeutics, 1*, 102. https://doi.org/10.4172/2572-0406.1000102

Kaul, T., Middleton, E., & Ogra, P. (1985) Antiviral effect of flavonoids on human viruses. J*ournal Medicinal Virology, 15*, 71-79. https://doi.org/10.1002/jmv.1890150110

Kwon, B-E., Song, J-H., Song, H-H., Kang, J. W., Hwang, S. N., Rhee, K-J., … Ko, H-J. (2016). Antiviral Activity of Oroxylin A against Coxsackievirus B3 Alleviates Virus-Induced Acute Pancreatic Damage in Mice. *PLoS ONE 11*, e0155784. https://doi.org/10.1371/journal.pone.0155784

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal.* Hindawi Publishing Corporation. http://doi.org/10.1155/2013/162750

Lani, R., Hassandarvish, P., Shu, M. H., Phoon, W. H., Chu, J. J. H., Higgs, S., Vanlandingham, D., Bakar, S. A., & Zandi, K. (2016). Antiviral activity of selected flavonoids against Chikungunya virus. *Antiviral Research, 133*, 50-61. https://doi.org/10.1016/j.antiviral.2016.07.009

Lee, J. Y., & Bowden, D. S. (2000). Rubella virus replication and links to teratogenicity. *Clinical Microbiology Reviews, 13*, 571-587.

Li, A., Xuan, H., Sun, A., Liu, R., & Cui, J. (2016). Preparative separation of polyphenols from water-soluble fraction of Chinese propolis using macroporous absorptive resin coupled with preparative high performance liquid chromatography. *Journal of Chromatography B Analytical Technological Biomedical Life Science*, *1012*, 42-49. https://doi.org/10.1016/j.jchromb.2015.12.038. Epub 2016 Jan 11.

Liu, A. L., Liu, B., Qin, H. L., Lee, S. M., Wang, Y. T., & Du, G. H. (2008). Anti-influenza virus activities of flavonoids from the medicinal plant Elsholtzia rugulosa. *Planta Medica*, *74*, 847-851. https://doi.org/10.1055/s-2008-1074558

Liu, S., Lu, H., Zhao, Q., He, Y., Niu, J., Debnath, A. K., Wu, S., & Jiang, S. (2005). Theaflavin derivatives in black tea and catechin derivatives in green tea inhibit HIV-1 entry by targeting gp41. *Biochimica et Biophysica Acta, 1723*, 270-281. https://doi.org/10.1016/j.bbagen.2005.02.012

Lv, X., Qiu, M., Chen, D., Zheng, N., Jin, Y., & Wu, Z. (2014). Apigenin inhibits enterovirus 71 replication through suppressing viral IRES activity and modulating cellular JNK pathway. *Antiviral Research*, *109*, 30-41. https://doi.org/10.1016/j.antiviral.2014.06.004.

Martínez-Torres, A. O., Mosquera, M. M., De Ory, F., González-Praetorius, A., & Echevarría, J. E. (2016). Genetic Characterization of Rubella Virus Strains Detected in Spain, 1998-2014. *PLoS One*, *13*, e0162403. https://doi.org/10.1371/journal.pone.0162403

Massaro, C. F., Katouli, M., Grkovic, T., Vu, H., Quinn, R. J., Heard, T. A., … Brooks, P. (2014). Anti-staphylococcal activity of C-methyl flavanones from propolis of Australian stingless bees (Tetragonula carbonaria) and fruit resins of Corymbia torelliana (Myrtaceae). *Fitoterapia*, *95*, 247-257. https://doi.org/10.1016/j.fitote.2014.03.024.

Mihajlovic, L., Radosavljevic, J., Burazer, L., Smiljanic, K., & Cirkovic, V. T. (2015). Composition of polyphenol and polyamide compounds in common ragweed (Ambrosia artemisiifolia L.) pollen and sub-pollen particles. *Phytochemistry*, *109*, 125-132. https://doi.org/10.1016/j.phytochem.2014.10.022.

Min, N., Leong, P. T., Lee, R. C. H., Khuan, J. S. E., & Chu, J. J. H. (2018). A flavonoid compound library screen revealed potent antiviral activity of plant-derived flavonoids on human enterovirus A71 replication. *Antiviral Research, 150*, 60-68. https://doi.org/10.1016/j.antiviral.2017.12.003.

Montenegro, G., & Mejías, E. (2013). Biological applications of honeys produced by *Apis mellifera*. *Biological Research*, *46*, 341-345. https://doi.org/10.4067/S0716-97602013000400005.

Moreira, R., Pereira, D. M., Valentão, P., & Andrade, P. B. (2018). Pyrrolizidine Alkaloids: Chemistry, Pharmacology, Toxicology and Food Safety. *International Journal Molecular Science, 19*, 1668. https://doi.org/10.3390/ijms19061668.

Muscat, M., Shefer, A., Ben Mamou, M., Spataru, R., Jankovic, D., Deshevoy, S., Butler, R., & Pfeifer, D.

(2014). The state of measles and rubella in the WHO European Region, 2013. *Clinical Microbiology and Infection*, *20*, 12-18. https://doi.org/10.1111/1469-0691.12584.

Negri, G., Teixeira, E. W., Alves, M. L., Moreti, A. C., Otsuk, I. P., Borguini, R. G., & Salatino, A. (2011). Hydroxycinnamic acid amide derivatives, phenolic compounds and antioxidant activities of extracts of pollen samples from Southeast Brazil. *Journal of Agricultural and Food Chemistry*, *59*, 5516-5522. https://doi.org/10.1021/jf200602k.

Negri, G., Carelli Barreto, L. M. R., Sper, F. L., de Carvalho, C., & Campos, M. G. R. (2018). Phytochemical analysis and botanical origin of *Apis mellifera* bee pollen from the municipality of Canavieiras, Bahia State, Brazil. *Brazilian Journal Food Technology*, *21*, e2016176. http://dx.doi.org/10.1590/1981-6723.17616

Nina, N., Quispe, C., Jiménez-Aspee, F., Theoduloz, C., Feresín, G. E., Lima, B., Leiva, E., & Schmeda-Hirschmann, G. (2015). Antibacterial Activity, Antioxidant Effect and Chemical Composition of Propolis from the Región del Maule, Central Chile. *Molecules*, *20*, 18144-67. https://doi.org/10.3390/molecules201018144.

Nolkemper, S., Reichling, J., Sensch, K. H., & Schnitzler, P. (2010). Mechanism of herpes simplex virus type 2 suppression by propolis extracts. *Phytomedicine*, *17*, 132-138. https://doi.org/10.1016/j.phymed.2009.07.006.

Oldoni, T. L. C., Oliveira, S. C., Andolfatto, S., Karling, M., Calegari, M. A., Sado, R. Y., … Lima, V. A. (2015). Chemical Characterization and Optimization of the Extraction Process of Bioactive Compounds from Propolis Produced by Selected Bees Apis mellifera. *Journal of the Brazilian Chemical Society*, *26*, 2054-2062. http://dx.doi.org/10.5935/0103-5053.20150186

Osés, S. M., Pascual-Maté, A., Fernández-Muiño, M. A., López-Díaz, T. M., & Sancho, M. T. (2015). Bioactive properties of honey with propolis. *Food Chemistry*, *196*, 1215-1223. https://doi.org/10.1016/j.foodchem.2015.10.050.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, *5*, e47, 1-15. https://doi.org/10.1017/jns.2016.41

Parkman, P. D. (1996). Togaviruses: Rubella Virus. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; Chapter 55.

Petrova, E. K., Dmitrieva, A. A., Trifonova, E. A., Nikitin, N. A., & Karpova, O. V. (2016). The key role of rubella virus glycoproteins in the formation of immune response, and perspectives on their use in the development of new recombinant vaccines. *Vaccine*, *34,* 1006-1111. https://doi.org/10.1016/j.vaccine.2016.01.010.

Pippi, B., Lana, A. J., Moraes, R. C., Güez, C. M., Machado, M., de Oliveira, L. F. S., Lino von Poser, G., & Fuentefria, A. M. (2015). *In vitro* evaluation of the acquisition of resistance, antifungal activity and synergism of Brazilian red propolis with antifungal drugs on *Candida* spp. *Journal of Applied Microbiology*, *118*, 839-850. https://doi.org/10.1111/jam.12746.

Plotkin, S. A. (2011). History of Rubella Vaccines and the Recent History of Cell Culture. In *History of Vaccine Development* (pp. 219-231). New York, NY: Springer New York.

Rasbach, A., Abel, T., Münch, R. C., Boller, K., Schneider-Schaulies, J., & Buchholz, C. J. 2013. The Receptor Attachment Function of Measles Virus Hemagglutinin Can Be Replaced with an Autonomous Protein That Binds Her2/neu While Maintaining Its Fusion-Helper Function. *Journal of Virology*, *87*, 6246-6256. https://doi.org/10.1128/JVI.03298-12.

Saeed, F., Ahmad, R. S., Arshad, M. U., Niaz, B., Batool, R., Naz, R., & Rasul, S. H. A. (2016) Propolis to Curb Lifestyle Related Disorders: An Overview. *International Journal of Food Properties, 19*, 420-437. https://doi.org/10.1080/10942912.2012.745131

Salas, A. L., Alberto, M. R., Zampini, I. C., Cuello, A. S., Maldonado, L., Ríos, J. L., Schmeda-Hirschmann, G., & Isla, M. I. (2016). Biological activities of polyphenols-enriched propolis from Argentina arid regions. *Phytomedicine*, *23*, 27-31. https://doi.org/10.1016/j.phymed.2015.11.007.

Santos, H. F. D., Campos, J. F., Santos, C. M. D., Balestieri, J. B. P., Silva, D. B., Carollo, C. A., … Dos, S. E. L. (2017). Chemical Profile and Antioxidant, Anti-Inflammatory, Antimutagenic and Antimicrobial Activities of Geopropolis from the Stingless Bee Melipona orbignyi. *International Journal Molecular Science, 18*, 953. https://doi.org/10.3390/ijms18050953.

Sartori, G., Pesarico, A. P., Pinton, S., Dobrachinski, F., Roman, S. S., Pauletto, F., Rodrigues, L. C. Jr., & Prigol, M. (2012). Protective effect of brown Brazilian propolis against acute vaginal lesions caused by herpes simplex virus type 2 in mice: involvement of antioxidant and anti-inflammatory mechanisms. *Cell Biochemistry and Function*, *30*, 1-10. https://doi.org/10.1002/cbf.1810.

Sawaya, A. C. H. F., Calado, J. C. P., Santos, L. C., Marcucci, M. C., Akatsu, I. P., Soares, A. E. E., … Eberlin, M. N. (2009). Composition and antioxidant activity of propolis from three species of Scaptotrigona stingless bees. *Journal of ApiProduct and ApiMedical Science, 1*, 37-42. https://doi.org/10.3896/IBRA.4.01.2.03

Schnitzler, P., Neuner, A., Nolkemper, S., Zundel, C., Nowack, H., Sensch, K. H., & Reichling, J. (2010). Antiviral Activity and Mode of Action of Propolis Extracts and Selected Compounds. *Phytotherapy Research*, *24*(S1), S20-S28. https://doi.org/10.1002/ptr.2868.

Seo, D. J., Jeon, S. B., Oh, H., Lee, B. H., Lee, S. Y., Oh, S. H., Jung, J. Y., & Choi, C. (2016). Comparison of the antiviral activity of flavonoids against murine norovirus and feline calicivirus. *Food Control*, *60*, 25-30. http://doi.org/10.1016/j.foodcont.2015.07.023

Shibata, C., Ohno, M., Otsuka, M., Kishikawa, T., Goto, K., Muroyama, R., Kato, N., Yoshikawa, T., Takata, A., & Koike, K. (2014). The flavonoid apigenin inhibits hepatitis C virus replication by decreasing mature microRNA122 levels. *Virology*, *462-463*, 42-48. http://doi.org/10.1016/j.virol.2014.05.024

Shimizu, T., Hino, A., Tsutsumi, A., Park, Y. K., Watanabe, W., & Kurokawa, M. (2008). Anti-influenza virus activity of propolis in vitro and its efficacy against influenza infection in mice. *Antiviral Chemistry & Chemotherapy*, *19*, 7-13. http://doi.org/10.1177/095632020801900102

Silva-Carvalho, R., Baltazar, F., Almeida-Aguiar, C., & Almeida-Aguiar, C. (2015). Propolis: A Complex Natural Product with a Plethora of Biological Activities That Can Be Explored for Drug Development. *Evidence-Based Complementary and Alternative Medicine*. https://doi.org/10.1155/2015/206439

Silva, N. N. T., Gardner, D., Hallwass, F., Leite, A. J. M., de Almeida, C. G., Silva, L. N., … Giordani, R. B. (2016). Activity of pyrrolizidine alkaloids against biofilm formation and Trichomonas vaginalis. *Biomedical Pharmacotherapy, 83*, 323-329. https://doi.org/10.1016/j.biopha.2016.06.033.

Song, J.-H., Kwon, B.-E., Jang, H., Kang, H., Cho, S., Park, K., Hyun-Jeong Ko, H-J., & Kim, H. (2015). Antiviral Activity of Chrysin Derivatives against Coxsackievirus B3 in vitro and in vivo. *Biomolecules & Therapeutics*, *23*, 465. http://doi.org/10.4062/biomolther.2015.095

Song, J. M., Lee, K. H., & Seong, B. L. (2005). Antiviral effect of catechins in green tea on influenza virus. *Antiviral Research*, *68*, 66-74. http://doi.org/10.1016/j.antiviral.2005.06.010

Souza, H. R., Corrêa, A. M. S., Cruz-Barros, M. A. V., & Albuquerque, P. M. C. (2015). Espectro polínico da própolis de Scaptotrigona aff. postica (Hymenoptera, Apidae, Meliponini) em Barra do Corda, MA, Brasil. *Acta Amazonica*, *45*, 307-316. http://doi.org/10.1590/1809-4392201403663

Silva, T. M. S., Souza S. A., Dias, T. L. M. F., Silva, T. M. G., Falcão, R. A., Alexandre, M. M. S., Silva, E. M. S., & Camara, C. A. (2014). Chemical composition, antinociceptive and free radical-scavenging activities of geopropolis from Melipona subnitida Ducke (Hymenoptera: Apidae: Meliponini). *Sociobiology*, *61*, 560-565. http://doi.org/10.13102/sociobiology.v61i4.560-565

Takemura, T., Urushisaki, T., Fukuoka, M., Muto, J-H., Hata, T., Okuda, Y., Hori, S., Tazawa, S., Araki, Y., & Kuwata, K. (2012). 3,4-Dicaffeoylquinic Acid, a Major Constituent of Brazilian Propolis, Increases TRAIL Expression and Extends the Lifetimes of Mice Infected with the Influenza A Virus. *Evidence-Based Complementary and Alternative Medicine : ECAM*. http://doi.org/10.1155/2012/946867

Urushisaki, T., Takemura, T., Tazawa, S., Fukuoka, M., Hosokawa-Muto, J., Araki, Y., & Kuwata, K. (2011). Caffeoylquinic acids are major constituents with potent anti-influenza effects in brazilian green propolis water extract. *Evidence-Based Complementary and Alternative Medicine*. http://doi.org/10.1155/2011/254914

Valenzuela-Barra, G., Castro, C., Figueroa, C., Barriga, A., Silva, X., de Las Heras, B., Hortelano, S., & Delporte, C. (2015). Anti-inflammatory activity and phenolic profile of propolis from two locations in Región Metropolitana de Santiago, Chile. *Journal of Ethnopharmacology*, *168*, 37-44. http://doi.org/10.1016/j.jep.2015.03.050

Visintini Jaime, M. F., Redko, F., Muschietti, L. V, Campos, R. H., Martino, V. S., & Cavallaro, L. V. (2013). In

vitro antiviral activity of plant extracts from Asteraceae medicinal plants. *Virology Journal*, *10*, 245. http://doi.org/10.1186/1743-422X-10-245

Wang, J., Zhang, T., Du, J., Cui, S., Yang, F., & Jin, Q. (2014). Anti-enterovirus 71 effects of chrysin and its phosphate ester. *PLoS ONE*, *9*, e89668. http://doi.org/10.1371/journal.pone.0089668

Wang, Y., Chen, M., Zhang, J., Zhang, X. L., Huang, X. J., Wu, X., Zhang, Q. W., Li, Y. L., & Ye, W. C. (2012). Flavone C-glycosides from the leaves of Lophatherum gracile and their in vitro antiviral activity. *Planta Medica*, *78*, 46-51. http://doi.org/10.1055/s-0031-1280128

World Health Organization. (2017). *WHO*. Retrieved from http://www.who.int/en/

Yildirim, A., Duran, G. G., Duran, N., Jenedi, K., Bolgul, B. S., Miraloglu, M., & Muz, M. (2016). Antiviral Activity of Hatay Propolis Against Replication of Herpes Simplex Virus Type 1 and Type 2. *Medical Science Monitor*, *22*, 422-430. http://doi.org/10.12659/MSM.897282

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Starch and Pectin Affect Hardness of Cooked Bananas

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Abstract

Texture is an important quality attribute of fresh and processed foods. In plant foods, texture is closely related with the structural integrity of the primary cell walls and middle lamella that is mainly composed of pectic substances. Bananas mainly contain water, starch, pectin and fibre which influence texture. Cooking bananas soften on cooking but harden on cooling. Despite many studies on retrogradation of starch and its effects on texture, little is known about the effect of added starch and/or breakdown of pectin on hardness of bananas upon cooking and cooling. In this study, the effects of added pectin and starch and structural elimination of pectin on hardness of bananas during cooking and cooling were investigated.

Hydrolysis of pectin resulted in significantly harder bananas during cooking and upon cooling ($P<0.05$). Hardness of starch-treated bananas increased significantly with increasing starch concentration upon cooking and cooling relative to the control ($P<0.05$). However, treatment of bananas with added pectin alone resulted in an insignificant increase in hardness relative to the control. Upon cooling, hardness of pectin-treated bananas decreased significantly with increasing pectin concentration ($P<0.05$). Hardness of cooked bananas treated with a combination of starch and pectin increased but was not significantly different from the control. Upon cooling, hardness of the starch-pectin treated bananas decreased with increasing concentration similar to the effect of pectin when added alone.

Current results showed that starch addition increases hardness of bananas upon cooking and cooling. However, pectin addition decreases hardness of cooked bananas upon cooling while structural elimination of pectin increases hardness upon cooking and cooling. These results imply that pectin contributes to a softer texture of bananas during cooking and cooling whereas starch increases hardness in cooked bananas. Pectin can therefore be added up to 5% to decrease hardness of cooked bananas.

Keywords: added starch, added pectin, cooked bananas, effect, textural hardness, structural pectin elimination

1. Introduction

1.1 Background

Texture is a group of physical characteristics that arise from the structural elements of a food perceived by the sense of touch, which are related to deformation, disintegration and flow of food under force, and measured objectively by functions of mass, time and distance (Bourne, 2002). It is a major sensory and quality attribute that plays an important role when assessing the quality of food. The texture of foods is related to the structure formed by micro and macro molecular elements forming the cell wall and other regions. The major challenge to cooking and consumption of bananas is their susceptibility to hardening of the texture after cooling. Changes in the texture of bananas affect other attributes such as flavor and /or taste which are lost upon cooling.

Changes in the texture of bananas during cooking are caused by structural changes in starch, pectin, cellulose and hemicellulose. The basic structure of the primary cell wall consists of a cellulose-hemicellulose network with pectin interwoven within this network forming the basis for structural integrity of the cell. In raw plant foods, pectin which is mainly found in the middle lamella, cements cell walls together and gives firmness and elasticity to plant tissues.

Cooking affects the structural integrity and biochemical composition of the cells and cell walls leading to loss of turgor pressure and the 'fresh' texture of bananas. Starch which is the most important carbohydrate and a major component in bananas undergoes gelatinization during cooking and retrogradation upon cooling which affect the

final hardness of cooked bananas. These changes are major determinants of starch's functional properties, quality attributes, nutritional and shelf-life of finished products (Wang & Copeland, 2013). According to Espinosa‐Solis, Jay‐lin, & Bello‐Perez (2009), gelatinized banana starch during storage has the largest thermal stability because its crystals dissociate at higher temperatures and with a larger enthalpy change; which implies that a higher degree of crystallinity is produced in banana starch than in the other starches. This means that retrograded banana starch is very stable hence when bananas are cooked and allowed to cool and harden, reversing the hardened texture is almost impossible. This is exactly what consumers of cooked bananas in Uganda experience upon reheating of cooled bananas. When cooked, bananas soften but upon cooling, they irreversibly become hard. The tendency of cooked banana texture to harden during cooling has been objectively proven by Gafuma, Byarugaba-Bazirake, & Mugampoza (2018).

The pectin content of raw green mature bananas consists majorly of protopectin which is characterized by a high degree of esterification (D.E) ranging between 88 to 96% (personal communication of unpublished results). Pectin consists of a linear chain of α- (1→4)-linked D-galacturonic acid units in which varying proportions of acid groups are esterified with methyl groups which are responsible for gel formation during heating in presence of sugar and divalent cations such as Ca^{2+}. This property affects water binding and holding properties of pectin which in turn may have significant influence on hardness of cooked bananas. Structural elimination of pectin can be performed by hydrolysis using pectinases majorly Polygalacturonase (PG) and Pectinmetylesterase (PME) which are the key enzymes responsible for pectin breakdown (Tapre & Jain, 2014) in plant tissues causing softening as fruits and vegetables mature and ripen. During ripening, PME removes methyl ester groups from the cell wall pectic substances, making them accessible to depolymerisation by PG which in turn reduces intercellular adhesiveness and tissue rigidity (Payasi, Mishra, Chaves, & Singh, 2009). Much as PME causes softening during ripening of fruits, it also increases firmness of fruits and vegetables via demethylation of endogenous pectin and subsequent chelation of divalent cations by ionized carboxyl groups on adjacent pectic acids (Jongen, 2002). Most commercial preparations of pectinases are produced from fungal sources (Sharma, Rathore, & Sharma, 2012) with the commonest source being *Aspergillus niger* (Gummadi & Panda, 2003). Depending on the need, these pectic enzymes can be used as processing aids particularly in the production of fruit juices. Due to the adhesive nature of pectin within plant tissues, it is not clear what would happen to the texture of bananas upon cooking and cooling if pectin is eliminated from their structural matrix. Currently, besides foods such as cheese where the effect of added pectin on texture has been studied, there is no information regarding the effect of structural elimination of pectin using pectinases, let alone added pectin on hardness of cooked bananas. Similarly, there is scant information on the effect of added starch on the hardness of cooked bananas. This study aimed to investigate the effects of added starch and pectin and structural elimination of pectin on hardness of bananas upon cooking and cooling.

Understanding these effects would promote innovation of cooked banana products that have more industrial and economic value. One of the challenges to cooked banana processing has been how to use recent advances in processing technologies to adjust raw banana composition, innovation of creative ingredient mix and processes to improve the texture of cooked bananas. As a result, future banana processors would produce banana products of a more desirable softer texture and flavor.

2. Materials and Methods

2.1 Materials

Three East African Highland Banana (AAA-EA) cultivars endemic to Uganda were selected for this study. These include *Kayinja* (KAY), a local juice banana cultivar from Central and Western Uganda under the Bluggoes ABB clone set and two cooking banana cultivars namely *Kazirakwe* (KAZ), a local cooking banana cultivar common in Western Uganda under the *Nakabululu* clone set; and *Nakitembe* (NAKT), a local cooking banana cultivar from Central Uganda under the *Nakitembe* clone set (Karamura, 1998). KAZ and NAKT were purchased from Kawanda Agricultural Research Institute (KARI) while KAY was bought from a farm near Kyengera trading centre in Wakiso district, Uganda. All samples were harvested and immediately transported to Makerere University Food Science and Nutrition laboratory for texture analysis. Pectin (CAS 9000-69-5; 75% D.E) and the enzyme pectinase (CAS: 9032-75-1; activity 1 U/mg) were purchased from Sigma Aldrich (USA) through Kobian Scientific, a local agent. Banana starch was locally extracted from NAKT banana cultivar using the alkaline starch extraction method (Zhang, Whistler, BeMiller, & Hamaker, 2005). Other materials were obtained from local supermarkets.

2.2 General Preparation of Banana Samples for Treatments

Banana fingers (21) were picked from green mature banana bunches (7 from the top, middle and bottom), then

washed, peeled and dipped in portable water to prevent browning. The peeled bananas were sliced to approx. 1 – 2 mm discs and subdivided into three equal portions. Each portion was placed in a glass beaker and covered with aluminium foil before being weighed.

2.3 Preparation of Pectinase-treated Bananas

Approx. 240 g of sliced banana sample was weighed into a beaker. Then, 1.5 g of pectinase (polygalacturonase) was weighed and mixed well with 410 ml of distilled water. The banana sample was mixed into the enzyme solution and the pH of the banana-enzyme mixture measured to be 5.3. The enzyme-banana mixture as well as the control were incubated at 50^{o}C for 2 h in a water bath with constant agitation. After incubation, the solutions in the enzyme-treated bananas and the control were drained and the banana slices lightly squeezed in a nylon cloth to remove excess water. Each of the control and the enzyme-treated bananas were placed in a small plastic bag of 30 microns which was perforated to allow normal exchange of air and moisture. The bag containing the banana sample was steamed in a 3 Tier Food Steamer (HS6000, Black & Decker, China) for 30 min, and thereafter removed and mashed. The mashed bananas were returned to the steamer and steamed for a further 20 min (50 min of cooking overall) before the first sample was taken for texture analysis. The bananas were steamed further for 70, 90, 110 and 130 min and appropriately sampled for texture analysis. The remaining banana masses for each replicate of the respective treatments were rolled together, cooled for 4 h at room temperature (25^{o}C) and used to measure texture of the cool samples. Mean values were tabulated and used to generate a profile of curves used for analysis of changes in textural hardness of the enzyme treated bananas.

2.4 Preparation of Starch and Pectin-treated Bananas

For each of starch and pectin treatments, 0 (0%), 1 (1.7%), 3 (5%), 5 (8.3%), and 7 g (11.7%) of banana starch extracted from *Nakitembe* according to the protocol of Gafuma et al. (2018) and pectin (Sigma) were weighed separately. Approx. 60 g of sliced bananas (section 2.2) were weighed into a plastic bag of 30 microns. The weighed starch or pectin was dispersed onto the banana slices carefully little by little from a plastic sieve by tapping the sieve while agitating the bananas to ensure uniform distribution. The plastic bag for each treatment was perforated at the upper side to prevent buildup of internal pressure and to allow normal exchange of air and moisture during steaming. A control for each treatment was prepared and comprised of bananas without added starch or pectin. The bananas were cooked by steaming in a Food Steamer for 30 min as in section 2.3 before being mashed. The mashed bananas were placed back into the steamer and steamed for a further 20 min (50 min of cooking overall) before sampling for texture analysis (Gafuma et al. 2018). The remaining banana masses were treated as in section 2.3 before being used to measure texture of the cool samples. Treatments were conducted in three independent replicates.

2.5 Preparation of Starch-pectin Composite Treated Bananas

Sliced bananas (60 g) from section 2.2 were weighed into a plastic bag as in section 2.3. Then, a starch-pectin composite was prepared by weighing 0, 1, 3, 5, and 7 g of banana starch and 0, 1, 3, 5, and 7 g of pectin (Sigma) separately. The starch and pectin powders were mixed thoroughly in a 1:1 ratio to achieve uniform composites of the different levels (0-11.7%) of treatment. The starch-pectin composites were applied onto the banana slices (3.33, 10, 16.7 and 23.3% composite) and the treated samples processed as in section 2.3.

2.6 Determination of Textural Hardness and Cohesiveness

Textural hardness analysis was performed using a Texture Analyzer (*TA.XT plus stable micro-systems, Surrey, UK*) according to the method of Gafuma et al. (2018). Cohesiveness was defined as the ratio of the area under the second penetration curve to the area under the first penetration and values were generated by the Texture Analyzer according to the manufacturer's instructions (Texture technologies Corp. and Stable Micro Systems, 2018).

2.7 Statistical Analysis

Data were analyzed using IBM (SPSS) package, version 23. All means were split and separated by group and analyzed using one-way Analysis of Variance (ANOVA) using Fisher's Least Significant Difference (LSD) and Turkey's test procedures. Means were tested for homogeneity of variance and significant differences were determined at $P < 0.05$.

3. Results

3.1 Introduction

Bananas were sliced to increase the surface area for enhanced enzyme activity and ensure uniform distribution of, and contact with, starch and pectin. Pectinase-treated bananas were incubated with pectinase (polygalacturonase)

to allow breakdown and structural elimination of pectin from the bananas. Treated bananas were steamed in a plastic bag to allow easy handling of the small sized samples during cooking and sampling. The effect of added pectin and starch on texture was examined using cooking bananas while the effect of structural elimination of pectin was investigated using cooking and juice bananas. The slicing of bananas in effect affected the texture by decreasing hardness of the samples meaning the texture of these samples (control) could not be directly compared with texture of the same intact (unsliced) banana samples.

3.2 Effect of Pectin-treatment on Hardness

Hardness of pectin-treated bananas was slightly higher than the control and increased with increasing pectin concentration up to 5% then it decreased as pectin concentration increased (Figure 1A, curve (b). Hardness increased from 0.93 N (control) through 1.04 to 1.24 N at 1.7 and 5% added pectin, respectively. Further increase in pectin concentration resulted in a decrease in hardness to 1.14 and 1.06 N at 8.3% and 11.7% added pectin, respectively with a weak positive correlation of 18.3%. Overall, there was no significant difference between hardness of pectin-treated bananas and the control ($P>0.05$). Upon cooling, hardness generally increased probably due to retrogradation of starch (Wang, Li, Copeland, Niu, & Wang, 2015). However, hardness of the control was profoundly higher than that of pectin-treated bananas and decreased with increasing pectin concentration from 4.50 (1.7%) to 1.87 N (8.3%) followed by a slight increase to 1.92 N at 11.7% added pectin (Figure 1A, curve (a) with a strong negative correlation of 80.4%. Hardness of the control and at 1.7% added pectin was not significantly different ($P>0.05$) but were significantly higher than hardness at other levels of added pectin (Figure 1A, curve (a).

Cohesiveness of pectin-treated bananas was higher than the control (0.84 N) and increased with increasing pectin concentration from 1.01 to 1.34 N upon cooking (Figure 1B, curve (b). Upon cooling, there was a general increase in cohesiveness of the control and pectin-treated bananas probably due to retrogradation of starch. However, cohesiveness of pectin-treated bananas (2.99 to 1.67 N) was lower than that of the control (3.02 N) and decreased with increasing added pectin concentration from 2.99 (1.7% pectin) to 1.67 N (8.3% pectin) and then slightly increased again to 1.81 N at 11.7% added pectin (Figure 1B, curve (a).

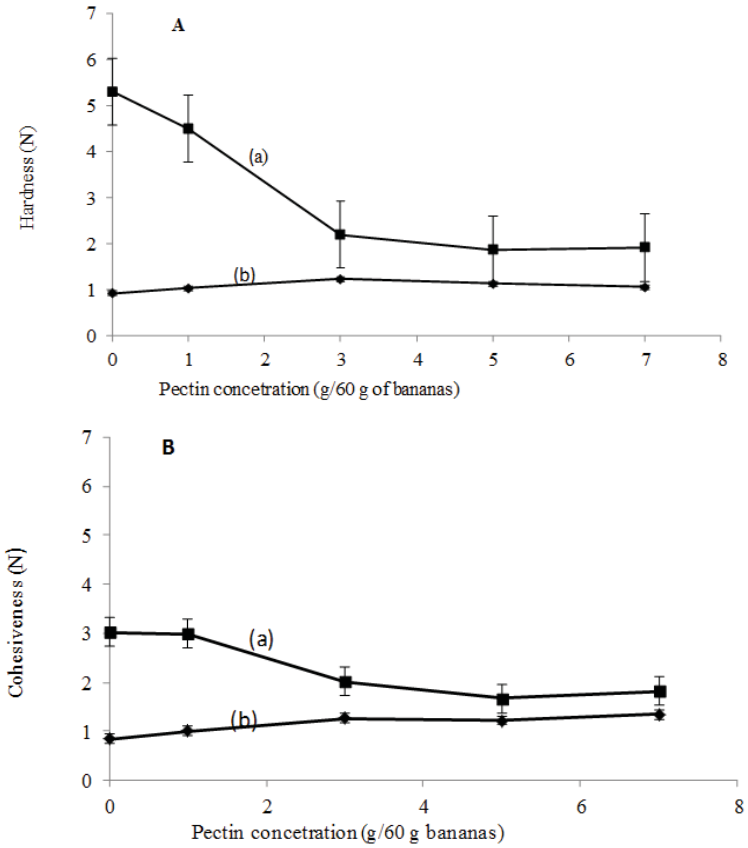

Figure 1. Effect of added pectin on (A) hardness and (B) cohesiveness of cooking bananas upon cooking and cooling. Curves: (a) cooled bananas (b) cooked bananas

3.3 Effect of Added Starch on Hardness

Upon cooking, hardness of starch-treated bananas (1.06 to 1.97 N) was higher than that of the control (0.86 N) and increased with increasing starch concentration (Figure 2A, curve (b). Hardness increased from 1.06 (1.7%) to 1.97 N (11.7% added starch) becoming significantly higher than the control beyond 5% added starch ($P<0.05$) with a strong positive correlation of 98.2%. Upon cooling, hardness increased for both the control (4.04 N) and starch-treated bananas (4.52 to 5.99 N) (Figure 2A, curve (a), which could be attributed to starch retrogradation. Hardness of cooled starch-treated bananas was higher than that of the control and increased significantly with increasing starch concentration from 4.52 at 1.7% to 5.99 N at 11.7% ($P<0.05$) with a strong positive correlation of 83.4%.

Cohesiveness of starch-treated bananas (0.91 to 1.56 N) was also higher than that of the control (0.84 N) and increased with increasing added-starch concentration from 0.91 (1.7%) to 1.56 N (11.7% added starch) upon cooking with a strong positive correlation of 98.3% (Figure 2B, curve (b). Upon cooling, cohesiveness of the control and starch-treated bananas generally increased due to starch retrogradation. However, cohesiveness of starch-treated bananas (3.20 to 4.49 N) was significantly higher than that of the control (2.34 N) and increased significantly with increasing starch concentration from 3.20 (1.7%) to 4.49 N (8.3% added starch) ($P<0.05$) before decreasing slightly to 3.65 N at 11.7% added starch with a correlation coefficient of 54% (Figure 2B, curve (a).

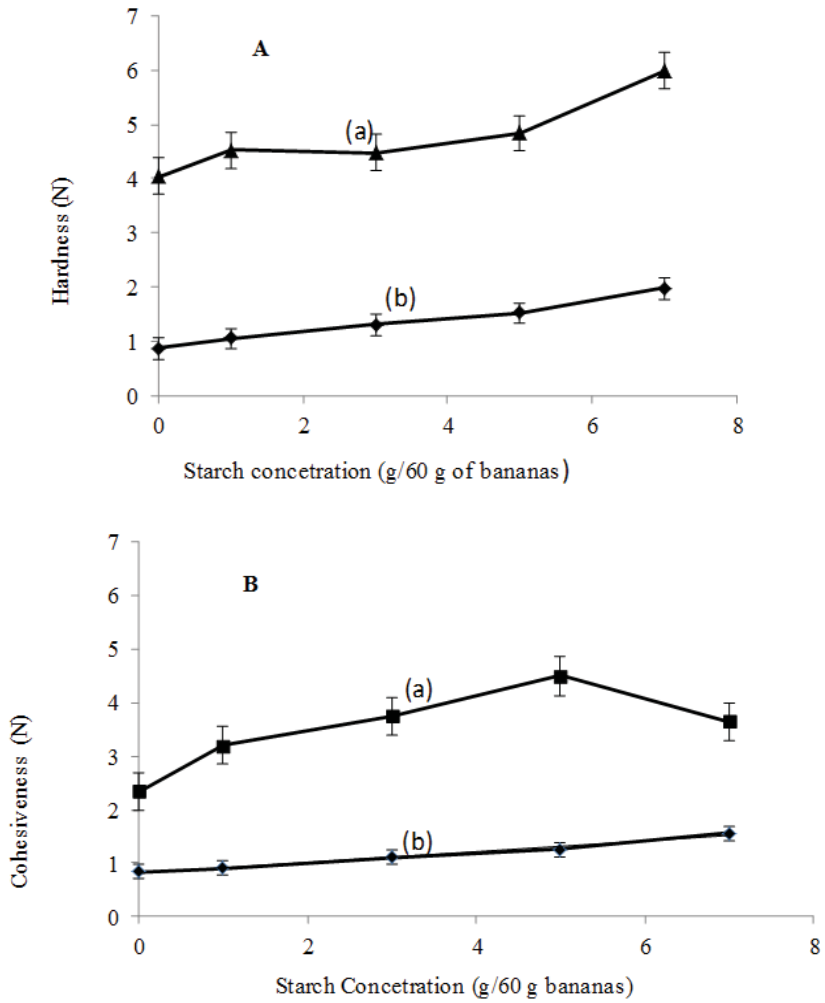

Figure 2. Effect of added starch on (A) hardness and (B) cohesiveness of cooking bananas upon cooking and cooling. Curves: (a) cooled bananas (b) cooked bananas

3.4 Effect of Starch-pectin Composite on Hardness

Upon cooking, hardness of starch-pectin composite treated bananas ranged between 1.42 and 1.15 N and was

generally harder than the control (0.89 N) but not significantly different (P >0.05) (Figure 3A, curve (b). Hardness was 1.42 N at 3.33% composite which was significantly higher than the control (P<0.05) and decreased to 1.15 N at 10% composite but then increased to 1.33 and 1.37 N at 16.6 and 23.4% composites, respectively. Upon cooling, hardness generally increased for each of the control and starch-pectin composite treated bananas due to starch retrogradation (Figure 3A, curve (a). Hardness of the control (4.76 N) was generally higher than that of the composite treated bananas (4.43 to 2.77 N) but not significantly different (P >0.05). Hardness of cooled bananas decreased from 4.43 at 3.33% to 2.77 N at 10% composite but then rose to 3.16 N at 16.6% and finally to 4.91 N at 23.4% composite.

Cohesiveness of the starch-pectin composite treated bananas (1.09 to 1.38 N) was higher than that of the control (0.86 N) upon cooking but not significantly different (P>0.05) (Figure 3B, curve (b). Cohesiveness decreased from 1.30 at 3.33% to 1.09 N at 10% composite and then increased to 1.26 and 1.38 N at 16.6 and 23.4% composite, respectively. Upon cooling, cohesiveness of the control (2.83 N) and that of the composite-treated bananas (2.13 to 3.76 N) generally increased due to starch retrogradation (Figure 3B, curve (a). Cohesiveness at 3.33% composite was 2.80 which decreased to 2.13 N at 10% composite but rose up to 2.47 and 3.76 N at 16.6 and 23.4% composite, respectively.

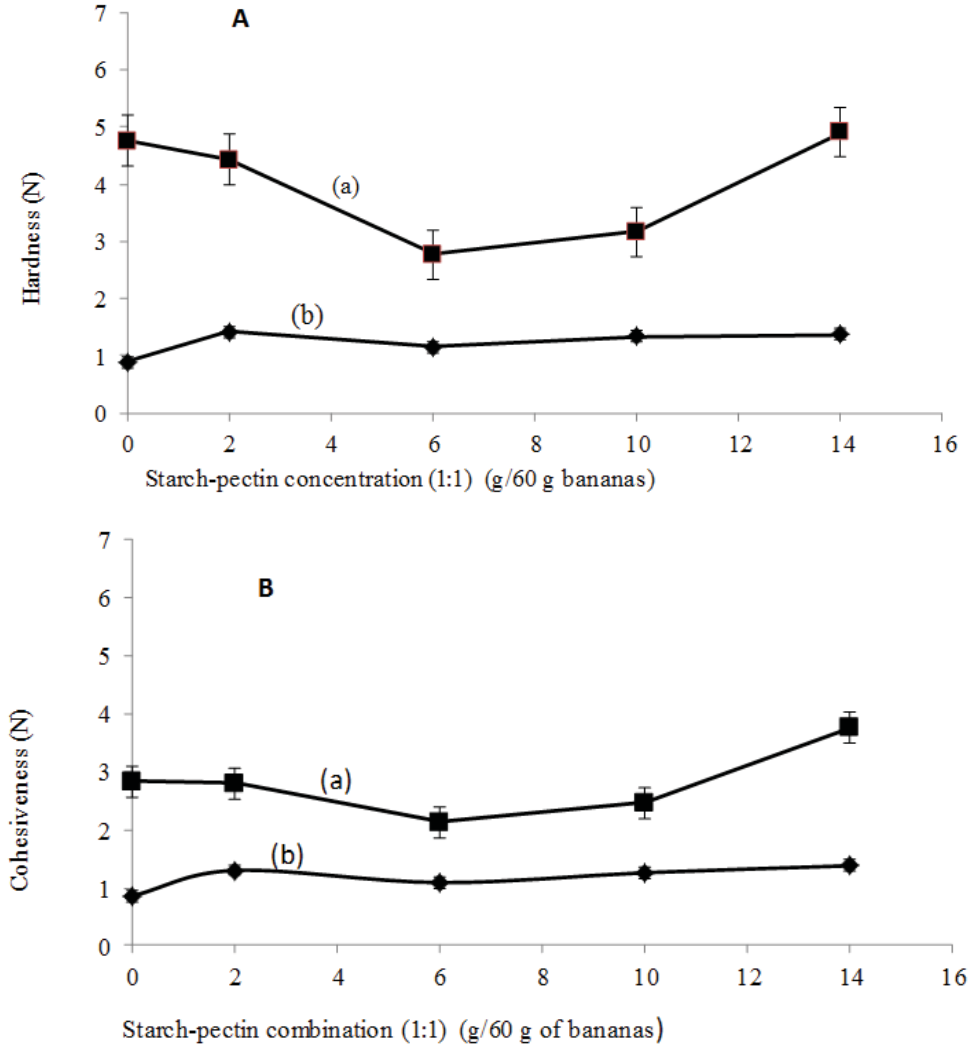

Figure 3. Effect of added starch-pectin composite on (A) hardness and (B) cohesiveness of cooking bananas upon cooking and cooling. Curves: (a) cooled bananas (b) cooked bananas

3.5 Effect of Pectinase (Polygalacturonase) Treatment on Hardness

The experiment was conducted within recommended optimum temperature and pH range for PG catalytic activity which vary between 45 and 55^{o}C and 4.4 to 7.2, respectively depending on the enzyme source and environment

(Duvetter, Sila, Van Buggenhout, Van Loey, & Hendrickx, 2009). The aim of this experiment was to establish if structural elimination of pectin from the bananas has a significant effect on hardness during cooking and upon cooling.

Results indicated that all pectinase-treated bananas were significantly harder than the controls ($P<0.05$) during cooking and upon cooling (Figures 4A and 5A). During cooking, hardness of pectinase-treated cooking bananas decreased with cooking time from 1.82 to 1.23 N relative to that of the control which fluctuated between 0.56 and 0.61 N (Figure 4A, curve (b). Hardness of pectinase treated juice bananas was also significantly higher than that of the control ($P<0.05$) and decreased from 3.49 to 1.80 N relative to the control which decreased from 1.78 to 1.57 during cooking (Figure 5A, curve (a). Upon cooling, hardness of pectinase-treated bananas and the controls increased significantly ($P<0.05$) probably due to starch retrogradation (Table 1). The cooled pectinase-treated bananas were significantly harder than their respective controls ($P<0.05$). Hardness of cooled pectinase-treated cooking bananas was 6.62 N while that of its corresponding control was 3.62 N. Hardness of cooled pectinase-treated juice bananas was 15.36 N while that of its corresponding control was 12.34 N.

Cohesiveness of pectinase-treated cooking bananas was significantly higher than that of the control and decreased from 1.47 to 1.10 N (Figure 4B, curve (a) while that of the control decreased from 0.61 to 0.57 N during cooking (Figure 4B, curve (b). Cohesiveness of the pectinase-treated juice bananas decreased from 2.47 to 1.26 N (Figure 5B, curve (a) while that of the control decreased from 1.47 to 1.23 N (Figure 5B, curve (b) and was not significantly different ($P>0.05$). Upon cooling, cohesiveness of the pectinase-treated bananas and the controls generally increased due to starch retrogradation. Cohesiveness of the cooled pectinase-treated cooking bananas was 5.12 N which was significantly higher than 2.63 N of the control (Table 1). Cohesiveness of cooled pectinase-treated juice bananas was 6.83 N which was not significantly different from 6.50 N of the control (Table 1).

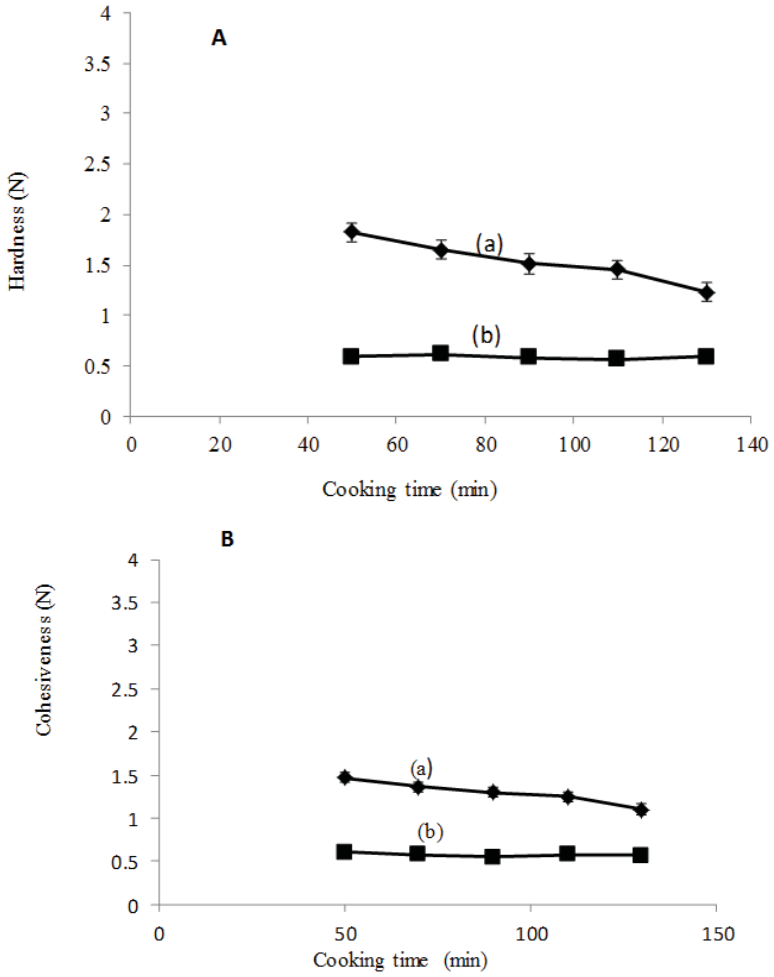

Figure 4. Effect of structural elimination of pectin on (A) hardness and (B) cohesiveness of cooking bananas (KAZ) during cooking. Curves: (a) Pectinase-treated bananas (b) Control

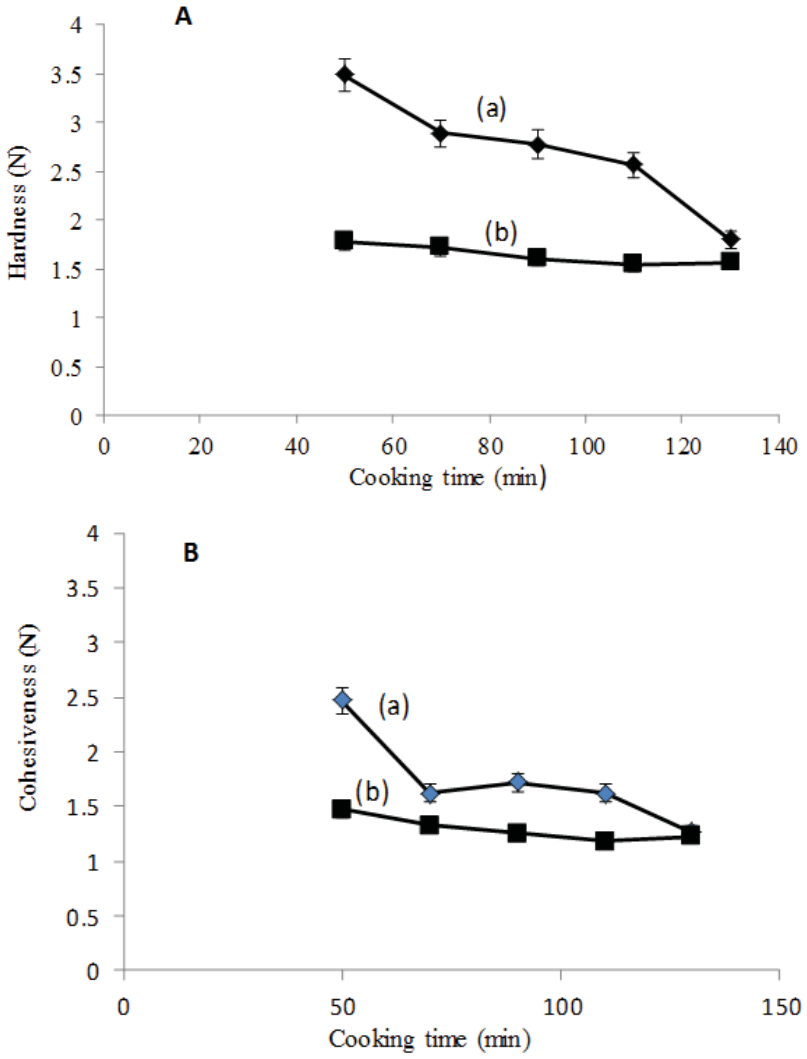

Figure 5. Effect of structural elimination of pectin on (**A**) hardness and (**B**) cohesiveness of juice bananas (KAY) during cooking. Curves: (a) cooled bananas (b) cooked bananas

Table 1. Effect of structural elimination of pectin on hardness of cooled bananas after cooking

Treatment	**Texture**			
	Hardness (N)		**Cohesiveness (N)**	
	Treated	Control	Treated	Control
Cooking bananas				
Cooked for 130 min	1.23±0.27^{a}	0.59±0.07^{b}	1.10±0.21^{a}	0.57±0.09^{b}
After cooling (4 h)	6.63±1.59^{a}	3.62 ± 0.59^{b}	5.12±0.56^{a}	2.63±0.15^{b}
Juice bananas				
Cooked for 130 min	1.80±0.21^{b}	1.57±0.10^{b}	1.26±0.31^{d}	1.23±0.11^{d}
After cooling (4 h)	15.36±a	12.34±0.88^{b}	6.83±1.45^{d}	6.50±0.13^{d}

Values with same superscript letters along the same row under the same subheading are not significantly different (P>0.05).

4. Discussion

4.1 Introduction

The changes that starch undergoes during gelatinization and retrogradation are major determinants of its functional properties for food processing, during digestion, and in industrial applications. These properties

determine the quality, acceptability, nutritional value, and shelf-life of the finished foods (Wang & Copeland, 2013). Starch retrogradation has been the subject of intensive research throughout the last half century resulting in a vast body of literature (Wang et al., 2015). The effects of starch retrogradation including its addition to certain foods have also been widely covered (Sozer, Dalgic, & Kaya, 2007; Liu, Nie, & Chen, 2013; Steeneken, Woortman, & Oudhuis, 2011). However, this has not been established on bananas before and hence the importance of the current study. On the other hand, pectin is associated with firmness of living plant tissues as well as processed products where its demethylation leads to divalent cations reacting with the carboxyl groups to form pectates which precipitate and cause increased firmness. However, the effect of adding pectin or its structural elimination on the textural hardness of bananas upon cooking and cooling has not been investigated before.

4.2 Effect of Pectin on Hardness and Cohesiveness of Bananas

Pectin of 75% D.E was applied on bananas at the rate of 0%, 1.7%, 5%, 8.3% and 11.7%. Results indicated that pectin-treated bananas were slightly harder than the control upon cooking but were not significantly different ($P>0.05$). However, upon cooling, hardness of pectin-treated bananas was lower than that of the control and decreased with increasing pectin concentration up to 5% and then rose again, implying that at lower concentrations, pectin enhances tenderness particularly during cooling of cooked bananas. The observed increase in hardness upon cooking of bananas could be attributed to a number of factors: adhesiveness of pectin leading to increased compactness of starch and other molecules; inadequate gelatinization of starch due to the high Water Binding Capacity (WBC) of pectin (Lobato-Calleros, Ramos-Solís, Santos-Moreno, & Rodríguez-Huezo, 2006) making it unavailable for starch to gelatinize adequately; the increased dry matter content due to added pectin. The weak correlation (18.3%) between hardness and added pectin is a manifestation that pectin has little effect on increasing hardness in cooked bananas. These results are in agreement with Tan, Ye, Singh, & Hemar (2007) who observed an increase in stiffness of rennet gels and a reduction in syneresis as the concentration of high methoxyl pectin increased from 0% to 0.1% attributed to a more compact microstructure. Kopjar, et al. (2009) also found that pectin increases the firmness and cohesiveness of strawberry jam which increases with increasing degree of esterification of pectin. These authors attributed this to increased adhesiveness and compactness of the molecules. Upon cooling, there was a general increase in hardness of pectin-treated bananas and the control which may be attributed to starch retrogradation (Wang et al., 2015). Retrogradation is accompanied by a series of physical changes such as exudation of water and increased crystallinity with the appearance of β-type crystalline polymorphs (Hoover, Hughes, Chung, & Liu, 2009). However, hardness decreased with increasing pectin concentration which was associated with a strong a negative correlation of 80%. The observed decrease in hardness of pectin-treated bananas upon cooling may be attributed to the High Water Retention Capacity (WRC) of pectin. Pectin is highly hygroscopic and absorbs water leading to formation of soft gels. The high water binding and holding capacity of pectin (Armstrong, Eastwood, & Brydon, 1993) may keep the cooked banana structural matrix moist, thus maintaining starch in a gelatinized state hence making pectin-treated bananas relatively soft in cooled form. Pectins absorb varying amounts of water, for instance sunflower seed pectin absorbs about 57 g of water per g of pectin (Miyamoto & Chang, 1992). Apple pectin has high methoxyl content with DE higher than 50% and a water holding capacity of approx. 6.7 g water/g pectin (Sri Puvanesvari, Yogeshini, Noranizan, & Kharidah, 2012). According to Sri Puvanesvari et al. (2012), apple pectin also has a high emulsifying activity of 90% and above implying that pectin can prevent loss of water from the food matrix hence preventing extensive starch retrogradation. Studies on cheese with added pectin have found increased moisture content coupled with reduced hardness (Ibáñez, Waldron, & McSweeney, 2016; Lobato-Calleros et al., 2006). Added pectin may also interrupt interactions between starch molecules within the cooked banana matrix hence preventing re-crystallization of starch leading to a decrease in hardness.

Cohesiveness of pectin-treated bananas was higher than the control and increased slightly with increase in pectin concentration upon cooking coupled with a strong positive correlation of 81%. Pectin has adhesive properties which could keep particles in the cooked banana matrix cohesive. Upon cooling, the untreated control was more cohesive than all pectin-treated banana samples whose cohesiveness decreased with increasing pectin concentration coupled with a strong negative correlation 82%. This implies that pectin reduces cohesion and hence confers tenderness in cooked bananas. The decreasing cohesiveness in cooled bananas could be due to high moisture levels retained by added pectin. This may be true only in the short run. Kopjar, et al. (2009) found that the cohesiveness of all pectin treated jam samples had increased over the storage period which may be due to loss of moisture causing structural re-arrangement and increased adhesiveness and hence cohesiveness.

4.3 Effect of Added Starch on Hardness and Cohesiveness of Bananas

Starch-treated bananas were significantly harder than the control upon cooking and cooling ($P<0.05$) and

hardness increased with increasing starch concentration with a strong positive correlation of 98.2%. This implies that starch increases hardness in cooked bananas. Despite the high Water Binding Capacity (WBC) particularly during heating, starch has a low Water Holding Capacity (WHC) which tends to reduce the water activity (aw) of foods (Homayouni et al., 2013). This property coupled with increased dry matter content possibly enhances increased hardness during cooking of bananas. Upon cooling, there was a general increase in hardness of starch-treated bananas and the control which could be due to starch retrogradation. Retrogradation of starch involves re-association of gelatinized starch (amylose) molecules to form an ordered crystalline structure (Eliasson & Wahlgren, 2004) associated with increased solidity and hence hardness. Upon cooling, hardness increased with increasing starch concentration. These results are in agreement with findings by Liu et al.(2013) who found a significant increase in hardness of Surimi gels ($P<0.05$) prepared with added corn starch. The starch used in this study was freshly extracted from the *Nakitembe* banana cultivar at green mature stage to avoid wide variation. Due to differences in physicochemical properties, different starches may differ in their influence on hardness of cooked bananas. Noodles made from starches isolated from several specific potato varieties were harder and more cohesive than other starch noodles (Kim, Wiesenborn, Lorenzen, & Berglund, 1996; Singh, Singh, & Sodhi, 2002). According to Toyama, Miura, & Taneya (1997), use of sweet potato, corn, and cassava starches in noodles was found to increase firmness. They also found that noodles supplemented with potato starch were firmer than those supplemented with other starches.

Cohesiveness is a good indicator of how the banana sample holds together upon cooking and may be directly affected by adhesiveness of the sample which in turn relates to the amount of starch and starch gelatinization (Sozer et al., 2007). There was a high correlation (98.3%) between cohesiveness of the bananas and starch concentration upon cooking implying that starch strongly increases cohesiveness. This may be attributed to the adhesive nature of gelatinized starch being more in samples with more starch. Upon cooling, cohesiveness was observed to increase with starch concentration though with a reduced correlation of 54.3% implying less adhesiveness of starch as the bananas cooled. Upon cooling, starch loses water (or its gelatinized state) leading to retrogradation and this increases fragility of cooked bananas. Beyond 8.3% added starch, there was a sudden decrease in cohesiveness (Figure 2B, curve (a). These results are in agreement with findings by Kim et al. (1996) and Singh et al. (2002) who found noodles made from starches to be cohesive with those made using different potato starches being more cohesive than those made using other starches. However, these results contrast the findings by Liu et al. (2013) who did not find starch to have a significant influence on cohesiveness of Surimi gels ($p < 0.05$).

4.4 Effect of Starch-pectin Composite on Hardness and Cohesiveness of Bananas

Application of starch-pectin composite generally resulted in increased hardness of bananas upon cooking which was observed to decrease and then increase again with increasing starch-pectin composite concentration. However, hardness was not far from that of starch alone or pectin alone treated bananas and was not significantly different from the control ($P >0.05$). Upon cooling, there was a general increase in hardness of composite treated bananas and the control which could be attributed to starch retrogradation. Upon cooling, hardness decreased from 4.43 to 2.77 N at 3.33% and 10% but suddenly increased to 3.16 and 4.91 N at 16.6 and 23.4% composite, respectively. The trend of these results was similar to that observed for pectin-treated bananas. Upon cooling, hardness of the control and the composite-treated bananas was not significantly different ($P>0.05$).

The high water binding and holding capacity of pectin could have had a significant effect on the properties of the composite and hence hardness of the composite-treated bananas. Pectin remains gelatinized long enough to cause a considerable proportion of starch also to remain gelatinized which reduces the extent of starch retrogradation and firming. Pectin may also have a diluting effect on starch by occupying intermolecular spaces between starch molecules. This keeps starch molecules apart and possibly prevents extensive re-association that would cause formation of a crystalline structure hence reducing hardness. However, as the concentration of starch-pectin composite increases beyond 16.6%, there was increased hardness upon cooling. This could be due to increased competition for water between starch and pectin which becomes significantly depleted. As a result, both starch and pectin possibly start forming lumps that possibly increase hardness.

The increase in hardness upon cooling of the composite treated bananas was also accompanied by an increase in cohesiveness. Generally, cohesiveness of starch-pectin composite treated bananas was higher than the control and decreased initially but then increased with increasing composite concentration upon cooking. The increase in cohesiveness relative to the control may be attributed to increased adhesiveness of starch and pectin upon gelatinization. From starch and pectin single treatment results, starch appears to contribute more to cohesiveness than pectin. The cohesiveness values of the composite-treated bananas (1.09 to 1.38 N) were closer to those of starch-treated bananas (0.91 to 1.56 N) implying that starch may have more influence on cohesiveness than

pectin. Upon cooling, cohesiveness appears to decrease up to a certain level and then increase.

4.5 Effect of Pectinase (Polygalacturonase) on Hardness of Bananas

To better understand the effect of pectin on hardness of bananas, the molecule was structurally eliminated from the banana matrix using pectinase (Polygalacturinase) enzyme. All bananas that were treated with pectinase were found to be significantly harder than the control ($P<0.05$) upon cooking and cooling. This implies that the enzyme pectinase was able to hydrolyze and eliminate pectin from the structural matrix of bananas. Hydrolysis of pectin leaves starch and cellulose/hemicellulose as the main structural elements that profoundly affect texture. This could have resulted in a significant increase in hardness of all bananas from which pectin was hydrolyzed during cooking and upon cooling. Polygalacturonase (PG) cleaves the α-1, 4-D galacturonan linkages in the pectin homogalacturonan chain by hydrolysis preferring the non-esterified substrate with decreasing activity as the degree of methoxylation increases. ExoPG attacks the chain from the non-reducing end and removes terminally (1→)-linked GalA residues (Duvetter et al., 2009). Removal of pectin leaves starch as the main structural component which has been shown in the current study to increase hardness of bananas upon cooking and cooling. Starch has a high Water Binding Capacity (WBC) (upon heating) but also a low Water Holding Capacity (WHC) upon cooling which leads to reduced water activity (Homayouni et al., 2013) of the bananas. The absence of pectin in the structural matrix of bananas appears to reduce water activity which in turn reduces the extent of starch gelatinization during cooking leading to increased hardness. It was observed during sample preparation that pectinase-treated bananas appeared dryer than the control. Pectinase treated juice bananas were harder than cooking bananas. This is because juice bananas contain more starch than cooking bananas (Gafuma et al., 2018) which means the more starch in the bananas, the harder the texture as evidenced in this work. In all cases, hardness reduced with cooking time between 50 and 130 min but still the pectinase-treated bananas remained significantly harder than the controls. The decrease in hardness with cooking time could be due to the increasing degree of starch gelatinization as more starch takes up moisture from the steam. In general, these results imply that presence of pectin in the structural matrix of bananas significantly increases tenderness during cooking and cooling. These findings also re-enforce earlier results where we have shown that added pectin decreases hardness in cooked bananas particularly upon cooling. This could be explained by the high Water Binding and Holding Capacities of pectin which help to keep starch gelatinized longer leading to increased tenderness of cooked bananas.

Cohesiveness measures the extent to which a food product stays together after deformation or the extent to which a product adheres to itself under some compressive or tensile stress. Current results indicated that there was a significant increase in cohesiveness of bananas from which pectin was hydrolyzed which decreased with cooking time while that of the control remained more or less constant. This implies that pectin lowers cohesiveness relative to starch. The increased cohesiveness of bananas due to pectin elimination would probably improve their processability as the product becomes more tolerant of manufacturing, packaging and delivery stresses. This enhances presentation of the product to the consumers in its expected state (Texture technologies Corp. and Stable Micro Systems, Ltd, 2018). However, the increased hardness found in this study would make the product undesirable to consumers who prefer tender banana products. Upon cooling, final cohesiveness of the cooled pectinase-treated juice bananas was not significantly different from that of the control.

Generally, the cooked starch-treated bananas were 1.3 times (130%) harder than pectin treated bananas. Also, hardness of cooled starch-treated bananas was 1.89 times (189%) harder than the cooled pectin-treated bananas. This implies that starch increases hardness more than pectin does. Current results showed that pectin decreases hardness in bananas particularly during the cooling phase probably due to its high Water Binding, Water Holding and Emulsifying Capacities. According to these results, pectin contributes to tenderness of bananas as observed in cooked and cooled bananas. Unlike starch, pectin has a tendency of remaining gelatinized in heated and cooled forms which may enhance the gelatinized state of banana starch.

Based on these results, the optimal concentration of pectin that can be added to enhance tenderness of cooked bananas without profoundly affecting other properties is between 1.7 and 5%. Removal of pectin from bananas using pectinase increases hardness in cooked and cooled bananas. Absence of pectin in the structural matrix of bananas encourages starch-starch molecular interactions leading to increased hardness. It was observed in this work that cohesiveness and hardness are positively correlated under cooking and cooling conditions. Both starch and pectin were found to increase cohesiveness during cooking. However, upon cooling, pectin appears to decrease cohesiveness while starch increases it.

5. Conclusions and recommendations

Starch increases hardness of bananas during cooking and cooling. Pectin has minimal effect on hardness of

bananas upon cooking but strongly decreases it during cooling. This implies that pectin favors tenderness in cooled bananas after cooking. Hence pectin should be added to bananas at optimal levels (1 - 5%) to enhance tenderness. Co-presence of starch and pectin increases cohesiveness of bananas upon cooking while pectin alone decreases it upon cooling.

Structural hydrolysis and elimination of pectin from the banana matrix significantly increases their hardness during cooking and upon cooling. These findings highlight the importance of pectin in enhancing tenderness of cooked bananas. There is the need to optimize pectin levels (1.7-5%) during cooking for tenderer, tastier and better appealing banana meals.

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References

Armstrong, E., Eastwood, M., & Brydon, W. (1993). The influence of wheat bran and pectin on the distribution of water in rat caecal contents and faeces. *British Journal of Nutrition, 69*(3), 913-920. https://doi.org/10.1079/BJN19930091

Bourne, M. (2002). *Food texture and Viscosity: Concept and Measurement* (2nd Edition ed.). London: Elsevier.

Duvetter, T., Sila, D., Van Buggenhout, S., Jolie, R., Van Loey, A., & Hendrickx, M. (2009). Pectins in Processed Fruit and Vegetables: Part I—Stability and Catalytic Activity of Pectinases. *Comprehensive Reviews in Food Science and Food Safety. Willey Online Library, 8*(2), 75-85. https://doi.org/10.1111/j.1541-4337.2009.00070.x.

Eliasson, A., & Wahlgren, M. (2004). Starch-lipid interactions and their relevance in food products. In A. Eliasson, *Starch in Food: Structure, Function and Applications*, 441-460. https://doi.org/10.1533/9781855739093.3.441

Espinosa‐Solis, V., Jay‐lin, J., & Bello‐Perez, L. (2009). Physicochemical Characteristics of Starches from Unripe Fruits of Mango and Banana. *Starch, 61*(5), 291-299. https://doi.org/10.1002/star.200800103 .

Gafuma, S., Byarugaba-Bazirake, G., & Mugampoza, E. (2018). Textural Hardness of Selected Ugandan Banana Cultivars under Different Processing Treatments. *Journal of Food Research, 7*(5). https://doi.org/10.5539/jfr.v7n5p98.

Gummadi, S., & Panda, T. (2003). Purification and biochemical properties of microbial pectinases—a review. *Process Biochemistry. Elesevier, 38*(7), 987-996. https://doi.org/10.1016/S0032-9592(02)00203-0.

Homayouni, A., Amini, A., Keshtiban, A., Mortazavian, A., Esazadeh, K., & Pourmoradian, S. (2013). Resistant starch in food industry: A changing outlook for consumer and producer. A review. *Starch, 65*, 1-13. https://doi.org/10.1002 star.201300110.

Hoover, R., Hughes, T., Chung, H.-J., & Liu, Q. (2009). Composition, molecular structure, properties, and modification of pulse starches: A review. *Food Research International, 43*(2), 399-413. https://doi.org/10.1016/j.foodres.2009.09.001 .

Ibáñez, R., Waldron, D., & McSweeney, P. (2016). Effect of pectin on the composition, microbiology, texture, and functionality of reduced-fat Cheddar cheese. *Dairy Science & Technology , 96*, 297-316. doi:10.1007/s13594-015-0265-y.

Jongen, W. (2002). *Fruit and Vegetable Processing. Improving quality.* (Second Edition ed.). Woodhead Publishing, Elsevier.

Karamura, D. (1998). *Numeric Taxonomic studies of the East African Highland Bananas (Musa AAA-East Africa) in Uganda.* Montpellier, France .

Kim, Y., Wiesenborn, D., Lorenzen, J., & Berglund, P. (1996). Suitability of edible bean and potato starches for starch noodles. *Cereal Chem, 73*, 302-3028.

Kopjar, M., Piližota, V., Tiban, N., Šubarić, D., Babić, J., Ačkar, D., et al. (2009). Strawberry Jams: Influence of Different Pectins on Colour and Textural Properties. *Czech Journal of Food Science, 27*(1), 20-28. https://doi.org/10.17221/95/2008-CJFS

Liu, H., Nie, Y., & Chen, H. (2013). Effect of Different Starches on Colors and Textural Properties of Surimi-Starch Gels. *International Journal of Food Properties, 17*(7), 1439-1448. https://doi.org/10.1080/10942912.2012.680224.

Lobato-Calleros, C., Ramos-Solís, L., Santos-Moreno, A., & Rodríguez-Huezo, M. (2006). MICROSTRUCTURE AND TEXTURE OF PANELA TYPE CHEESE-LIKE PRODUCTS: USE OF LOW METHOXYL PECTIN AND CANOLA OIL AS MILK-FAT SUBSTITUTES . *Amidiq , 5*, 71-79 .

Miyamoto, A., & Chang, K. (1992). Extraction and Physicochemical Characterization of Pectin from Sunflower Head Residues. *Journal of Food Science, 57*(6), 1439-1443. https://doi.org/10.1111/j.1365-2621.1992.tb06878.x .

Payasi, A., Mishra, N., Chaves, A., & Singh, R. (2009). Biochemistry of fruit softening: An overview. *Physiology and Molecular biology of Plants, 15*(2), 103-13. https://doi.org/10.1007/s12298-009-0012-z .

Sharma, N., Rathore, M., & Sharma, M. (2012). Microbial pectinase: sources, characterization and applications. . *Reviews in Environmental Science and Bio/Technology*. https://doi.org/10.1007/s11157-012-9276-9.

Singh, N., Singh, J., & Sodhi, N. (2002). Morphological, thermal, rheological and noodle-making properties of potato and corn-starch. *J. Sci. Food Agric., 82*, 1376-1383. https://doi.org/10.1002/jsfa.1194

Sozer, N., Dalgıc, A., & Kaya, A. (2007). Thermal, textural and cooking properties of spaghetti enriched with resistant starch. *Journal of Food Engineering, 81*, 476-484. https://doi.org/10.1016/j.jfoodeng.2006.11.026.

Sri Puvanesvari, G., Yogeshini, R., Noranizan, M., & Kharidah, M. (2012). Functional and Preliminary Characterisation of Hydrocolloids from Tamarillo (Solanum betaceum Cav.) Puree. *Molecules, 17*, 6869-6885. https://doi.org/10.3390/molecules17066869 .

Steeneken, P., Woortman, A., & Oudhuis, A. (2011). Processing stability of cross-linked starches in acid sauce applications and identification of some of the molecular factors involved. *Food Hydrocolloids, 25*(3), 410-418. https://doi.org/10.1016/j.foodhyd.2010.07.009

Tan, Y., Ye, A., Singh, H., & Hemar, Y. (2007, May). Effects of Biopolymer addition on the dynamic rheology and microstructure of Renneted skim milk systems. *Journal of textural Studies, 38*(3), 404-422. https://doi.org/10.1111/j.1745-4603.2007.00104.x .

Tapre, A. R., & Jain, R. K. (2014). Pectinases: Enzymes for fruit processing industry. Mini Review. *International Food Research Journal, 21*(2), 447-453.

Texture technologies Corp. and Stable Micro Systems, Ltd. (2018). *Overview of Texture Profile Analysis.* Godalming, Surrey. United Kingdom: Stable Micro Systems. Retrieved from http://texturetechnologies.com/resources/texture-profile-analysis.

Toyama, R., Miura, M., & Taneya, S. (1997). Effects of starch origin and contents on sensory properties of "Reimen" (Korean noodles). *Nippon Chori Kagaku Kaishi, 30*, 213-225 (in Japanese).

Wang, S., & Copeland, L. (2013). Molecular disassembly of starch granules during gelatinization and its effect on starch digestibility: a review. *Food Function, 4*(11), 1564-80. https://doi.org/10.1039/c3fo60258c.

Wang, S., Li, C., Copeland, L., Niu, Q., & Wang, S. (2015). Starch retrogradation: A comprehensive Review. *Institute of Food Technologists, 14*(5), 568-585. https://doi.org/10.1111/1541-4337.12143.

Zhang, P., Whistler, R., BeMiller, J., & Hamaker, B. (2005). Banana starch: Production, physicochemical properties, and digestibility - A review. *Carbohydrate Polymers, 59*(4), 443-458. https://doi.org/10.1016/j.carbpol.2004.10.014.

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Effects of Promolux Platinum LED on Shelf-life of Ground Beef Patties

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Abstract

The objective of this study was to determine the effects of three light sources, Promolux platinum LED (PPLED), fluorescent (FLS) lighting, and no light (control), on shelf-life properties of ground beef patties. Treatments were evaluated for % drip loss, pH value, % moisture content, visual and instrumental color (L*, a* and b* values), lipid stability (TBARS), aerobic plate count, yeast/mold, *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. every 3 days for 9 days. At day 9, drip loss was lowest ($P<0.05$) in patties under the control treatment. No difference was found in visual color appraisal between treatments based on evaluations by trained color panelists ($N=15$) from days 1 to 5. Patties under PPLED had the greatest ($P<0.05$) redness a* values at day 9 of the experiment. When patties were not exposed to light they had the lowest TBARs values at day 7 and 9. Aerobic plate counts were lowest in patties under the control treatment throughout the experimental patties. There was no yeast/mold, *E. coli*, *Salmonella* spp. and *Listeria* spp. found in this study from days 1 to 5 and minimal counts at day 7 and 9.

Keyword: light source, meat color, microorganism

1. Introduction

Color is an important factor in the marketing of meat because it influences consumers buying decisions (Banović, Aguiar, Barreira, & Grunert, 2012; Suman & Joseph, 2013). When the meat changes to discoloration or a brownish color in refrigerated display, consumers begin rejecting products (Mancini & Hunt, 2005), resulting in discounted or discarded meat and causing the retailers loss up to $1 billion dollar annually (Smith, Belk, Sofos, Tatum, & Williams, 2000).

Myoglobin is the primary pigment for meat color and it can exist as deoxymyoglobin, oxymyoglobin, or metmyoglobin. Deoxymyoglobin and metmyoglobin present a brown color to meat while oxymyoglobin shows a bright red color. These forms depend upon the pigment concentration, the state of iron molecule, and the occupation of the sixth ligand (Faustman & Cassens, 1990). In addition, they may be affected by several factors including absorbing properties (Kropf, 1998), temperature (Martin et al., 2013), the length of retail display (Jeremiah & Gibson, 2001; Martin et al., 2013), and lighting type (Steele et al., 2016). Lighting type and intensity have a major impact on the appearance and shelf-life of fresh beef in refrigerated retail display (Smith et al., 2000; Steele et al., 2016; Cooper, Suman, Wiegand, Schumacher, & Lorenzen, 2017). The previous studies reported that fluorescent lighting can increase the temperatures in the displayed cases and increase the rate of discoloration (Steele et al., 2016). Meat is displayed under refrigeration temperatures 3-5°C retards discoloration (MacDougall, 1982). Specifically, ground beef with an oxygen-permeable overwrapped film has a shelf-life of 2-3 days (Robert, 2009). The shelf-life of beef is important in the retail marketplace which determines the length of time that passes before meat becomes unpalatable or unpleasant for human consumption because of the discoloration or growth of spoilage microorganisms is important in the beef retail market place (Smith et al., 2000). Previous research (Marriott, Naumann, Stringer, & Hedrick, 1967) reported that beef short loin steaks stored in the dark at 27°F for 10 days changed only slightly in visual color, while steak kept under 120 foot-candles of soft white fluorescent light discolored markedly after 5 days. Similarly, beef under lighting at 254 nm and 3230 lux of UV radiation accelerated discoloration (Hood, 1980).

Light emitting diode (LED) is commonly used in the meat industry which is more energy efficient and reduces heat generation throughout display (Steele et al., 2016). Previous studies demonstrated that LED light sources

promoted redness retention in ground beef patties during retail display (Cooper et al., 2016). In contrast, Steele et al (2016) showed that there was no difference in a* values between ground beef displayed under LED and fluorescent. Therefore, evaluating the impact of light sources during retail display on beef patties is still inconclusive.

Recently, newer technologies in lighting offer the ability to enhance meat color and to reduce energy costs for meat retail display. Promolux Platinum LED (PPLED) offers advantages for display because it is more energy-efficient and generates less heat than fluorescent lights. These advantages may be beneficial for fresh meat color stability. The objective of this study was to determine the effects of PPLED on visual and instrumental meat color and shelf-life properties of ground beef patties.

2. Method

2.1 Preparation of Beef Patties

Ground beef (80% lean and 20% fat) was obtained from the Center for Advancement of Meat Production and Processing (CAMPP) at McNeese State University in Lake Charles, Louisiana at 48 h postmortem. Beef patties (115 g) were made with a hamburger mold, placed on 20.96 x 14.61 x 1.59 cm foam tray with an absorbent pad, and wrapped with polyvinyl chloride (PVC) film. Patties (n = 81) were randomly assigned to three packaging treatments and stored in a 2.2°C cooler under three types of lighting conditions: 1) Control (no light), 2) fluorescent (FLS) and 3) Promolux platinum LED (PPLED) for 9 days. Three replicates of each treatment (n = 243) were analyzed for % drip loss, pH value, % moisture content, visual and instrumental color (L*, a* and b* values), lipid oxidation (thiobarbituric acid-reactive substances (TBARS) protocol), aerobic plate count (APC), yeast/mold, *Escherichia coli* (*E. coli*), *Salmonella* spp. and *Listeria* spp. every 3 days for 9 days.

2.2 Sensory Analysis

Visual color was determined following the American Meat Science Association protocol (AMSA, 2012). Fifteen trained visual color panelists from McNeese State University evaluated beef patty color every 3 days for 9 days using hedonic 8-point scales unique to each product (1 = very bright red, 2 = bright red, 3 = dull red, 4 = slightly dark red, 5 = moderately dark red, 6 = dark red to tannish-red, 7 = dark reddish-tan, 8 = tan to brown).

2.3 pH Test

Each treatment was replicated three times (n = 243) and evaluated for pH with a probe electrode portable meter (Model 2000 VWR Scientific) and results are expressed as the mean and standard error of the mean (SEM). Calibration of the pH meter was accomplished using pH 7 and pH 4 standardization buffers before use.

2.4 Moisture Content

Moisture content was determined according to the design method of the Association of Official Analytical Chemists (AOAC, 2000). Crucibles were heated at 102°C for 3 h and transferred to a desiccator to cool and record dry crucible weight. Each 3 g sample (n = 243) was weighed and dried in a hot air oven (Model 26 Precision Thelco) at 102°C for 24 h. After drying, crucibles were moved to the desiccator to cool and obtain dry sample weight. The total moisture content was determined by dividing the difference between the initial weight (IW) and dry weight (DW) and dividing by initial weight.

$$[(IW-DW)/IW] \quad (1)$$

2.5 Drip Loss Analysis

For determination of exudation and weight retention during storage, all treatment samples (n = 243) were weighed separately at the time of initial sampling at days 1, 3, 5, 7, and 9. Weight loss was calculated as the difference of final sample weight and initial sample weight divided by the initial weight for ground beef patties.

2.6 Color Test

Instrumental color was determined following the American Meat Science Association protocol (AMSA, 2012). On each sampling day, each package was opened and exposed to the air for a maximum of 10 seconds. Color was measured at three different locations (n = 243) and was averaged to obtain single values for each sample using a Minolta spectrophotometer (Model CR-10 portable) with an 8 mm aperture, 10° observer angle, D65 illuminant source in terms of L* (100 = white, 0 = black), a* (+40 = red, -40 = green), b* (+40 = yellow, -40 = blue).

2.7 TBARS Test

The 2-thiobarbituric acid (TBARS) method was used to measure the lipid oxidation for each sample designated for TBARS analysis (Tarladgis, Watts, Younathan, & Jr. Dugan, 1964). A fifteen gram sample of each beef patty

(n = 243) was blended with 30 mL of trichloroacetic acid solution. The sample solution was filtered through Whatman No. 1 filter paper. Five ml aliquots of the filtrate were transferred to separate test tubes (in duplicate) and mixed with 5 mL of 0.02 M TBA. The mixture was vigorously agitated in a vortex and was heated in a boiling water bath (100°C) for 45 min to develop a pink color. After cooling the reaction mixture under running water the absorbance was determined at 530 nm using a Beckman Du-640 spectrophotometer against a blank containing 5 mL of distilled water and 5 mL of TBA reagent. The TBA value used to express the results were calculated from standard curves and known dilutions of tetraethoxypropane (TEP). The result was expressed by the mg malonaldehyde (MDA)/kg tissue.

2.8 Microbial Counts

The microorganisms were determined following the standards of the Association of Official Analytical Chemists (AOAC, 2000). Buffered peptone water (BPW) was added as a diluent option for serial dilutions. All samples were plated on 3MTM Petrifilm to determine the enumeration (log CFU/g) of APC, yeast/mold, and *E. coli*. *Salmonella* was isolated with xylose lysine deoxycholate (XLD) agar and ACTERO™ *Listeria* enrichment media agar was used for *Listeria* spp. Plates were incubated in a horizontal position, clear side up in stacks of no more than 20 plates at 37°C for 24-48 h. Results were obtained by selecting a countable plate (30-300 colonies) and the colonies were counted and reported as CFU/g.

2.9 Statistical Analysis

The Proc GLM procedures of SAS windows (SAS, 2003) were used to evaluate the significance of differences of the obtained data. The PDIFF option of LSMEANS was employed to determine significance among treatments. All data are presented as means with standard error (SD) and a significance level of $P<0.05$ was used in ANOVA technique for statistical analysis of means from treatments.

3. Results and Discussion

3.1 Sensory Analysis

Using the hedonic scale, fifteen trained visual color panelists from McNeese State University evaluated beef patty color every 3 days for 9 days (Figure 1). No difference was found in visual color appraisal between treatments based on evaluations by trained color panelists from days 1 to 5. The trained panelists scored all treatments 1.8 (bright red) at d 1. On d 5, the average color scores ranged from 3.27 to 3.87 (dull red). Over 9 d storage, all treatments increased in discoloration ($P<0.05$). As expected, control patties had the lowest ($P<0.05$) scores 4.83 (moderate dark red) and 5.83 (dark red) at days 7 and 9, respectively. There were significant differences between FLS and PPLED treatments throughout the 9 d storage period. These results are consistent with those reported by Lentz (1971) and Barbut (2002) who reported that the panelists found dark red or brown color of ground beef under FLS lighting. In addition, Bertelson and Skibsted (1987) indicated that the beef retains an acceptable color under LED light for 3 days.

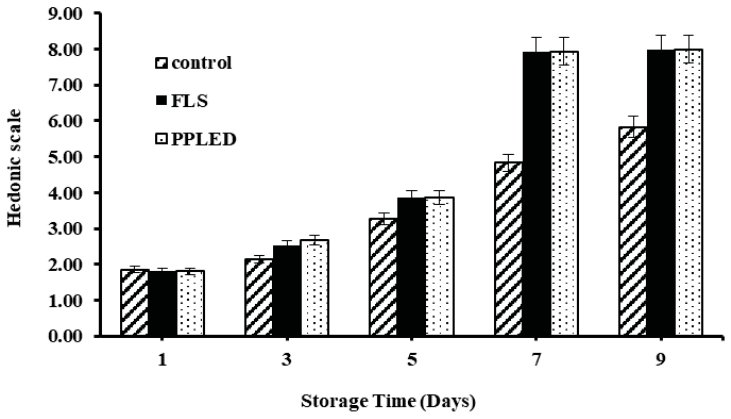

Figure 1. Least squares means for hedonic scales from trained panelists of beef patties at 2.2°C for 9 days

3.2 pH

Initial pH values of each treatment ranged from 7.60 to 7.64 which are similar to previous studies (Tangkham,

Rushing, & LeMieux, 2016). Over the 9-day experimental period, the pH values of beef patties were similar as all treatments decreased (Figure 2). The pH values of control patties declined from 7.60 to 7.46, from 7.63 to 7.38 in the FLS treatment, and from 7.64 to 7.46 in the PPLED treatment. Decreasing pH may lead to unacceptable discoloration of beef patties. Cornforth (1994) indicated that pH value affects the rate of formation of MMb pigment in brownish color. Specifically, our results showed that the pH values were lower in the beef patties under FLS lighting (7.38) than PPLED (7.46) over 9-day storage. Therefore, the pH values of beef patties at 2.2°C for 9 days are impacted by light source.

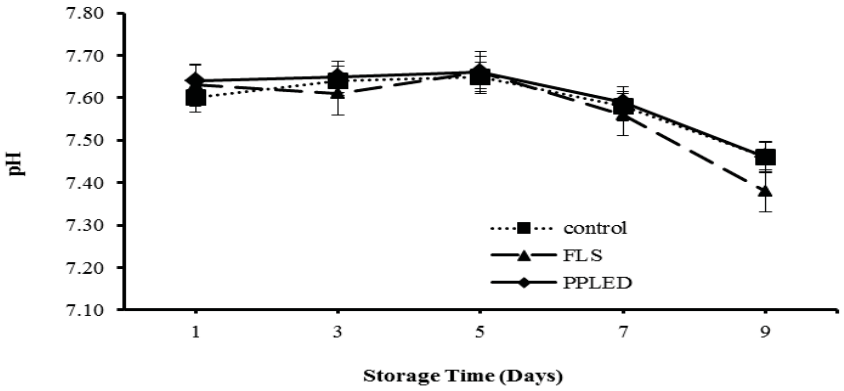

Figure 2. Least squares means for pH value of beef patties at 2.2°C for 9 days

3.3 Moisture Content

Moisture content of the three beef patty treatments are shown in Figure 3. The average initial moisture content of the three treatments ranged between 51.60%-54.82%. This value was similar to the study of USDA (2011). Moisture content declined from days 1 through 9 regardless of treatment. Beef patties under FLS treatment decreased from 54.82 to 48.49 and from 51.60 to 48.65 in the PPLED treatment. The control treatment had the lowest moisture content at 48.07% on day 9. These results are consistent with those reported by Zamudio-Flores et al. (2015). These results suggest that the lighting type affects moisture loss from the meat surface during the 9 d storage period.

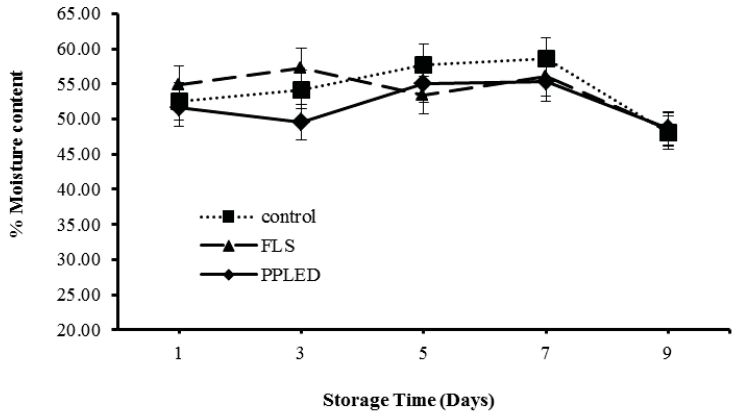

Figure 3. Least squares means for moisture content (%) of beef patties at 2.2°C for 9 days

3.4 Drip Loss

Percent drip loss was affected ($P<0.05$) by lighting treatments and storage time (Figure 2). All treatments increased in % drip loss ($P<0.05$) over 9 d storage. Beef patties under FLS treatment increased from 5.36% to 10.31% and from 5.26% to 11.17% in the PPLED treatment. On d 9, beef patties under control treatment had the lowest ($P<0.05$) percent drip loss at 6.72%. Our results suggest that the percent drip loss of beef patties at 2.2°C

for 9 days are influenced by lighting type.

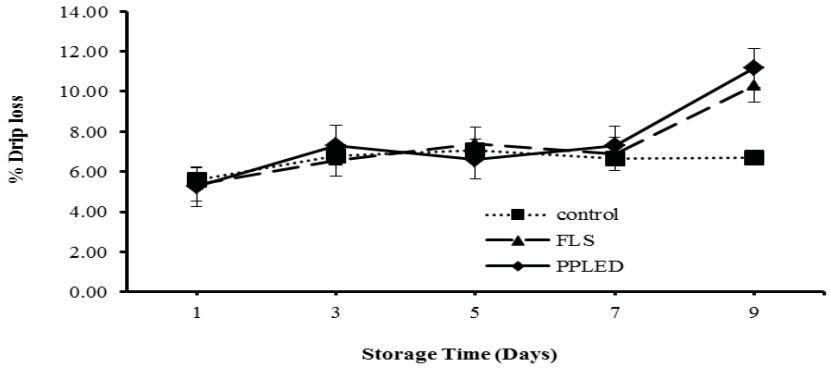

Figure 4. Least squares means for drip loss (%) of beef patties at 2.2°C for 9 days

3.5 Color Test

Meat color is major factor when consumers select meat at a retail outlet. Lighting type and intensity become important on meat appearance in the retail display. These may be beneficial to enhance meat color and reduce heat generation throughout the display. Over a 9 d experimental period, lighting type had an effect ($P<0.05$) on the instrumental color in terms of redness a* and yellowness b* values (Table 1). No difference was found in the lightness L* values. These results are consistent with those reported by Jade et al. (2017) who found that fresh steaks from beef triceps brachii under LED had no effect in lightness (L*) values over 7 day of retail display. However, our results disagreed with the findings by King, Shackelford, and Wheeler (2011) who reported that triceps brachii steaks consistently decreased in L* values from d 0 through d 6 of retail display.

We found that the redness a* and yellowness b* values declined during the experiment regardless of lighting technique which was similar to the study of Jade et al. (2017) which could be attributed to the formation of brown MMb on the beef patty surface. Data on surface redness agrees with previous studies (Hamling, Jenschke, & Calkins, 2008; Steele et al., 2016; Canto et al., 2016), which showed a decrease in a* values and a decline in red color retention with increasing retail display time. At day 9, the redness a* value was greater in beef patties under PPLED (18.16) lighting than FLS (17.11). According to Holman, et al. (2017), a threshold for consumer acceptance of redness is 14.5. In our experiment, all treatments had a* redness values above this threshold which is acceptable for consumers. This is similar to Cooper et al. (2016) who found that LED light sources promoted redness retention in ground beef patties during retail display. Our results indicate that beef patties under PPLED increased the redness value. These results are consistent with those reported by Steele et al. (2016) for beef patties. At day 7, control patties had the highest yellowness b* value at 15.20. Samples under FLS treatment had the yellowness b* value at 12.64 in the FLS treatment and 13.07 in PPLED treatment. Therefore, PPLED light source and retail display length impact a* and b* values of beef patties which help to minimize surface discoloration in low color stability beef muscles.

Table 1. Least squares means for HunterLab L*, a*, and b* values (n = 243) of beef patties at 2.2°C for 9 days

Parameter	Treatment	Storage time (d)				
		1	3	5	7	9
L*	Control	47.29^{a}	47.43^{a}	49.24^{a}	49.57^{a}	44.78^{a}
	FLS	49.33^{a}	48.01^{a}	49.44^{a}	51.70^{a}	42.56^{a}
	PPLED	45.77^{a}	47.83^{a}	48.60^{a}	51.69^{a}	41.71^{a}
a*	Control	26.78^{a}	26.53^{a}	21.92^{a}	19.57^{a}	16.39^{a}
	FLS	25.47^{a}	24.56^{a}	20.48^{a}	19.14^{a}	17.11^{b}
	PPLED	27.64^{a}	23.88^{a}	20.64^{a}	19.32^{a}	18.16^{c}
b*	Control	17.56^{a}	17.38^{a}	15.39^{a}	15.20^{a}	12.92^{a}
	FLS	17.72^{a}	16.71^{a}	15.06^{a}	12.82^{b}	12.64^{a}
	PPLED	17.81^{a}	16.09^{a}	14.87^{a}	12.62^{bc}	13.07^{a}

^{a,b,c}LSMeans with different superscripts within a same column is significantly different ($P<0.05$). Data are means from three replications. SEM for L* value = 0.972, SEM for a* value = 0.712, SEM for b* value = 0.346

3.6 Lipid Stability (TBARS)

Lipid oxidation is determined by the malonaldehyde concentration as a secondary bi-product of the propagation step. This measurement is used to detect oxidative deterioration of beef patties which correlate between the accumulation of malonaldehyde and MMb buildup (Hutchins, Lui, & Watts, 1967). The degree of lipid oxidation is dependent on several factors: the composition of the phospholipids, the amount of polyunsaturated fatty acids, and the concentration of pro-oxidants (Calkins & Hodgen, 2007). Lipid oxidation can be related to many different meat quality factors including loss of color, development of off-flavor, odors and loss of nutritional value.

The initial TBARS values of beef patties in this experiment ranged from 1.31 to 1.42 mg MDA/kg. As expected, TBARS values increased ($P<0.05$) throughout the storage time which is similar to the study of Jade, Surendranat, Bryon, Leon, and Carol (2017). In addition, other studies (Esmer, Irkin, Degirmencioglu, & Degirmencioglu, 2011; Martin et al., 2013; Steele et al., 2016) were similar in their findings. This is expected as the autoxidation of lipids and pigment oxidation lead to increase the amount of lipid oxidation. However, PPLED treatment exhibited higher TBARS values ($P<0.05$) at 2.90 mg MDA/kg than the remaining treatments at day 9. This is supported by a previous study (Steele et al., 2016).

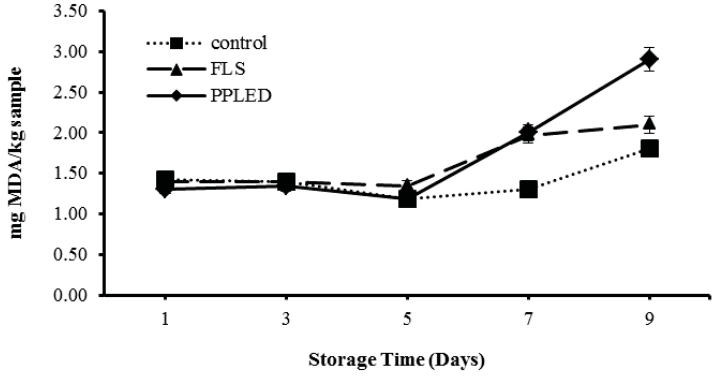

Figure 5. Least squares means for TBARS values of beef patties at 2.2°C for 9 days

3.7 Microbial Counts

Microorganism populations increased as display time increased for beef patties (Table 2) which similar to findings of Steele (2016) who reported that APC populations increased as display time increased for ground beef. Specifically, APC in the beef patties under PPLED lighting (5.60 log CFU/g) were lower than FLS (5.77 log CFU/g). These results are consistent with those reported by Steel, 2016 who reported that pork chops under LED lighting had lower ($P<0.05$) APC populations than FLS by the end of display.

Previous studies indicated that FLS influences the discoloration of meat which related to bacteria growth as the logarithmic growth phase of aerobic bacteria (Renerre & Labadie, 1993; Seideman, Cross, Smith, & Durland, 1984). Using proper packaging and storage temperatures can control bacteria growth in meat. Higher temperatures and oxygen permeable film will lead to an increase in bacterial growth on meat products (Seideman et al., 1984). At the end of display, the beef patties under FLS lighting had lower number of *Salmonella*, *Listeria* and yeast/mold as compared to PPLED. No *E. coli*, *Salmonella*, *Listeria* and yeast/mold were found in this study from days 1 to 5.

Table 2. Least squares means for microorganisms (n = 243) of beef patties at 2.2°C for 9 days

Microorganisms	Treatment	Storage time (d)				
		1	3	5	7	9
APC	Control	3.30[a]	3.39[a]	3.00[a]	4.29[a]	4.87[a]
	FLS	3.20[a]	3.50[a]	4.76[b]	4.83[ab]	5.77[b]
	PPLED	3.00[a]	4.24[b]	4.78[b]	4.99[b]	5.60[b]
E. coli	Control	ND	ND	ND	ND	ND
	FLS	ND	ND	ND	1.67[a]	3.82[a]
	PPLED	ND	ND	ND	2.13[b]	3.59[b]
Salmonella spp.	Control	ND	ND	ND	4.25[a]	5.18[a]
	FLS	ND	ND	ND	5.59[b]	6.59[b]
	PPLED	ND	ND	ND	5.53[b]	7.18[c]
Listeria spp.	Control	ND	ND	ND	3.90[a]	3.99[a]
	FLS	ND	ND	ND	5.30[b]	5.79[b]
	PPLED	ND	ND	ND	5.30[b]	5.99[c]
Yeast/mold	Control	ND	ND	ND	3.31[a]	4.18[a]
	FLS	ND	ND	ND	4.60[b]	5.60[b]
	PPLED	ND	ND	ND	4.49[c]	6.00[c]

a,b,cLSMeans with different superscripts within a same column is significantly different ($P<0.05$). ND = nondetectable. Data are means from three replications. SEM for APC = 0.208, SEM for *E. coli* = 0.050, SEM for *Salmonella* = 0.013, SEM for *Listeria* = 0.004, SEM for yeast/mold = 0.013.

4. Conclusions

Light source influenced surface discoloration (a* and b* values), pH, drip loss, lipid oxidation and microorganisms.

Our findings suggest that PPLED lighting is an effective light source for maintaining color stability in the redness a* and yellowness b* values. Control patties had lower drip loss, TBARS values, and the counts of APC compared to experimental treatments. Beef patties under FLS lighting had the lowest pH value.

Acknowledgement

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References

American meat science association (AMSA). (2012). Meat color measurement guidelines. Retrieved from http://www.meatscience.org Accessed 10 August 2015.

Association of official analytical chemists (AOAC). (2000). Official Methods of Analysis. 17th ed. Gaithersburg, MD, USA.

Barbut, S. (2002). Effect of lighting on ground beef acceptability. *Canadian Journal of Animal Science, 82*, 305-309. http://www.nrcresearchpress.com/doi/pdf/10.4141/A01-0

Banović, M., Aguiar F. M., Barreira, M. M., & Grunert, K. G. (2012). Impact of product familiarity on beef quality perception. *Agribusiness, 28*, 157-172. https://doi.org/10.1002/agr.21290

Bertelsen, G., & Skibsted, L. H. (1987). Photooxidation of oxymyoglobin. Wavelength dependence of quantum yields in relation to light discoloration of meat. *Meat Science, 19*, 243-251. https://doi.org/10.1016/0309-1740(87)90070-2

Calkins, C. R., & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science, 77*, 63-80. https://doi.org/10.1016/j.meatsci.2007.04.016

Canto, A. C. V. C. S., Costa-Lima, B. R. C., Suman, S. P., Monteiro, M. L. G., Viana, F. M., Salim, A. P. A. A., … Conte-Junior, C. A. (2016). Color attributes and oxidative stability of longissimus lumborum and psoas major muscles from Nellore bulls. *Meat Science, 121*, 19-26. https://doi.org/10.1016/j.meatsci.2016.05.015

Cooper, J. V., Wiegand, B. R., Koc, A. B., Schumacher, L., Gruën, I., & Lorenzen, C. L. (2016). Impact of contemporary light sources on oxidation of fresh ground beef. *Journal of Animal Science, 94*, 4457-4462.

https://doi.org/10.2527./jas2016-0728

Cooper, J. V., Suman, S. P., Wiegand, B. R., Schumacher, L., & Lorenzen, C. L. (2017). Light source influences color stability and lipid oxidation in steaks from low color stability beef triceps brachii muscle. *Meat and Muscle Biology, 1*, 149-156. https://doi.org/10.22175/mmb2017.06.0029

Cornforth, D. P. (1994). Color: its basis and importance. In: Pearson AM, Dutson TR (eds) Quality attributes and their measurement in meat, poultry and fish products. Aspen Publishers Inc, US, pp 34-39

Djenane, D., Sánchez-Escalante, A., Beltrán, J. A., & Roncalés, P. (2001). Extension of the retail display life of fresh beef packaged in modified atmosphere by varying lighting conditions. *Journal of Food Science, 66*(1), 181-186. https://doi.org/10.1111/j.1365-2621.2001.tb15603.x

Esmer, O. K., Irkin, R., Degirmencioglu, N., & Degirmencioglu, A. (2011). The effects of modified atmosphere gad composition on microbiological criteria, color and oxidation values of minced beef meat. *Meat Science, 88*, 221-226. https://doi.org/10.1016/j.meatsci.2010.12.021

Faustman, C., & Cassens, R. G. (1990). The biochemical basis for discoloration in fresh meat: a review. *Journal of Muscle Foods, 1*, 217-243. https://doi.org/10.1111/j.1745-4573.1990.tb00366.x

Hamling, A. E., Jenschke, B. E., & Calkins, C. R. (2008). Effects of dark storage and retail display on beef chuck and round muscles enhanced with ammonium hydroxide, salt, and carbon monoxide. *Journal of Animal Science, 86,* 972-981. https://doi.org/10.2527/jas.2007-0394

Holman, B. W. B., Van De Ven, R. J., Mao, Y., Coombs, C. E. O., & Hopkins, D. L. (2017). Using instrumental (CIE and reflectance) measures to predict consumers acceptance of beef colour. *Meat Science, 127*, 57-62. https://doi.org/10.1016/j.meatsci.2017.01.005

Hood, D. E. (1980). Factors affecting the rate of metmyoglobin accumulation in pre-packaged beef. *Meat Science, 4*, 247-265. https://doi.org/10.1016/0309-1740(80)90026-1

Hutchins, B. K., Lui, T. H. P., & Watts B. M. (1967). Effects of additives and refrigeration on reducing activity, metmyoglobin and malonaldehyde of raw ground beef. *Journal of Food Science, 32,* 214-217. https://doi.org/10.1111/j.1365-2621.1967.tb01297.x

Jade, V. C., Surendranat, P. S., Bryon, R. W., Leon, S., & Carol, L. L. (2017). Impact of light source on color and lipid oxidative stabilities from a moderately color-stable beef muscle during retail display. *Meat and Muscle Biology, 2*(1), 102-110. https://doi:10.22175/mmb2017.07.0040

Jeremiah, L. E., & Gibson, L. L. (2001). The influence of storage temperature and storage time on color stability retail properties and case-life of retail-ready ground beef. *Food Research International, 34*, 815-826. https://doi.org/10.1016/S0963-9969(01)00104-1

King, D. A., Shackelford, S. D., & Wheeler, T. L. (2011). Relative contributions of animal and muscle effects to variation in beef lean color stability. *Journal of Animal Science, 89*, 1434-1451. https://doi:10.2527/jas.2010-3595.

Kropf, D. H. (1998). Meat display lighting. Facts Pork Quality. National Pork Board, Des Moines, IA.

Lentz, C. P. (1971). Effect of light and temperature on color and flavor of prepackaged frozen beef. *Canadian Institute Food Technology Journal, 4*, 166-168. https://doi.org/10.1016/S0008-3860(71)74223-8

MacDougall, D. B. (1982). Changes in the color and opacity of meat. *Food Chemistry, 9*, 75-88. https://doi.org/10.1016/0308-8146(82)90070-X

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Sci., 71*, 100-121. https://doi.org/10.1016/j.meatsci.2005.03.003

Marriott, N. G., Naumann, H. D., Stringer, W. C., & Hedrick, H. B. (1967). Color stability of prepackaged fresh beef as influenced by pre-display environments. *Food Technology, 21*, 104.

Martin, J. N., Brooks, J. C., Brooks, T. A., Legako, J. F., Starkey, J. D., Jackson, S. P., & Miller, M. F. (2013). Storage length, storage temperature, and lean formulation influence the shelf-life and stability of traditionally packaged ground beef. *Meat Science, 95*, 495-502. https://doi.org/10.1016/j.meatsci.2013.05.032

Renerre, M., & Labadie, J. (1993). Review: fresh meat packaging and meat quality. In: Proceedings of the 39th International Congress of Meat Science and Technology. 361-387. Calgary, Canada.

Robert, J. D. (2009). Beef shelf-life. Beef facts, product enhancement. Retrieved from

http://www.beefresearch.org/cmdocs/beefresearch/beef%20shelf-life.pdf. Accessed 10 August 2015.

Statistical Analysis Software (SAS). (2003). SAS User's Guide Version 9.1.3. North Carolina, NC: Cary.

Seideman, S. C., Cross, H. R., Smith, G. C., & Durland, P. R. (1984). Factors associated with fresh meat color: a review. *Journal of Food Quality, 6*, 211-237. https://doi.org/10.1111/j.1745-4557.1984.tb00826.x

Smith, G. C., Belk, K. E., Sofos, J. N., Tatum, J. D., & Williams, S. N. (2000). Economic implications of improved color stability in beef. 397-426. https://www.cabdirect.org/cabdirect/abstract/20013089987

Suman, S. P., & Joseph, P. (2013). Myoglobin chemistry and meat color. *Annual Review of Food Science and Technology, 4*, 79-99. https://doi.org/10.1146/annurev-food-030212-182623

Steele, K. S., Webber, M. J., Boyle, E. A. E., Hunt, M. C., Lobaton-Sulabo, A. S., Cundith, C., … Roenbaugh, T. L. (2016). Shelf life of fresh meat products under LED or fluorescent lighting. *Meat Science, 117*, 75-84. https://doi.org/10.1016/j.meatsci.2016.02.032

Tangkham, W., Rushing, A., & LeMieux, F. (2016). Effects of L-Arginine on the Sensory, Physicochemical and Microbiological Characteristics of Beef Jerky. ICoMST2016: 62nd International Congress of Meat Science and Technology. Bangkok, Thailand, August 14-19.

Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Jr. Dugan, L. (1964). Chemistry of the 2-thiobarbituric acid test for determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists' Society, 37*, 44-48. https://doi.org/10.1007/BF02633347

USDA. (2011). Water in meat and poultry. United States Department of Agriculture, Food Safety and Inspection Service. Retrieved from https://www.fsis.usda.gov/wps/wcm/connect/42a903e2-451d-40ea-897a-22dc74ef6e1c/Water_in_Meats.pdf?MOD=AJPERES

Zamudio-Flores, P. B., Ochoa-Reyes, E., De Ornelas-Paz, J., Aparicio-Saguilán, A., Vargas-Torres, A., Bello, P. … Cárdenas-Félix, R. G. (2015). Effect of storage time on physicochemical and textural properties of sausages covered with oxidized banana starch film with and without betalains. *J. Food, 13*, 3. https://doi.org/10.1080/19476337.2014.998713

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Occurrence of Antibiotic Residues and Antibiotic-Resistant Bacteria in Nile Tilapia Sold in Accra, Ghana: Public Health Implications

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Abstract

In Ghana there are concerns that antibiotics may be used inappropriately to boost fish production, though no study has investigated this problem. To provide preliminary insights into public health aspects of the problem, we investigated the occurrence of antibiotic residues and antibiotic-resistant bacteria in Nile tilapia (*Oreochromis niloticus*), a fish commonly cultivated and consumed in Ghana. Two hundred Nile Tilapia fish were randomly sampled from four major markets in Accra, the capital city of Ghana. One hundred samples were screened for antibiotic residues using a microbial inhibition plate test that detects sixteen different antibiotics commonly used in animal husbandry and aquaculture. The other 100 samples were cultured for bacteria using direct culture methods, and the isolates were tested against seven antibiotics by the Kirby Bauer method. The overall prevalence of antibiotic residues in the fish samples was 7%. Bacteria that were isolated from the fish samples were *Shigella sonnei* (10%), *Enterobacter cloacae* (7%), *Escherichia coli* (6%), *Salmonella* Typhi (3%) and *Klebsiella pneumoniae* and *Proteus mirabilis* (2%). All bacteria isolated were susceptible to gentamicin and ciprofloxacin but resistant to ampicillin. Multi-drug resistance (ie resistance to three or more different classes of antibiotics) occurred in 86.7% of the isolates. Nile Tilapia sold in Accra is a source of multi-drug resistant bacteria. Consumption of the fish can also lead to significant exposure to antibiotic residues.

Keywords: antibiotic residues, multi-drug resistance, fish, *Shigella sonnei*

1. Introduction

Antibiotics are used in aquaculture for several reasons including treatment and prevention of fish diseases, increment in fish reproduction and tranquilization such as in weighing (Cabello, 2006; Kim *et al*., 2011). Common antibiotics used in aquaculture include tetracyclines, penicillins, chloramphenicol, fluoroquinolones, sulfonamides and macrolides (Cabello, 2006; Kim *et al*., 2011; Samanidou & Evaggelopoulou, 2007). The dependency on antibiotics in aquaculture can have negative effects on the health of humans, as well as damage to the environment (Kim *et al*., 2011; Buschmann *et al*., 2006; Sorum, 2006). Certain antibiotics in fish products, particularly penicillin can evoke allergic reactions even if small amounts are ingested; it is known that about 10% and 3.4% of humans have allergic sensitivity to penicillin and sulphonamide respectively (Wassenaar, 2005; Sundlof, 2004). Chloramphenicol can cause aplastic anaemia, while broad-spectrum antibiotics such as metronidazole may adversely affect a wide range of intestinal flora and consequently cause gastrointestinal disturbance (Wassenaar, 2005; Sundlof, 2004). To protect public health against toxicological risk of antibiotics used in animal husbandry and aquaculture, maximum residue limits have been established for various antibiotics as well as acceptable daily intakes (FAO, 2005; JECFA, 2005). Additionally, withdrawal periods have been defined for these antibiotics to prevent animal source food contamination.

The widespread use of antibiotics in aquaculture has resulted in the emergence of antibiotic-resistant bacteria in aquaculture environments. In Australia, a study on fish and fish ponds showed widespread resistance among *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* (Ndi & Barton, 2012). L'Abée-Lund & Sørum (2001) have reported the presence of tetracycline resistance associated with *tet* (A) and *tet* (E) resistance genes in tranposons and integrons in multi-resistant *Aeromonas salmonicida* isolated from fishes from Finland, France,

Japan, Norway, Scotland, Switzerland, and the United States. In Turkey, a study showed varying levels of multi-resistance in some gram-negative bacteria from wastewater river containing fishes (Toroglu & Korkmaz, 2005). A study in Nigeria with *Salmonella Enteritidis* recovered from fishes revealed varying levels of resistance (Raufu *et al*., 2014).

Commercial aquaculture now exists in a huge scale in many developed and developing countries. In Ghana, the aquaculture industry is relatively young and faced with significant challenges involving production intensification and disease control (ATFALCO, 2012). In order to address some of these issues, antibiotics are sometimes used in fish cultivation. Currently, there are concerns that antibiotics may be used inappropriately in aquaculture in Ghana, though there has been no study to investigate the problem. To provide preliminary insights into the public health aspects of the problem, we investigated the occurrence of antibiotic residues and antibiotic resistant bacteria in Nile Tilapia (*Oreochromis niloticus*), a fish commonly cultivated and consumed in Ghana.

2. Methods

2.1 Study Area, Design and Sampling

The study was carried out in four markets in Accra, including Agbobgloshie, Kaneshie, Makola and Weija markets from May 2014 to July 2015. Accra is the capital city of Ghana and has a population of 2.5 million people (Population and Housing Census, 2010). The four sites selected for the study are markets where residents of Accra commonly purchase fish. A wide range of fishes is sold on these markets, however only a few types including tilapia and catfish are from aquaculture sources. In Ghana, Tilapia is the major fish species cultivated and constitutes over 80 percent of aquaculture production (ATFALCO, 2012).

Two hundred fresh fish samples of Nile Tilapia (*Oreochromis niloticus*) were randomly sampled from the four markets. The fish samples were transported to the bacteriology laboratory of School of Biomedical and Allied Health Sciences of University of Ghana in Accra for analysis. The first 100 fish samples collected were screened for antibiotic residues while the other 100 samples were cultured for bacteria followed by antimicrobial susceptibility testing of the isolates.

2.2 Determination of Antibiotic Residues in Fish Samples

Fish samples were screened for antibiotic residues using the microbial inhibition method described by Koenen-Dierick *et al.* (1995). Briefly, 0.5 McFarland's standard suspension of *Bacillus subtilis* ATCC 6633 was prepared and used to inoculate the surface of Muller-Hinton agar (MHA) plates. A sterile cork borer of 3 mm in diameter was used to create four wells on the agar plates. Using a sterile forceps and scalpel blade, 3mm of the gill of each tilapia sample was cut and transferred aseptically into the cut wells. For each MHA plate, positive and negative controls were set up with penicillin and sterile distilled water respectively. The agar plates were incubated at 37°C for 18–24 hr. After incubation, the plates were examined for zones of inhibition. Any well containing fish gills with ≥1mm zone of inhibition was considered positive for antibiotic residue (Koenen-Dierick *et al*., 1995)

2.3 Isolation and Identification of Bacteria from Fish Samples

Sterile swab sticks were used to swab the gut regions of the fish samples and inoculated into Brain-heart infusion broth and *Rappaport-Vassiliadis* broth. The inoculated broths were incubated at 37^{o}C for 18-24 hours and subcultured onto MacConkey, Blood and Xylose Lysine Deoxycholate agar plates. After incubation at 37^{o}C for 18-24 hours, bacterial isolates were identified based on colonial morphology, Gram stain and a battery of biochemical tests such as the triple sugar iron test, catalase test, urease test, indole test and citrate utilization test (Baron *et al*., 1994).

2.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of bacterial isolates were determined by the Kirby Bauer method (Bauer *et al*., 1966; CLSI, 2010). On the agar plate of the test organism, a sterile loop was used to touch four to five morphologically similar colonies and inoculate 5 mL sterile peptone water until the turbidity was comparable to 0.5% McFarland's standard. A loopful of the suspension was transferred to a Mueller-Hinton agar plate, and a sterile cotton swab was used to streak the entire surface of the plate. The lid of the agar plate was left ajar for 3 to 5 minutes to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks. The antibiotics tested included ceftriaxone (30µg), gentamicin (10µg), cefuroxime (30µg), meropenem (10µg), amikacin (30µg), cotrimaxole (25µg) and ciprofloxacin (5µg), ampicillin (10µg), cefotaxime (30µg), chloramphenicol (30µg). The agar plates were incubated at 37°C for 18–24 hours, after which zone diameters around the antibiotic discs were measured and classified as sensitive or resistant based on the CLSI break point system (CLSI, 2010). Quality control testing was done by using *Escherichia coli* ATCC25922 strain for

validation of the susceptibility testing process.

2.5 Data Analysis

The data were analysed using STATA 11 (Strata Corp., College Station. TX, USA). Descriptive analysis including frequencies and percentages were computed for antibiotic residues and various bacterial organisms in the fish samples. The Chi-square test was used to compare these parameters among the various markets and a p value <0.05 was considered to be significant. Analysis of frequencies and percentages were also done for resistant and multi-drug resistance isolates. Multidrug resistance was defined as resistance to three or more classes of antibiotics.

3. Results

3.1 Prevalence of Antibiotic Residues in Fish Samples

The prevalence of antibiotic residues in fish sampled from the markets is shown in Table 1. While none of the fish samples from Agbobloshie market tested positive, prevalence of antibiotic residues in samples from Kaneshie and Makola markets were 11.8% and 9.1% respectively. The overall prevalence of antibiotic residues in the fish samples was 7%; generally, there was no significant difference in the prevalence of fish contamination among the different markets at $p<0.05$.

Table 1. Prevalence of antibiotic residues in fish sampled from markets in Accra (Ghana)

Name of Market	Number of samples tested	Number of positive samples	% of positive samples
Agbobloshie	33	0	0
Kaneshie	34	4	11.8
Makola	33	3	9.1
Weija	0	0	0
Overall	100	7	7

No fish samples from Weija market were tested for antibiotic residues; No significant difference in positive samples from the different markets.

3.2 Bacteria Isolated from Fish Samples

For the 100 fish samples screened by culture, 6 different types of bacterial pathogens were isolated from 46 samples. As shown in Table 2, the most prevalent organism was *Shigella sonnei* (10%), followed by *Enterobacter cloacae* (7%), *Escherichia coli* (6%), *Salmonella* Typhi (3%) and *Klebsiella pneumoniae*/*Proteus mirabilis* (2%).

Unlike the other study markets, fish samples from Makola market harboured all the 6 bacterial pathogens while samples from Agbobloshie market were least contaminated. Generally, there was no significant difference in the types of bacteria isolated from the different markets ($p>0.05$).

Table 2. Bacteria isolated from fish sampled in markets in Accra (Ghana)

Bacteria	Market				
	Agbobloshie	Kaneshie	Makola	Weija	Overall
	n (%)	n (%)	n (%)	n (%)	n (%)
Shigella sonnei	0 (0)	5 (20)	4 (16)	1 (4)	10 (10)
Enterobacter cloacae	0 (0)	4 (16)	2 (8)	1 (4)	7 (7)
Escherichia coli	1 (4)	0 (0)	2 (8)	3 (12)	6 (6)
Salmonella Typhi	0 (0)	0 (0)	1 (4)	2 (8)	3 (3)
Klebsiella pneumoniae	0 (0)	1 (4)	1 (4)	0 (0)	2 (2)
Proteus mirabilis	0 (0)	0 (0)	2 (8)	0 (0)	2 (2)

n indicates number of samples that tested positive for bacteria

% indicates corresponding percentage of positive samples

3.3 Antibiotic Resistance of Bacteria Isolated from Fish Samples

All the 30 isolates from fish samples were susceptible to gentamicin and ciprofloxacin but resistant to ampicillin. Prevalence of multi-drug resistance among the 30 isolates was 86.7%. Multi-drug resistance occurred among all the different bacterial pathogens (Table 3). In many cases, resistance profiles of the multi-drug resistant isolates

were highly varied within the same species (Table 4). Further analysis on the prevalence of resistance to various antibiotics was done for only *Shigella sonnei* as isolates of the other bacterial pathogens were insufficient for analysis. For *S. sonnei*, antibiotic resistance was observed for eight of the ten drugs tested, increasing across chloramphenicol/ceftriaxone/cefuroxime (25%), amikacin (33%), cefotaxime (42%), meropenem (67%), cotrimoxazole (83%) and ampicillin (100%) (Figure 1).

Table 3. Prevalence of multi-drug resistance among bacteria isolated from fish samples

Bacteria	Number of isolates	Number of Multi-drug resistant isolates (%)
Enterobacter cloacae	7	4 (57.1)
Escherichia coli	6	6 (100)
Klebsiella pneumoniae	2	2 (100)
Proteus mirabilis	2	1 (50)
Salmonella Typhi	3	3 (100)
Shigella sonnei	10	10 (100)

4. Discussion

This study is probably the first to report on contamination of antibiotic residues in fish in Ghana. By comparison, studies on fish in Nigeria and Vietnam reported antibiotic residue contamination of 61.3% (Olatoye & Basiru, 2013) and 29.4% (Pham *et al*., 2015) respectively. In Ghana, prevalence of antibiotic residues has been reported in several animal foods including beef (30.8%), chevon (29.3%), pork (28.6%), mutton (24%), and egg (6.8%) (Donkor *et al*., 2011). Generally, in the developed world, antibiotic residue contamination of animal source food is low, and in Europe prevalence rates of <1% are generally reported (Commission of the European Communities, 2005). The relatively lower prevalence of antibiotic residues in food in the developed world can be attributed to quality assurance programmes such as educational programmes, widespread testing of food for antimicrobial drugs, and financial penalties (Commission of the European Communities, 2005; Mckenzie & Hathway 2006). The aquaculture industry in Ghana is still young and the concept of using antibiotics in cultivation may not be widespread among farmers yet, which may explain the relatively lower prevalence of antibiotic residues in fish in this study.

Table 4. Resistance profile of multi-drug resistant bacteria isolated from fish

Isolates	Number of isolates	Resistance pattern
Enterobacter cloacae	2	TET-COT-MEM-AMP
	2	CTR-MEM-AMP
Escherichia coli	4	TET-CRX-CTX-MEM-AM
	1	CRX-MEM-AMP
	1	CRX-CTX-MEM-AMP
Klebsiella pneumoniae	1	TET-COT-CTX-CTR-AMP
	1	TET-COT-CTX-CTR-MEM-AMP
Proteus mirabilis	1	TET-COT-MEM-AMP
Salmonella Typhi	1	TET-COT-CRX-CTX-MEM-AMP
	1	CRXT-CTX-MEM-AMP
	1	COT-CRX-MEM-AMP
Shigella sonnei	2	TET-COT-CRX-CTX-MEM-AMP
	3	COT-TET-AMK-AMP
	1	TET-AMK-AMP
	1	TET-COT-CRX-CHL-MEM-AMP
	1	TET-CRX-CTX-MEM-AM
	1	TET-COT-CHL-CTX-MEM-AMP
	1	COT-CRX-MEM-AMK-AMP

TET= tetracycline COT= cotrimoxazole GEN= gentamicin CRX= cefuroxime CHL= chloramphenicol CTX= cefotaxime CTR= ceftriaxone MEM= meropenem AMK= amikacin CIP= ciprofloxacin AMP= ampicillin

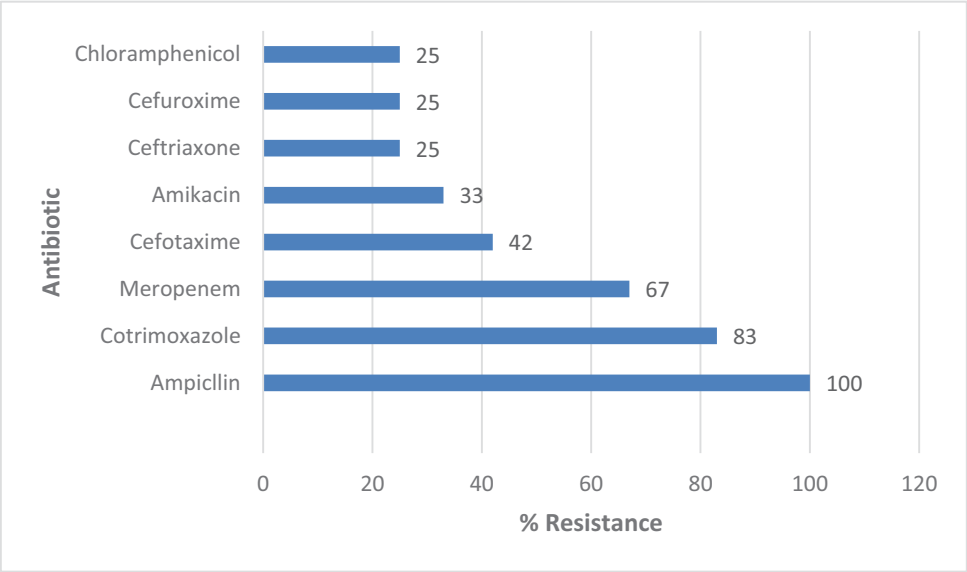

Figure 1. Percentage resistance of *Shigella sonnei* to various antibiotics

The microbial inhibition plate test used to screen fish for antibiotic residues in this study has a low sensitivity for macrolides but has a high sensitivity for several other antibiotics that are known to be used in animal husbandry in Ghana, such as b-lactams, tetracyclines, aminoglycosides, sulphonamides, and quinolones (Koenen-Dierick, *et al*., 1995; Sekyere, 2014). Overall, the test used can detect sixteen different antibiotics commonly used in animal husbandry and aquaculture. With such a wide coverage of antibiotics, we expect that our screening captured to a large extent any antibiotics used in aquaculture in Ghana. Though the test used detects a wide range of antibiotic residues in food above maximum residue limits, it is unable to differentiate the residues. Assays that detect specific antibiotics in animal food are rather expensive and include chemical methods such as chromatography and mass spectrometry (Samanidou & Evaggelopoulou, 2007).

An overall tilapia contamination of 7% with antibiotic residues in this study translates into an average risk of exposure every fifteenth time a consumer takes in the commodity. The risk of exposure of a human population to food contaminated with antibiotic residues depends mainly on the extent of food contamination and the consumption rate of the food (Lu & Sam, 2002; MacNeil, 2005). In this study, because we did not quantify specific drugs in the fish samples and evaluate consumption trends of the commodity, it is difficult to measure the actual risk of specific drugs to consumers. Consumption rate of tilapia in Ghana is known to be very high and some of the samples, which tested positive for antibiotic residues in this study, had large zones sizes (>31mm) reflecting high concentration of antibiotic residues. These observations along with a fish contamination rate of 7% reflect a potentially high risk of exposure to antibiotic residues in Tilapia, though further studies are needed to throw more light on this. In Ghana, there are no national limits for antibiotic residues in fish currently. With the increasing public health concerns of antibiotic residues in fish sold in the country, it is about time for legislative establishment of such limits.

The various bacteria reported in this study have been previously isolated from fish (Onyango *et al*., 2009; Marcel *et al*., 2013, Plumb, 1999). All the organisms isolated are enteric, suggesting that faecal pollution may have played a role in the microbial contamination of the fish samples (Ashbolt *et al*., 2015). Consumption of fish contaminated by some of these organisms particularly, *Shigella sonnei* and *Salmonella typhi* could pose serious threat to public health. *Shigella* causes shigellosis or bacillary dysentery and is specific host-adapted to humans (Shears, 1996). It has a low infectious dose and some of its outbreaks have been linked to fish consumption (Shears, 1996). Recent epidemiological evidence indicates that *S. sonnei,* which has historically been more commonly isolated in developed countries, is undergoing an unprecedented expansion across industrializing regions (Thompson *et al*., 2015). Consequently, it is interesting to note that *S. sonn*ei was the predominant *Shigella* spp. isolated in this study, though it was previously reported to be relatively uncommon in Ghana compared to several other *Shigella* spp. (Opintan & Newman, 2007). Further studies are needed to evaluate the public health significance of *S. sonnei* in Ghana in light of its changing epidemiology. *S.* Typhi causes typhoid fever, an infection, which is endemic in some parts of Ghana (Marks *et al*., 2010)]. Though *S.* Typhi has been isolated from several types of food in Ghana, the current study is probably the first report on fish. Generally, *Salmonella* is responsible

for a huge public health problem associated with fish and fishery products and its presence in food reflects poor standard of hygiene (Heinitz *et al*., 2000). In light of this study, the recent efforts to expand aquaculture in Ghana could be associated with an increased risk of typhoid fever unless precautions are taken to protect public health.

Though fish samples from Agbogbloshie market were least contaminated by bacteria and were also free of antibiotic residues, it is difficult to attribute these observations to better food safety and hygienic practices at this market. This is because fish samples on the different markets originated from farm sources and may have moved through various channels before reaching the markets. For example, cross-contamination of food with microbes commonly occurs during food transportation (Stier, 2004), while drug residue contamination of food is mainly a farm level issue (Aning *et al*., 2007).

The extent of antibiotic resistance observed among bacteria isolated from fish is high and similar to what has been reported for clinical isolates in Ghana especially for tetracycline, chloramphenicol, ampicillin and cotrimoxazole (Opintan & Newman, 2007; Newman *et al*., 2011; Labi *et al*., 2014). These antibiotics have been on the market in Ghana for a long time and have been subjected to a high rate of usage leading to emergence and spread of resistant strains at a high level (Donkor *et al*., 2012; Donkor *et al*., 2011; Tetteh-Quarcoo *et al*., 2013). Of concern is the extremely high prevalence of multi-drug resistance in pathogens of public health significance such as *Shigella sonnei* and *Salmonella* Typhi. This could have serious public health implications, especially in developing countries like Ghana where antibiotic treatment options are limited. Multi-drug resistance in enteric bacteria is mediated by mobile genetic elements such as plasmids and transposons that carry resistant genes (von Wintersdorff, et al., 2016; AbdelRahim *et al*., 2015; Codjoe & Donkor, 2017). Several lines of evidence indicate that such genetic elements can be transferred from indigenous microbial flora of fish to human pathogens (Marshall & Levy, 2011; Sorum, 2006; Akinbowale *et al*., 2006). It is important to note the high susceptibility of enteric bacteria to ciprofloxacin and gentamicin in this study, despite the high levels of resistance to several antibiotics in this study. This could be because these antibiotics are hardly used in aquaculture in Ghana. There is the need to limit the use of these drugs in Ghana through their prudent usage. The design of this study did not permit us to collect data on antibiotic use in aquaculture and thus it is difficult to relate this to the pattern of resistance observed. There is the need for studies to understand the epidemiology of antibiotic use in aquaculture in Ghana, as this will be an important step in solving the huge problem of antibiotic resistance in the acquaculture environment that has been exposed by this study.

There are a few limitations of the study. Firstly, the method used to screen fish samples for antibiotic residues could not identify and quantify specific antibiotics. Secondly, since the study was done at the market level, we do not have information on the origin of the fish samples. Additionally, we did not collect data on certain biological parameters of the fish samples such as sex, weight and length.

In conclusion, Nile Tilapia sold in Accra is a source of multi-drug resistant bacteria and consumption of the fish could also lead to significant exposure to antibiotic residues. Though fish is normally consumed in the cooked state thus decreasing the potential of infection, they may play a role in spreading multi-drug resistant bacteria to other food sources in the environment. The study has revealed a potentially serious public health problem. There is the need to create awareness about this among policy makers and also test for drug residues at fish distribution centres. There is also the need to include the acquaculture environment in antibiotic-resistance management and surveillance programmes in Ghana.

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References

AbdelRahim, K. A., Hassanein, A. M., & Abd El Azeiz, H. A. (2015). Prevalence, plasmids and antibiotic resistance correlation of enteric bacteria in different drinking water resources in sohag, Egypt. *Jundishapur Journal of Microbiology, 8*(1), e18648.

Akinbowale, O. L., Peng, H., & Barton, M. D. (2006). Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. *Journal of Applied Microbiology, 100*, 1103-1113. https://doi.org/10.1111/j.1365-2672.2006.02812.x

Aning, K. G., Donkor, E. S., Omore, A., Nurah, G. K., Osafo, E. L. K., & Staal, S. (2007). Risk of exposure to marketed milk with antimicrobial drug residues in Ghana. *The Open Food Science Journal, 1*, 1-5. https://doi.org/10.2174/1874256400701010001

Ashbolt, N. J. (2015). Microbial contamination of drinking water and human health from community water

systems. *Current Environmental Health Reports, 2*, 95-106. https://doi.org/10.1007/s40572-014-0037-5

ATFALCO (2012). The Ministerial Conference on Fisheries Cooperation among African States Bordering the Atlantic Ocean, Fishery and aquaculture industry in Ghana. Available from http://www.comhafat.org/fr/files/publications/112102014102624AM.pdf,

Baron, J. E., Peterson, L. R., & Finegold, S. M. (1994). *Bailey and Scott Diagnostic Microbiology*. 9th ed. Maryland Heights, MO: C V Mosby Co.

Bauer, A. W., Kikrby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*(4), 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Buschmann, A. H., Riquelme, V. A., Hernandez Gonzalez, M. C., Varela, D., Jimenez, J. E., Henriquez, L. A., Vergara, P. A., Guíñez, R., & Filun, L. (2006). A review of the impacts of salmonid farming on marine coastal ecosystems in the southeast Pacific. *ICES Journal of Marine Science, 63*. 1338-1345. https://doi.org/10.1016/j.icesjms.2006.04.021

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology, 8*(7), 1137-1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Codjoe, F. S., & Donkor, E. S. (2017). Carbapenem resistance: a review. *Medical Sciences*, *6* (1), pii, E1.

Clinical Laboratory Standard Institute (CLSI) (2010). Performance Standards for Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement Wayne: Clinical and Laboratory Standards Institute.

Commission of the European Communities (2005). Commission Staff Working Paper On The Implementation Of National Residue Monitoring Plans In The Member States In 2003 (Council Directive 96/23/Ec), Retrieved from http://www.uni-mannheim.de/edz/pdf/sek/2005/sek-2005-0025-en.pdf

Donkor, E. S., Newman, M. J., & Yeboah-Manu, D. (2012). Epidemiological aspects of non-human antibiotic usage and resistance: implications for the control of antibiotic resistance in Ghana. *Tropical Medicine and International Health, 17*(4), 462-468. https://doi.org/10.1111/j.1365-3156.2012.02955.x

Donkor, E. S., Newman, M. J., Tay, S. C. K., Dayie, N. T. K. D., Bannerman, E., & Olu-Taiwo, M. (2011) Investigation into the risk of exposure to antibiotic residues contaminating meat and egg in Ghana. *Food Control, 22*, 869-873. https://doi.org/10.1016/j.foodcont.2010.11.014

FAO (Food and Agriculture Organization of the United Nations), (2002). The State of World Fisheries and Aquaculture - 2002 (SOFIA). Retrieved from http://www.fao.org/docrep/005/y7300e/y7300e06.htm,

Heinitz, M. L., Ruble, R. D., Wagner, D. E., & Tatini, S. R. (2000). Incidence of Salmonella in fish and seafood. *Journal of Food Protection, 63*(5), 579-592. https://doi.org/10.4315/0362-028X-63.5.579

JECFA (2005). Joint FAO/WHO Expert Committee on Food Additives. Dietary exposure assessment of chemicals in food, Annapolis, Maryland, USA. Retrieved from http://apps.who.int/iris/bitstream/10665/44027/1/9789241597470_eng.pdf

Kim, K. R., Owens, G., Kwon, S. I., So, K. H., Lee, D. B., & Ok, Y. S. (2011). Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. *Water Air Soil Pollution, 214*, 163-174. http://dx.doi.org/10.1007/s11270-010-0412-2.

Koenen-Dierick, L., Okerman, L., De Zutter, L., Degroodt, J. M., Hoof, J. V., & Srebrnik, S. A. (1995). One-plate microbiological screening testing in kidney tissue and meat; an alternative to the EEC four plate method?. *Food Additives & Contaminants, 12*(1), 77-82. https://doi.org/10.1080/02652039509374281

L'Abée-Lund, M. T., & Sørum, H. (2001). Class 1 Integrons Mediate Antibiotic Resistance in the Fish Pathogen Aeromonas salmonicida Worldwide. *Microbial Drug Resistance, 7*(3), 263-272. https://doi.org/10.1089/10766290152652819

Labi, A. K., Obeng-Nkrumah, N., Addison, N., & Donkor, E. S. (2014). *Salmonella* blood stream infections in a tertiary care setting in Ghana. *BMC Infectious Diseases, 21*(14), 3857. https://doi.org/10.1186/s12879-014-0697-7

Lu, F. C., & Sam, K. (2002). Lu's basic toxicology: fundamentals, target organs and risk assessment. *Taylor & Francis, 364*. https://doi.org/10.1201/9781420055986

MacNeil, J. D. (2005). The Joint food and Agriculture Organization of the United Nations/World health

Organization Expert Committee on food additives and its role in the Evaluation of the safety of veterinary drug residues in foods. *The American Association of Pharmaceutical Scientist Journal, 7*(2), E274-E280. https://doi.org/10.1208/aapsj070228

Marcel, G., Sabri, M. Y., Siti-Zahrah, A., & Emikpe, B. O. (2013). Water condition and identification of potential pathogenic bacteria from red tilapia reared in cage-cultured system in two different water bodies in Malaysia. *African Journal of Microbiology Research, 7*(47), 5330-5337. https://doi.org/10.5897/AJMR12.1468

Marks, F., Adu-Sarkodie, Y., Hünger, F., Sarpong, N., Ekuban, S., Agyekum, A., Nkrumah, B., Schwarz, N. G., Favorov, M. O., Meyer, C. G., & May, J. (2010). Typhoid fever among children, Ghana. *Emerging Infectious Diseases journal, 16*(11), 1796-1797. https://doi.org/10.3201/eid1611.100388

Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: impacts on human health. *Clinical Microbiology Reviews, 24*(4), 718-33. https://doi.org/10.1128/CMR.00002-11

Mckenzie, A. I., & Hathway, S. C. (2006). The role and functionality of veterinary services in food safety throughout the food chain. *Scientific and Technical Review of the Office International des Epizooties (Paris), 25*(2), 837-848. https://doi.org/10.20506/rst.25.2.1697

Ndi, L. O., & Barton, M. D. (2012). Resistance determinants of Pseudomonas species from aquaculture in Australia. *Journal of Aquaculture Research and Development, 3*, 1-6. https://doi.org/10.4172/2155-9546.1000119

Newman, M. J., Frimpong, E., Donkor, E. S., Opintan, J. A, & Asamoah-Adu, A. (2011). Resistance to antimicrobial drugs in Ghana. *Infection and Drug Resistance, 4*, 215-220.

Olatoye, O. I., & Basiru, A. (2013). Antibiotic usage and oxytetracycline residues in African catfish (Clarias gariepinus in Ibadan Nigeria). *World journal of fish and marine sciences, 5*(3), 302-309.

Onyango, D. M., Wandili, S., Kakai, R., & Waindi, E. N. (2009) Isolation of *Salmonella* and *Shigella* from fish harvested from the Winam Gulf of Lake Victoria, Kenya. The *Journal of Infection in Developing Countries, 3*(2), 99-104.

Opintan, J., & Newman, M. J. (2007). Distribution of serogroups and serotypes of multiple drug resistant *Shigella* isolates. *Ghana Medical Journal, 41*(1), 4-8.

Pham, D. K., Chu, J., Do, N. T., Brose, F., Degand, G., Delahaut, P., De Pauw, E., Douny, C., Van Nguyen, K., Vu, T. D., Scippo, M. L., & Wertheim, H. F. (2015). Monitoring Antibiotic Use and Residue in Freshwater Aquaculture for Domestic Use in Vietnam. *Ecohealth, 12*(3), 480-489. https://doi.org/10.1007/s10393-014-1006-z

Plumb, J. A. (1999). Tilapia Bacterial Diseases. In: Health Maintenance and Principal Microbial Diseases Cultured Fishes, Plumb, J.A. (Ed.). Iowa State University Press, Ames, IA., USA. 297-305.

Population and Housing Census: Accra: Ghana Statistical Service. (2010). Retrieved from http://www.stasghana.gov.gh.

Raufu, I. A., Lawan, F. A., Bello, H. S., Musa, A. S., Ameh, J. A., & Ambali, A. G. (2014). Occurrence and antimicrobial susceptibility profilesof Salmonella serovars from fish in Maiduguri, sub-Saharah, Nigeria. *Egyptian Journal of Aquatic Research, 40*, 59-63. https://doi.org/10.1016/j.ejar.2014.01.003

Samanidou, V. F., & Evaggelopoulou, E. N. (2007). Analytical strategies to determine antibiotic residues in fish. *Journal of Separation Science, 30*(16), 2549-69. https://doi.org/10.1002/jssc.200700252

Sekyere, J. O. (2014). Antibiotic Types and Handling Practices in Disease Management among Pig Farms in Ashanti Region, Ghana. *Journal of Veterinary Medicine*, 1-8. http://dx.doi.org/10.1155/2014/531952.

Shears P. (1996). *Shigella* infections. *Annals of Tropical Medicine & Parasitology, 90*(2), 105-114. https://doi.org/10.1080/00034983.1996.11813034

Sorum, H. (2006). Antimicrobial drug resistance in fish pathogens. In Aarestrup F. M., editor. (ed.), *Antimicrobial resistance in bacteria of animal origin*. ASM Press, Washington, DC. 213-238.

Stier, R. F. (2004). Say goodbye to the weakest link. *Food Engineering, 76*(6), 20-21.

Sundlof, S. F. (1994). Human risks associated with drug residues in animal derived food. *Journal of Agromedicine, 1*, 5-22. https://doi.org/10.1300/J096v01n02_02

Tetteh-Quarcoo, P. B., Donkor, E. S., Attah, S. K., Duedu, K. O., Afutu, E., Boamah, I., Olu-Taiwo, M.,

Anim-Baidoo, I., & Ayeh-Kumi, P. F. (2013). Microbial carriage of cockroaches in a tertiary hospital in Ghana: public health implications. *Environmental Health Insights*, *7*, 59-66.

Thompson, C. N., Duy, P. T., & Bake, S. (2015) The Rising Dominance of Shigella sonnei: An Intercontinental Shift in the Etiology of Bacillary Dysentery. *PLOS Neglected Tropical Diseases, 9*(6), e0003708. https://doi.org/10.1371/journal.pntd.0003708.

Toroglu, S., & Korkmaz, H. (2005) Antibiotic resistance in Gram negative bacteria isolated from Aksu River in (Kahramanmaras) Turkey. *Annals of Microbiology, 55*(3), 229-233.

von Wintersdorff, C. J., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B., Savelkoul, P. H., & Wolffs, P. F. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Frontiers in Microbiology, 7*, 173. https://doi.org/10.3389/fmicb.2016.00173

Wassenaar, T. M. (2005). The use of antimicrobial agents in veterinary medicine and implications for human health. *Critical Reviews in Microbiology, 31*(3), 155-169. https://doi.org/10.1080/10408410591005110

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