

OPEN ACCESS



African Journal of **Food Science**

April 2020
ISSN 1996-0794
DOI: 10.5897/AJFS
www.academicjournals.org



**ACADEMIC
JOURNALS**
expand your knowledge

ABOUT AJFS

The African Journal of Food Science (AJFS) (ISSN 1996-0794) is published monthly (one volume per year) by Academic Journals.

African Journal of Food Science (AJFS) provides rapid publication of articles in all areas of Food Science such as Sensory analysis, Molecular gastronomy, Food safety, Food technology etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in AJFS are peer-reviewed.

Contact Us

Editorial Office: ajfs@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: <http://www.academicjournals.org/journal/AJFS>

Submit manuscript online <http://ms.academicjournals.me/>

Editors

Dr. Thaddeus Chukwuemeka Ezeji

Ohio State University and
Ohio State Agricultural and Development
Center (OARDC)
Department of Animal Sciences
USA.

Prof. Kofi E. Aidoo

Department of Biological and Biomedical
Sciences
Glasgow Caledonian University
Glasgow
Scotland.

Dr. Barakat S.M. Mahmoud

Food Safety/Microbiology
Experimental Seafood Processing Laboratory
Costal Research and Extension Centre
Mississippi State University
USA.

Dr. Neela Badrie

Department of Food Production,
Faculty of Science and Agriculture,
University of the West Indies,
Trinidad and Tobago.

Dr. Hu Xiao-Qing

State Key Lab of Food Science and Technology,
Jiangnan University,
China.

Dr. Dominic Agyei

Department of Food Science/Te Tari Pūtaiao Kai
University of Otago,
Dunedin,
New Zealand.

Dr. Fook Yee Chye

Faculty of Food Science and Nutrition,
Universiti Malaysia Sabah,
Malaysia.

Dr. Adel Shatta

Department of Food Technology,
Faculty of Agriculture,
Egypt.

Dr. Tendekayi Henry Gadaga

Department of Environmental Health Science
University of Swaziland
Swaziland.

Editorial Board Members

Dr. K. Pandima Devi

Department of Biotechnology
Alagappa University
Tamil Nadu
India.

Dr. Ashish Kumar Singh

Dairy Technology Division
National Dairy Research Institute,
Haryana,
India.

Prof. Rui Cruz

Department of Food Engineering
Institute of Engineering
University of Algarve, Faro
Portugal.

Table of Content

Fruit juices in polysaccharides edible films Hulda Noemi Mamani Chambi, Bianca Souza da Costa, Wiliene Camila de Lima, Daniel Consul Kassardjian and Flávio Luís Schmidt	53
Variability of nutrients in Parkia biglobosa kernels from three geographical regions in Burkina Faso Aimée W. D. B. Guissou, Charles Parkouda, Barbara Vinceti, Esther M. A. Traoré, Aboubacar S. Dao, Céline Termote, Mattia Manica and Aly Savadogo	63
Effect of the incorporation of graded levels of turmeric (<i>Curcuma longa</i>) on different qualities of stirred yoghurt Eze Chinazom Martina, Aremu Kehinde Oludayo, Nnamani Chidera Linda, Omeje Patience Chinasa, Omelagu Chizoba Ambrose and Okonkwo Thomas Muoneme	71

Full Length Research Paper

Fruit juices in polysaccharides edible films

Hulda Noemi Mamani Chambi, Bianca Souza da Costa, Wiliene Camila de Lima, Daniel Consul Kassardjian and Flávio Luís Schmidt*

Department of Food Technology, School of Food Engineering, State University of Campinas, UNICAMP, Campinas, SP 13083-862, Brazil.

Received 12 February, 2020; Accepted 2 April, 2020

In this study, jambolan and grape juices were used to produce polysaccharide-based edible films by the solvent-casting technique. The polysaccharides used were carboxymethyl cellulose, hydroxypropyl methyl cellulose, high-methoxyl pectin, low-methoxyl pectin, sodium alginate, and locust bean gum. The films exhibited good mechanical resistance and flexibility, with tensile strength (8 to 28 Mpa), elongation at break (6 to 36%), adhesion force (0.4 to 1.4 N), swelling index (1.0 to 2.3), and disintegration time (0.5 to 60 min) that varied as a function of the polysaccharide and the fruit juice used. The surface pH was, respectively, ~5.5 and ~4.6 for the films produced with grape and jambolan juices, regardless of the polysaccharide used. All films presented the typical color of the fruit juices, which was characterized by the L*, a* and b* parameters. The films produced with jambolan juice had the higher anthocyanin content (3.4 mg/g, d.b.) and antioxidant capacity (198 µMol Trolox equivalent/g, d.b.) when compared to those produced with grape juice (0.28 mg/g and 85 µMol Trolox equivalent/g, d.b.). The results are interesting for the food industry, specifically in edible or biodegradable films production, since alternative fruit juices can be used in food formulations and their natural compounds can replace synthetic additives.

Key words: Alternatives fruits, *Syzygium cumini* L., *Vitis vinifera* L., antioxidant, anthocyanin, natural colorants.

INTRODUCTION

Edible films can be produced from polysaccharides as cellulose or starch derivatives, pectin, pullulan and gelatin (Borges et al., 2015; Prajapati et al., 2018; Tedesco et al., 2017). Many synthetic additives can be used for the formulation of edible films, including plasticizers, colorants, stabilizers, salivary secretion stimulants, buffer systems, sweeteners, taste masking agents, and palatability enhancers (Borges et al., 2015; Silva et al., 2015). Fruit juices contain mainly sugars, organic acids,

phenolic compounds, vitamins and mineral elements, among others (Coelho et al., 2016; Gurak et al., 2010) and can be used in the formulation of edible films. Thus, glucose and fructose can contribute to the film sweetness and act as a matrix plasticizer, due to its low molecular weight (Olivas and Barbosa-Cánovas, 2008; Santacruz et al., 2015). Organic acids, besides contributing to the organoleptic characteristics of the films, can also act as salivary secretory stimulants. Phenolic compounds and

*Corresponding author. E-mail: schmidt@unicamp.br. Tel: +55 19 35214017.

Table 1. Physicochemical characteristics of jambolan and grape juices.

Characteristics*	Jambolan juice	Grape juice
pH	3.88±0.01	3.03±0.02
Acidity (g/g, d.b.)	0.051±0.001	0.034±0.001
Reducing sugars (g glucose/g, d.b.)	0.24±0.01	0.80±0.02
Anthocyanins (mg/g, d.b.)	5.48±0.02	0.40±0.01
Antioxidant activity (µMol TE/g, d.b.)	241.27±17.39	89.83±0.49

*Acidity expressed as grams of tartaric acid for grape juice, and grams of citric acid for jambolan juice. TE - Trolox Equivalent.

anthocyanins can confer an attractive color and astringency, and provide antioxidant properties to the films.

Jambolan (*Syzygium cumini* L.) is an Asian native fruit, found in Brazil as an ornamental tree (Tavares et al., 2016). Its high anthocyanins content imparts an intense and attractive purple color to the bark (Silva et al., 2018), as well as potential biological activities, including antioxidant capacity, anti-inflammatory properties, antibacterial, anti-ulcerogenic, cardioprotective, anticancer, anti-allergic properties, anti-diabetes effect, among others (Singh et al., 2018; Tavares et al., 2016). Grape juice is a product with high antioxidant potential, capable of combating the oxidative processes in the body (Mendes Lopes et al., 2016; Cosme et al., 2018). Studies have shown that its consumption can positively affect the risk factors associated with cardiovascular health, cancer, neurodegenerative diseases, and age-related cognitive decline (Blumberg et al., 2015; Vislocky and Fernandez, 2010; Wightman and Heuberger, 2015). Thus, the aim of this study was to use fruit juices (jambolan and grape) in the development of polysaccharides edible films.

MATERIALS AND METHODS

Polysaccharides

Sodium carboxymethylcellulose (CEKOL 150, CPKelco, Brazil); hydroxypropyl methylcellulose (Benecel K4M PHARM, Ashland, Brazil); high-methoxyl pectin (GENU Pectin D Slow Set Z, CPKelco, Brazil); low-methoxyl pectin (GENU Pectin LM-102-AS-Z, CPKelco, Brazil); sodium Alginate (CAS 9005-38-3, Dinâmica Química, Brazil); and locust bean gum (GENU-GUM RL 200 Z, CPKelco, Brazil) were used.

Fruit juice extraction

Jambolan juice in natura was extracted from ripened jambolan fruits collected at State University of Campinas, Campinas - SP, Brazil, 22° 81' 95" S, 47° 06' 49". The fruits were sanitized with sodium hypochlorite solution (50 mg/L; 15 min) with a subsequent water rinse. The fruits were pulped in a brush-type pulping machine (Sterling Power System Inc. Lionel Corporation), packed in polyethylene bags (500 g) and stored at -20°C. The thawed pulp was filtrated in a 270 mesh sieve and centrifuged (Dammom IEC Model HN-S, USA) at 1100 x g for 15 min to obtain a clear juice

(~10 °Brix).

Grape juice concentrate (68 °Brix) was purchased from Golden Sucos, Brazil. The fruit juices were characterized for pH, acidity, and reducing sugars according to the AOAC methods (AOAC, 1997a, b, 2000) in triplicate, and the results were expressed on a dry basis (d.b.) (Table 1). The anthocyanins content and antioxidant capacity of the juices were also determined (Brand-Williams et al., 1995; Lee et al., 2005) and the determinations are described in subsequently.

Film production

The films were produced using the solvent-casting technique that consists in the dispersion of a film-forming solution (solution casting) on a plate surface followed by the evaporation of the solvent. The film-forming solutions (FFS) were prepared to contain 2% (w/w) and 1.5% (w/w) soluble solids from jambolan and grape juices, respectively. These concentrations were established to form a structural matrix sufficiently cohesive so that the film can be easily removed from the support without breaking. Jambolan or grape juices were mixed with the polysaccharides solution was prepared according to Table 2, using a magnetic stirrer until complete homogenization (10 min). To improve the grape juice dispersion into the polysaccharide solution, the concentrate grape juice was diluted to 20 °Brix, while jambolan juice was kept at 10°Brix. The FFS were dispersed in polyester plates (13.7 cm in diameter) and dried in an oven with air circulation at 30°C/16 h. The films were conditioned at 23±0.5°C and 33% relative humidity for 5 days before characterization. After conditioning, the moisture contents were 9.1±0.8 and 8.1±0.5% for the films produced with grape and jambolan juices, respectively. This film production process was repeated three times for each formulation.

Film characterization

Mechanical properties

The mechanical properties were determined using a TA.xT2i (TA Instruments, New Castle, USA) texture analyzer, 25 kg cell loading, according to the ASTM (1995) method D882-95, and carried out at 23°C and 40 to 50% relative humidity (RH). A grip separation and crosshead speed of 50 mm and 1 mm/s, respectively, were applied to the films (25 mm wide and 10 cm long). Film thickness was determined from the mean of five measurements across the films using a digital micrometer (Mitutoyo, Japan) with a range of 0 to 12.7 mm and an accuracy of 0.001 mm. The mean values were used for calculation of tensile strength, Young's modulus, and elongation at rupture. The typical film thickness was 0.044±0.004 and 0.055±0.007 mm for the films prepared with grape and jambolan juices, respectively. Mechanical measurements were done in triplicate.

Table 2. Solubilization methods of polysaccharides.

Polysaccharides	Conc. (% w/w)	Solubilization methods
HMP, LMP, CMC SA	1 0.8	Slow dispersion in distilled water at room temperature until complete solubilization.
LBG	0.8	Rapid dispersion in distilled water at 5°C followed by heating to 70°C.
HPMC	1	Rapid dispersion in 1/3 of the total volume of distilled water at 90°C and addition of 2/3 of the volume of water at 5°C.

HMP - high-methoxyl pectin; LMP - low-methoxyl pectin; CMC - carboxymethylcellulose; SA - sodium alginate; LBG - locust bean gum; HPMC - hydroxypropyl methylcellulose.

**Figure 1.** Acrylic cell, 4.5 cm in diameter, 5 cm height.

Adhesion strength *in vitro*

The adhesion strength of the films was determined in a TA.xT2i texture analyzer (25 kg cell loading, TA Instruments, New Castle, USA) using gelatin gel as a buccal model (Okeke and Boateng, 2016) upon which the films were allowed to adhere. Gelatin (GELITA, 150 BLOM/30 MESH) was solubilized in water (10%, 90°C), placed in the Petri plate (5.2 cm in diameter and 1 cm height), cooled down, and stored under refrigerated storage overnight for gelling (5°C). The film (20 mm in diameter) was attached with double-sided adhesive tape to the Perspex support (20 mm), connected to the mobile arm of the texture analyzer. Artificial saliva (40 μ L) was added onto the gelatin surface, and the film and the gelatin gel were allowed to adhere. The adhesion strength was measured as the maximum applied force (N) to detach the film from the gelatin gel. The contact force, contact time, and the speed of probe withdrawal during the adhesion experiment was fixed at 1 N, 10 s, and 0.5 mm/s, respectively.

Swelling index

Films with 20 mm in diameter were placed on a Petri plate (9.5 cm in diameter) containing 30 mL of artificial saliva, and the changes were measured at different time intervals up to constant diameter.

The swelling index was measured in triplicate and calculated using Equation 1 (Nair et al., 2013), where A_t is the area of the film at time t , and A_0 is the area of the film at time zero.

$$\text{Swelling index} = A_t/A_0 \quad (1)$$

In vitro disintegration time

The film was fixed on an acrylic cell (Figure 1) and 200 μ L of artificial saliva was added onto the center of the support. The time the drop takes to disintegrate the film and reach the interior base of the cell was defined as the *in vitro* disintegration time (measured in triplicate).

Surface pH

The surface pH of the films was determined using artificial saliva, according to Prabhu et al. (2011) with modifications. The artificial saliva was prepared with phosphate buffer solution (pH = 7.1-7.2) and mucin from porcine stomach (SIGMA-ALDRICH, TYPE II) at 2 mg/mL (Sánchez et al., 2011). The film was placed inside a 5 mL flask (at the bottom and at the sides). The artificial saliva (~ 0.5 mL) was spread on the film surface. The electrode was placed in

Table 3. Swelling index, disintegration time, and surface pH of the films made with different polysaccharides and fruit juices.

Films	Swelling index	Disintegration time (minutes)	Surface pH
With grape juice			
CMC	1.55±0.04 ^b	0.5±0.03 ^c	5.7±0.1 ^{ab}
HPMC	1.78±0.05 ^a	3.2±0.1 ^d	5.5±0.2 ^{ab}
HMP	1.48±0.10 ^b	5.3±0.3 ^b	5.3±0.4 ^b
LMP	1.51±0.04 ^b	39.7±1.1 ^a	5.6±0.2 ^{ab}
AS	1.76±0.10 ^a	>60	6.0±0.1 ^a
LGB	1.11±0.01 ^c	>60	5.7±0.1 ^{ab}
With jambolan juice			
CMC	1.95±0.11 ^b	1.4±0.01 ^d	4.6±0.3 ^b
HPMC	1.64±0.02 ^c	5.1±0.1 ^c	4.6±0.1 ^b
HMP	1.48±0.14 ^c	1.2±0.1 ^d	4.6±0.1 ^b
LMP	2.25±0.05 ^a	12.9±0.2 ^b	5.2±0.1 ^a
AS	1.56±0.01 ^c	58.4±0.3 ^a	4.6±0.1 ^b
LGB	1.04±0.02 ^d	>60	5.1±0.1 ^a

Different lowercase letters in the same column and in the same block (Films with Grape juice or with Jambolan juice) indicate a significant difference ($p < 0.05$).

contact with the wetted film for 10 s for stabilization, and the pH was measured (in triplicate).

Color and opacity

Film color was evaluated by L^* , a^* , and b^* parameters, measured by transmittance using CIELab color scale. Film opacity was determined by reflectance, and calculated from the relationship between the opacity of the film over the black (Y_b) and white (Y_w) reflectance color standard. Determinations were carried out in triplicate, using a spectrophotometer UltraScan PRO D65 Hunterlab (Reston, USA) and the software EasyMatch QC. All determinations were made by placing the film surface in contact with the air in the direction of light. The color difference (ΔE) was calculated using Equation 2, where L_s^* , a_s^* , b_s^* are the color parameters of the locust bean gum (LBG) film (Table 3) made with grape juice or jambolan juice. The LBG film containing grape and jambolan juices showed high a^* and low b^* values, which allowed determining the differences (ΔE) between the samples.

$$\Delta E = [(L^* - L_s^*)^2 + (a^* - a_s^*)^2 + (b^* - b_s^*)^2]^{1/2} \quad (2)$$

Anthocyanins content

Anthocyanins content was determined by the differential method (Lee et al., 2005), in triplicate, in a dark environment at 20°C. The fruit juices were adjusted to 20 and 10 °Brix for grape and jambolan, respectively. While the films (0.3 g) made with or without fruit juices were dispersed in 30 g distilled water. When necessary, the samples were diluted with distilled water to obtain absorbance readings lower than 0.9. The samples were mixed with two buffer solutions separately: 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). The absorbance of the buffer solutions was measured at 520 and 700 nm. Anthocyanin content was expressed as cyanidin-3-glucoside equivalents according to Equation (3), and then converted to a dry basis:

$$\text{Anthocyanin content (mg/L)} = [A \times MW \times DF \times 10^3] / [\epsilon \times PL] \quad (3)$$

Where $A = [(A_{520nm} - A_{700nm})_{pH 1.0} - (A_{520nm} - A_{700nm})_{pH 4.5}]$; MW (molecular weight) = 449.2 $g \cdot mol^{-1}$, for cyaniding-3-glucoside (cyd-3-glu); DF = dilution factor; PL = path length, 1 cm; $\epsilon = 26,900$ molar extinction coefficient ($L \cdot mol^{-1} \cdot cm^{-1}$), for cyd-3-glu; and $10^3 =$ conversion factor from g to mg.

Antioxidant capacity

The antioxidant capacity was determined by DPPH assay (Brand-Williams et al., 1995), in triplicate, in a dark environment at 20°C. Films (0.3 g) were dispersed in 30 g distilled water, and the fruit juices were adjusted to 20 and 10 °Brix for grape and jambolan, respectively. When necessary, the samples were diluted with distilled water to obtain absorbance readings lower than 0.9. Sample or Trolox standard (0 – 2000 μM in ethanol) aliquots of 50 μL were mixed to 4.95 mL of 0.06 mM DPPH solution (in ethanol) in dark and kept for 16 h to achieve a constant concentration of remaining DPPH for grape juice concentrate. After the reaction, the absorbance was measured at 517 nm, and the results were expressed as Trolox equivalent antioxidant capacity.

Statistical analysis

The results were submitted to analysis of variance (ANOVA) and Tukey's comparison test to identify the differences at a 5% level of significance, using the software SAS 9.4.

RESULTS AND DISCUSSION

Mechanical properties

The edible films made with different polysaccharides with

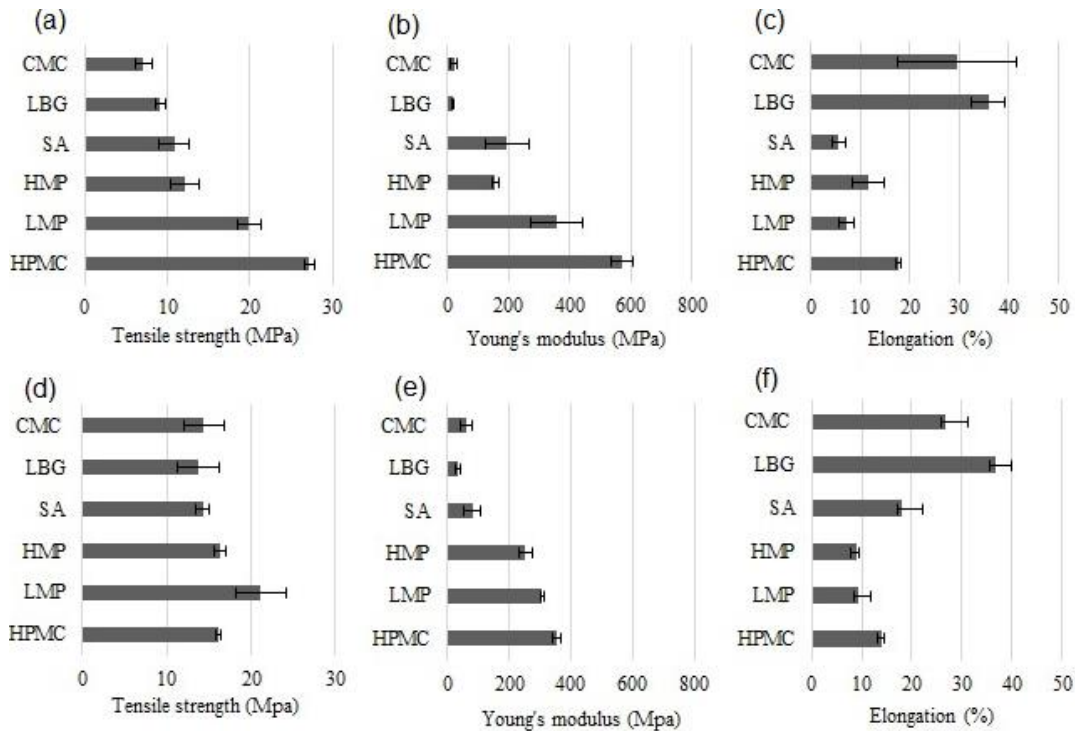


Figure 2. Mechanical properties of films based on polysaccharides and fruit juices: (a-c) grape, (d-f) jambolan.

the addition of the grape and jambolan juices were visually homogeneous and with no insoluble particles. In general, the mechanical properties values of the films varied from 7 to 27 Mpa (tension at rupture - TR), 20 to 570 MPa (Young's Modulus - MY), and from 6 to 36% (elongation at rupture - ER) (Figure 2). It is recommended that these films have sufficient tension to be handled without breaking during packaging or handling, but not so flexible to easily extend and deform during cutting in the production line (Borges et al., 2015). The present results were close to those reported for films made with hydroxypropylmethylcellulose or hydroxypropylcellulose with different medicinal plants extracts (TR = 0.2 - 2.5 Mpa; ER = 6 - 70%; MY = 23 - 321 Mpa, thickness = 69 - 192 μm) (Visser et al., 2016), and those reported for films made with high methoxyl pectin, glucomannan, methylcellulose and their blends (TR = 37 - 73 Mpa; ER = 2 - 10%; thickness = 19 - 28 μm) (Chambi and Grosso, 2011a).

Regardless of the type of juice used in the formulation, the films produced with SA, HMP, LMP, and HPMC were resistant to handling, without damages during the characterization process carried out at 23°C and 40 - 50% RH. Above 50% RH, the films were sticky, due to the presence of sugar from the fruits that are hygroscopic in high RH, which should be considered for future applications. Therefore, all characterizations were carried out at 40 to 50% RH (Figure 2).

Adhesion strength

The results of maximum adhesive strength of the films varied between 0.4 and 1.4 N (Figure 3), depending on the polysaccharide and the fruit juice used. The films made with jambolan juice had higher adhesion strength than those made with grape juice (Figure 4). The films made with jambolan juice had lower soluble solids when compared to the films made with grape juice. Moreover, the jambolan juice presented the lowest reducing sugar content (Table 1). The results are interesting from the technological point of view, once a smaller amount of jambolan juice was required to produce films with good resistance and flexibility, and high adhesive strength. Studies have shown that the higher the amount of additives the higher the adhesive strength (Perumal et al., 2008).

The films made with CMC and SA exhibited a greater adhesion to the buccal surface model, while a lower adhesion was observed for the films made with HPMC and LBG (Figure 4). During the contact process, the film adsorbs the artificial saliva on the buccal mucosa model and hydrates, initiating the interpenetration of polymer chains within the model buccal layer and *vice versa*. The adhesion effect is probably due to the high number of hydroxyl and carboxyl groups of polysaccharides, which improves the hydrogen bonding to the mucosa. Electrostatic interactions may also be formed with

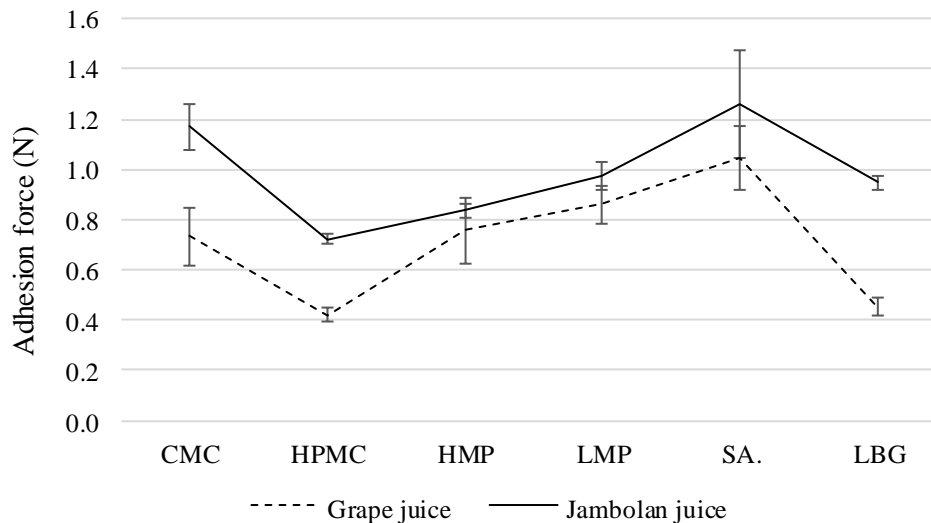


Figure 3. Adhesives properties of films based on polysaccharides and fruit juices: (a) grape, (b) jambolan.



Figure 4. Jambolan fruit and its high-methoxyl pectin film.

polysaccharides, loaded as CMC and SA. These polysaccharides facilitate strong interactions with the mucosa, resulting in high adhesive strength. Studied have shown that charged polymers increase the adhesion by leading to stronger bonds between the film and the buccal surface (Morales and McConville, 2011; Okeke and Boateng, 2016).

Swelling index

The swelling index of the films was dependent on the type of juice and the polysaccharide used in the formulation (Table 3). Films made from LMP and jambolan juice had the highest swelling index (2.3), while

the LBG films exhibited the lowest index (1.1) for both juices. These results were similar to those reported for films made with methylcellulose and hydroxypropylmethylcellulose blends, which presented a swelling index from 1 to 1.5 (Attama et al., 2008). A higher swelling index indicates a greater hydration capacity of the polymer film. The hydration capacity of the film is an important characteristic in the manufacture of films, once it is related to the adhesive strength and the ease of release of compounds naturally present in juices (Mahcene et al., 2020; Piñeros-Hernandez et al., 2017).

In general, the higher the swelling index, the greater the adhesive strength (Mortazavian et al., 2014). For the films produced with grape juice, the adhesive strength (Figure 3) was directly related to the swelling index

Table 4. Color parameters and opacity of the films made with polysaccharides and fruit juices.

Film	ΔE^*	L^*	a^*	b^*	Opacity
Grape juice					
CMC	3.8±0.5 ^b	81.1±1.1 ^b	7.2±0.5 ^b	5.8±0.4 ^a	21.2±1.5 ^a
HPMC	2.5±0.7 ^c	81.1±2.5 ^b	10.2±1.6 ^a	5.3±0.7 ^a	20.7±1.5 ^a
HMP	1.3±0.6 ^c	82.1±0.5 ^{ab}	9.9±0.4 ^a	5.2±0.2 ^a	21.6±2.0 ^a
LMP	5.9±0.4 ^a	84.8±0.4 ^a	6.1±0.2 ^b	5.3±0.2 ^a	17.9±1.7 ^a
SA	3.9±0.3 ^b	80.6±1.4 ^b	7.3±0.6 ^b	5.6±0.4 ^a	21.8±1.0 ^a
LBG	0	81.2±0.2 ^b	10.9±0.2 ^a	5.2±0.1 ^a	20.9±0.9 ^a
Jambolan juice					
CMC	33.3±1.2 ^a	75.4±0.7 ^a	17.0±0.9 ^d	-16.8±0.8 ^c	24.0±1.9 ^c
HPMC	35.4±0.5 ^a	72.0±3.4 ^{ab}	15.6±0.5 ^d	-9.8±0.4 ^a	26.0±3.1 ^c
HMP	17.0±2.0 ^c	70.7±1.3 ^{ab}	35.8±1.7 ^b	-15.2±0.5 ^b	25.9±1.9 ^c
LMP	21.3±1.9 ^b	69.5±1.4 ^b	29.2±1.3 ^c	-14.1±0.6 ^b	27.8±1.7 ^{bc}
SA	3.9±0.1 ^d	57.5±0.8 ^c	42.6±0.6 ^a	-24.7±0.3 ^d	41.2±3.7 ^a
LBG	0	59.8±1.5 ^c	45.5±1.4 ^a	-23.9±0.6 ^d	32.9±2.0 ^b

Different lowercase letters in the same column and in the same block (films + grape juice or films + jambolan juice) indicate a significant difference ($p < 0.05$).

(Table 4), with an exception for the film made with HPMC. This relationship was not observed for the films made with jambolan juice. Although sugars and organic acids from fruit juices act as additives in the film production, the minor components as anthocyanins (present in a higher proportion in the jambolan juice) also contribute with the different interactions in the polymeric matrix, resulting in films with different values of swelling index. Each polysaccharide has a particular structure with hydroxyl groups that in its turn could form hydrogen bonding with the anthocyanin hydroxyl groups. Starch-BBE (anthocyanin-rich bayberry extract) films had their properties modified (water vapor permeability, tensile strength, UV-vis light barrier, among others) due to the presence of anthocyanins in BBE (Yun et al., 2019).

Disintegration time

The disintegration time of the films changed according to the type of polysaccharide used (Table 3). Films made with CMC, HPMC and HMP had the lower disintegration times (0.5 to 5.3 min), while those made with SA and LBG took longer to disintegrate (60 min). Both fast and slow disintegrating films can have pharmaceutical applications. Due to the rapid disintegration time (30 s) of the CMC films made with grape juice, they can be easily administered in people with dysphagia, nausea, vomiting and mental disorders (Sudhakar et al., 2006). HPMC and HMP films can be used when the continuous release of the active ingredient is required within a few minutes. On the other hand, the SA and LBG films can be used as patches for applications requiring long periods of time,

which should be removed at the end of the application. The use of SA and LBG in the production of adhesive films has the advantage of preventing the use of organic solvents generally used in the solubilization of polymers for the manufacture of insoluble films.

Surface pH

The polysaccharide films containing both grape and jambolan juice presented a surface pH (Table 4) near the pH of saliva (5-7) (Sudhakar et al., 2006). Thus, the consumption of these films should not cause irritation to the oral mucosa. Oral films produced from NaCMC (matrix), glycerol (plasticizer) and nystatin (an antifungal agent) had similar results, pH = 5 to 5.4 (Gajdošová et al., 2016).

Color and opacity

All films presented the typical color of the fruit juice used in their formulations. Figure 4 illustrates the jambolan fruit, and the film resulting from the addition of juice to the film formulation. The films with jambolan juice exhibited a bright purple color, while those made with the addition of grape juice were purplish blue.

The color of the films was determined by the parameters L^* , a^* , b^* and ΔE^* (Table 4), which varied according to the polysaccharide and the fruit juice used in the formulation. The natural pigments present in the juices and the polysaccharides used in the formulation can provide great differences in color between the films,

Table 5. Anthocyanins content and antioxidant capacity of the films made with polysaccharides and fruit juices.

Film	CMC	HPMC	HMP	LMP	SA	LBG
Anthocyanins (mg/g, d.b.)						
Polysaccharide ¹ (P)	0.02±0.003 ^a	0.04±0.01 ^a	0.03±0.003 ^a	0.02±0.01 ^a	0.03±0.004 ^a	0.02±0.01 ^a
P + Grape juice ²	0.27±0.01 ^a	0.24±0.05 ^a	0.33±0.02 ^a	0.30±0.01 ^a	0.29±0.03 ^a	0.28±0.05 ^a
P + Jambolan juice ³	3.10±0.07 ^b	3.38±0.15 ^{ab}	3.70±0.01 ^a	3.41±0.08 ^{ab}	3.26±0.38 ^{ab}	3.28±0.01 ^{ab}
Antioxidant capacity (μMol TE/g, d.b.)						
Polysaccharide ¹ (P)	16.84±1.86 ^{abc}	16.96±0.79 ^{abc}	21.20±2.50 ^a	19.86±0.32 ^{ab}	16.34±1.15 ^{bc}	13.06±2.17 ^c
P + Grape juice ²	83.23±2.38 ^a	87.86±1.46 ^a	85.14±6.37 ^a	85.67±0.86 ^a	84.78±5.50 ^a	84.66±6.15 ^a
P + Jambolan juice ³	193.71±1.45 ^{bc}	187.40±2.68 ^c	206.35±1.51 ^a	207.43±2.67 ^a	193.46±1.07 ^{bc}	199.80±6.44 ^{ab}

Different lowercase letters in the same line and in the same block (anthocyanins or antioxidant) indicate a significant difference ($p < 0.05$); ¹blank; ²grape juice (anthocyanins=0.4±0.01; antioxidant=89.8±0.5); ³jambolan juice (anthocyanins=5.5±0.02; antioxidant=241.3±17.4).

resulting in differences in consumer perception. The films made with grape juice presented no significant variations in L^* and b^* , as a function of the polysaccharide, with small variations for a^* and ΔE^* . In contrast, the films made with jambolan juice presented the most significant variations in L^* , a^* , b^* and ΔE^* depending on the polysaccharide used.

Anthocyanins are a group of water-soluble flavonoids that are responsible for the bright red, blue and purple colors in fruits like jambolan (Jampani et al., 2014). The anthocyanin concentration in jambolan juices was higher when compared to the grape juice (Table 1), thus significant differences were observed for the films produced with jambolan juices, probably due to the anthocyanins stability in the film matrix. The anthocyanin stability is affected by several factors such as pH, temperature, light, presence of copigments, metal ions, oxygen, enzymes, ascorbic acid, sugars, and their degradation products (Fang et al., 2020). The surface pH values were similar for the films containing jambolan juices (Table 4), which affected the color to a lesser extent. All films were prepared under the same conditions, thus the parameters temperature and light did not affect the color of the films produced.

In nature, anthocyanin molecules are normally associated with colorless molecules (copigments) that affects the plant color (Falcão et al., 2003; Fan et al., 2019). The different interactions between anthocyanins (present in a higher proportion in the jambolan juice) and the polysaccharides (copigments) resulted in different color parameters and intensity of bright purple color. This type of copigmentation is known as being intermolecular (Lopes et al., 2007).

The film opacity was dependent on both the type of polysaccharide and the fruit juice. In general, the films were translucent, presenting low opacity, with values around 20 and 25 for the films containing grape juice and jambolan juice, respectively. Only the LBG and SA films had high opacity values (33 and 41, respectively), which resulted in an unattractive color for the films made with jambolan juice. Different values of film transparency are

related to their internal structure that is defined by a component rearrangement in the film matrix during the drying process (Chambi and Grosso, 2011b).

Anthocyanins content and antioxidant capacity

The total monomeric anthocyanins content and the antioxidant capacity of the films were similar to the values found for the fruit juices used in the formulations (Table 5). These results indicate that the manufacturing process did not lead to significant losses of the functional properties. The anthocyanins content and the antioxidant capacity of the films made with jambolan juice (Table 5) were higher than those observed for the films made with grape juice (Table 1), due to the higher anthocyanins levels of jambolan juice. The antioxidant capacity of the films produced only with polysaccharides was related to their ability to remove free radicals such as DPPH (Wang et al., 2018) acting as antioxidants to protect living organism from oxidative damage (Wang et al., 2016a). This antioxidant ability will vary depending on the type of polysaccharide (Wang et al., 2018). Polysaccharides such as pectins from grapefruit peel, apple pomace and citrus peel presented antioxidant capacity that would be related to the hydroxyl groups presents in the pectin structure (Wang et al., 2016b, 2014). The anthocyanin concentration was similar for all films prepared with the same fruit juice, with a mean anthocyanin content of 3.4 ± 0.2 for the films containing jambolan juice (Table 5). Therefore, it was possible to produce films with different color intensities with similar anthocyanin concentrations (Table 4). The films showed antioxidant capacity, which together with the attractive color make the fruit juices potential ingredients for the production of edible films.

Conclusion

In the polysaccharide based edible films, glucose, fructose, organic acids, phenolics, and anthocyanins from

fruit juices acted as synthetic additives replacer as well as active ingredients due to their antioxidant potential. The properties of the films were mainly modulated by the different polysaccharides used, and allow several applications, including those that require a rapid disintegration (CMC and HPMC, 0.5 and 3.2 min, respectively) and long application (SA and LGB, 58.4 and > 60 min, respectively) of the active ingredient. The films presented an attractive color, and those made with jambolan juice stood out among the others. The results are useful for the food and pharmaceutical industry since alternative fruit juices can be used in food formulations or as drug delivery matrices in the oral cavity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors extends their appreciation to coordination for the Improvement of Higher Education Personnel (CAPES) for the Postdoctoral Fellowship of the first author and to the State University of Campinas for the Undergraduate Research Fellowship of the third and fourth authors as well as for the installation and equipment used.

REFERENCES

- AOAC (1997a). Method 981.12: pH of Acidified Foods. Washington, USA: Association of Official Analytical Chemist.
- AOAC (1997b). Method 942.15: Acidity (Titratable) of Fruit Products. Washington, USA: Association of Official Analytical Chemist.
- AOAC (2000). Method 923.09: Invert Sugar in Sugars and Syrups. Lane-Eynon General Volumetric Method, Washington, USA: Association of Official Analytical Chemist.
- ASTM (1995). D882-95a. Standard Test Method for Tensile Properties of Thin Plastic Sheeting. New York, United States.
- Attama AA, Akpa PA, Onugwu LE, Igwilo G (2008). Novel buccoadhesive delivery system of hydrochlorothiazide formulated with ethyl cellulose- hydroxypropyl methylcellulose interpolymer complex. *Scientific Research and Essay* 3(6):343-347.
- Blumberg JB, Vita JA, Oliver Chen CY (2015). Concord grape juice polyphenols and cardiovascular risk factors: Dose-response relationships. *Nutrients* 7(12):10032-10052.
- Borges AF, Siva C, Coelho JFJ, Simões S (2015). Oral films: Current status and future perspectives I — Galenical development and quality attributes. *Journal of Controlled Release* 206:1-19.
- Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology* 28(1):25-30.
- Chambi HNM, Grosso CRF (2011a). Mechanical and water vapor permeability properties of biodegradable films based on methylcellulose, glucomannan, pectin and gelatin. *Food Science and Technology* 31(3):739-746.
- Chambi HNM, Grosso CRF (2011b). Effect of surfactants on the functional properties of gelatin-polysaccharide-based films. *European Food Research and Technology* 232:63-69.
- Coelho EM, de Azevêdo LC, Corrêa LC, Bordignon-Luiz MT, Lima MDS (2016). Phenolic Profile, Organic Acids and Antioxidant Activity of Frozen Pulp and Juice of the Jambolan (*Syzygium Cumini*). *Journal of Food Biochemistry* 40(2):211-219.
- Cosme F, Pinto T, Vilela A (2018). Phenolic compounds and antioxidant activity in grape juices: A chemical and sensory view (Review). *Beverages* 4:1-14
- Falcão LD, Barros DM, Gauche C, Luiz MTB (2003). Copigmentação entre e intermolecular de antocianinas: Uma revisão. *Boletim Centro de Pesquisa de Processamento de Alimentos* 21:351-366.
- Fan L, Wang Y, Xie P, Zhang L, Li Y, Zhou J (2019). Copigmentation effects of phenolics on color enhancement and stability of blackberry wine residue anthocyanins: Chromaticity, kinetics and structural simulation. *Food Chemistry* 275:299-308.
- Fang J, Luo Y, Yuan K, Guo Y, Jin S (2020). Preparation and evaluation of an encapsulated anthocyanin complex for enhancing the stability of anthocyanin. *LWT - Food Science and Technology* 117:108543.
- Gajdošová M, Vetchý D, Doležel P, Gajdziok J, Landová H, Muselík J, Jekl V (2016). Evaluation of mucoadhesive oral films containing nystatin. *Journal of Applied Biomedicine* 14(4):247-256.
- Gurak PD, Cabral LMC, Rocha-Leão MHM, Matta VM, Freitas SP (2010). Quality evaluation of grape juice concentrated by reverse osmosis. *Journal of Food Engineering* 96(3):421-426.
- Jampani C, Naik A, Raghavarao KSMS (2014). Purification of anthocyanins from jamun (*Syzygium cumini* L.) employing adsorption. *Separation and Purification Technology* 125:170-178.
- Lee J, Durst RW, Wrolstad RE (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International* 88(5):1269-1278.
- Lopes TJ, Xavier MF, Gabriela M, Quadri N, Quadri MB (2007). Antocianinas: Uma breve revisão das características estruturais e da estabilidade. *Revista Brasileira de Agrociência* 13:291-297.
- Mahcene Z, Khelil A, Hasni S, Kubra P, Bozkurt F, Birech K, Tornuk F (2020). Development and characterization of sodium alginate based active edible films incorporated with essential oils of some medicinal plants. *International Journal of Biological Macromolecules* 145:124-132.
- Mendes Lopes ML, Miguel MAL, Fialho E, Valente-Mesquita VL (2016). Grape juice obtained using steam extraction and other small-scale extraction methods: phenolic content, antioxidant capacity and stability during storage. *International Journal of Food Science and Technology* 51(7):1696-1702.
- Morales JO, McConville JT (2011). Manufacture and characterization of mucoadhesive buccal films. *European Journal of Pharmaceutics and Biopharmaceutics* 77(2):187-199.
- Mortazavian E, Dorkoosh FA, Rafiee-Tehrani M (2014). Design, characterization and ex vivo evaluation of chitosan film integrating of insulin nanoparticles composed of thiolated chitosan derivative for buccal delivery of insulin. *Drug Development and Industrial Pharmacy* 40(5):691-8.
- Nair AB, Kumria R, Harsha S, Attimarad M, Al-Dhubiab BE, Alhaider IA (2013). *In vitro* techniques to evaluate buccal films. *Journal of Controlled Release* 166(1):10-21.
- Okeke OC, Boateng JS (2016). Composite HPMC and sodium alginate based buccal formulations for nicotine replacement therapy. *International Journal of Biological Macromolecules* 91:31-44.
- Olivas GI, Barbosa-Cánovas GV (2008). Alginate-calcium films: Water vapor permeability and mechanical properties as affected by plasticizer and relative humidity. *LWT - Food Science and Technology* 41(2):359-366.
- Perumal VA, Lutchman D, Mackraj I, Govender T (2008). Formulation of monolayered films with drug and polymers of opposing solubilities. *International Journal of Pharmaceutics* 358(1-2):184-191.
- Piñeros-Hernandez D, Medina-Jaramillo C, López-Cordoba A, Goyanes S (2017). Edible cassava starch films carrying rosemary antioxidant extracts for potential use as active food packaging. *Food Hydrocolloids* 63:488-495.
- Prabhu P, Malli R, Koland M, Vijaynarayana K, D'Souza U, Harish N, Charyulu R (2011). Formulation and evaluation of fast dissolving films of levocitrizine di hydrochloride. *International Journal of Pharmaceutical Investigation* 1(2):99-104.
- Prajapati VD, Chaudhari AM, Gandhi AK, Maheriya P (2018). Pullulan based oral thin film formulation of zolmitriptan: Development and

- optimization using factorial design. *International Journal of Biological Macromolecules* 107:2075-2085.
- Sánchez GA, Miozza V, Delgado A, Busch L (2011). Determination of salivary levels of mucin and amylase in chronic periodontitis patients. *Journal of Periodontal Research* 46(2):221-227.
- Santacruz S, Rivadeneira C, Castro M (2015). Edible films based on starch and chitosan. Effect of starch source and concentration, plasticizer, surfactant's hydrophobic tail and mechanical treatment. *Food Hydrocolloids* 49:89-94.
- Silva BMA, Borges AF, Silva C, Coelho JFJ, Simões S (2015). Mucoadhesive oral films: The potential for unmet needs. *International Journal of Pharmaceutics* 494(1):537-551.
- Silva WP, Nunes JS, Gomes JP, Silva CMDPS (2018). Obtaining anthocyanin from jambolan fruit: kinetics, extraction rate, and prediction of process time for different agitation frequencies. *Food Science and Nutrition* 6:1664-1669.
- Singh B, Singh JP, Kaur A, Singh N (2018). Insights into the phenolic compounds present in jambolan (*Syzygium Cumini*) along with their health-promoting effects. *International Journal of Food Science and Technology* 53:2431-2447.
- Sudhakar Y, Kuotsu K, Bandyopadhyay AK (2006). Buccal bioadhesive drug delivery - A promising option for orally less efficient drugs. *Journal of Controlled Release* 114(1):15-40.
- Tavares IMDC, Lago-Vanzela ES, Rebello LPG, Ramos AM, Gómez-Alonso S, García-Romero E, Hermosín-Gutiérrez I (2016). Comprehensive study of the phenolic composition of the edible parts of jambolan fruit (*Syzygium cumini* L. Skeels). *Food Research International* 82:1-13.
- Tedesco MP, Monaco-lourenço CA, Carvalho RA (2017). Characterization of oral disintegrating film of peanut skin extract — Potential route for buccal delivery of phenolic compounds. *International Journal of Biological Macromolecules* 97:418-425.
- Vislocky LM, Fernandez ML (2010). Biomedical effects of grape products. *Nutrition Reviews* 68(11):656-670.
- Visser JC, Eugresya G, Hinrichs WLJ, Tjandrawinata RR, Avanti C, Frijlink HW, Woerdenbag HJ (2016). Development of orodispersible films with selected Indonesian medicinal plant extracts. *Journal of Herbal Medicine* 7:37-46.
- Wang X, Chen Q, Lü X (2014). Pectin extracted from apple pomace and citrus peel by subcritical water. *Food Hydrocolloids* 38:129-137.
- Wang J, Hu S, Nie S, Yu Q, Xie M (2016a). Reviews on mechanism of *in vitro* antioxidant activity of polysaccharides. *Oxidative Medicine and Cellular Longevity* 2016:1-13.
- Wang W, Ma X, Jiang P, Hu L, Zhi Z, Chen J, Liu D (2016b). Characterization of pectin from grapefruit peel: A comparison of ultrasound-assisted and conventional heating extractions. *Food Hydrocolloids* 61:730-739.
- Wang JM, Sun XY, Ouyang JM (2018). Structural characterization, antioxidant activity, and biomedical application of *Astragalus* polysaccharide degradation products. *International Journal of Polymer Science* 2018:1-13.
- Wightman JD, Heuberger RA (2015). Effect of grape and other berries on cardiovascular health. *Journal of the Science of Food and Agriculture* 95(8):1584-1597.
- Yun D, Cai H, Liu Y, Xiao L, Song J, Liu J (2019). Development of active and intelligent films based on cassava starch and chinese bayberry (*Myrica rubra* Sieb. Et Zucc.) anthocyanins. *Royal Society of Chemistry* 9:30905-30916.

Full Length Research Paper

Variability of nutrients in *Parkia biglobosa* kernels from three geographical regions in Burkina Faso

Aimée W. D. B. Guissou^{1,2}, Charles Parkouda^{1*}, Barbara Vinceti³, Esther M. A. Traoré¹,
Aboubacar S. Dao¹, Céline Termote⁴, Mattia Manica⁵ and Aly Savadogo²

¹Département Technologie Alimentaire, Institut de Recherche en Sciences Appliquées et Technologies, CNRST, Ouagadougou, Burkina Faso.

²Laboratoire de Biochimie et d'Immunologie Appliquée, Département de Biochimie-Microbiologie, Université Joseph Ki-Zerbo, Ouagadougou, Burkina Faso.

³Bioversity International, Rome, Italy.

⁴Bioversity International, Nairobi, Kenya.

⁵Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy.

Received 15 February, 2020; Accepted 18 March, 2020

This study aimed to determine the nutritional composition of the kernels of the African locust bean, *Parkia biglobosa*, collected in three populations (Louda, Nobéré and Péni) representative of different environmental conditions, from more arid in the northern population (Louda) to wetter in the most southern population (Péni). Biochemical analyses were carried out using standard methods. Results expressed on a dry basis showed that glutamic acid was the amino acid with highest values in kernels (2.72 - 6.44%). Methionine was the amino acid with lowest values (0.11 - 0.33%). The kernels had an interesting amount of minerals, Fe (4.15 - 18.48 mg), K (662.55 - 2801.69 mg), Mg (178.19 - 1624.83 mg), Zn (2.58 - 4.86 mg) and Ca (316.13 - 1731.41 mg). Moisture content varied from 4.49 to 7.56%, carbohydrates from 10.85 to 19.81%, proteins from 30.32 to 43.91%, ashes and lipids respectively from 4.19 to 5.80% and from 21.64 to 30.77%. Samples from Louda contained the highest mean values for proximate composition. Samples from Nobéré contained higher amounts of mineral and amino acids compared to the other two populations. The most important variation between samples of the same location was observed in the mineral composition (CV% > 20% except Zn); Louda was the location with highest dispersion of values. Samples from Péni in the South-Soudanian zone showed overall lower nutritional value. The nutritional composition of *P. biglobosa* kernels varied significantly according to the location where seeds were collected.

Key words: *Parkia biglobosa*, kernels, nutrients, variability, Burkina Faso.

INTRODUCTION

In most of the African countries wild fruit trees constitute an important part of the diet and play an important role in

*Corresponding author. E-mail: cparkouda@gmail.com. Tel: +226 70308930.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

income generation. *Parkia biglobosa*, commonly known as African locust bean tree or néré, is one of these species. Like in the case of other plants, for example melon (*Citrullus vulgaris*), castor (*Ricinus* spp.) and soybean (*Glycine max*), its seeds are fermented to produce condiments with high content of proteins (Omafuvbe et al., 2004). 'Soumbala' is the most commonly known natural plant-derived, food condiment used in the savannah regions of West and Central Africa (Adeyeye, 2006). Initially consumed in some West African countries, such as Burkina Faso, Ghana, and Benin, 'Soumbala' became increasingly popular due to its high nutritional value and other medicinal properties, such as the lowering of high blood pressure (Pelig-Ba, 2009). 'Soumbala' is a tasty condiment, a flavor intensifier obtained by traditional alkaline fermentation of *Parkia biglobosa* seeds. The preparation takes place in steps including first boiling, followed by dehulling, a second boiling, fermentation, air drying and molding (Wang and Fung, 1996; Ouoba et al., 2005). The introduction of mechanical dehullers has made the laborious process of dehulling, traditionally done manually, more producer-friendly. With the use of a mechanical device, *P. biglobosa* seeds are dehulled before boiling. 'Soumbala' producers have preferences for raw material coming from specific locations because of the higher quality. The location of the source thus seems to have an influence on the quality and possibly also on the nutritional composition of the seeds (Olujobi, 2012).

Several studies have reported on the high nutritional quality of *P. biglobosa* seeds and kernels with a proximate composition ranging from 21 to 33% for protein, 15 to 22.5% for fat, 3.5 to 5% for ashes and 35 to 49% for carbohydrates (Adeyeye, 2006; Esenwah and Ikenebomeh, 2008; Odebunmi et al., 2010; Elemo et al., 2011; Koura et al., 2014; Nyadanu et al., 2016). Comparing data between studies is not recommended given the different sampling, processing (seeds analyzed with or without hull, boiled or not, etc) and analytical methods applied in different studies. However, comparative studies examining the variation in nutritional composition of *P. biglobosa* kernels from different locations are rare. One of the very few examples is a research conducted by Olujobi (2012).

The objective of this study was to assess the range of variation in nutrient content of the kernels across three different populations (Louda in the South-sahelian zone, Nobéré in the North-Soudanian zone, Péni in the South-soudanian zone) and to assess if differences between tree populations were significant.

MATERIALS AND METHODS

Sampling

The *P. biglobosa* pods were collected from three geographical areas in Burkina Faso, selected across the distribution range of the tree species, along a rainfall gradient: Louda (12°58'54,64"-

1°4'56,59") in the South-Sahelian zone (400-600 mm/year), Nobéré (11°32'31,17"-1°12'35,57") in the North-Soudanian zone (900-1100 mm/year) and Péni (10°57'22,97"-4°32'21,76") in the South-Soudanian zone (\geq 1100 mm/year). The three locations have different soil characteristics: Ferruginous soil in Louda (L), eutrophic brown soil in Nobéré (N) and ferralitic soil in Péni (P). Each selected population had to have a minimum of 50 adult trees, all in the reproductive stage, thus able to produce fruits. Twenty to twenty-five healthy trees were randomly identified in each location and mature fruits collected for the characterization. On each tree, a quantity of 6-8 kg of dry pods was collected across the entire crown surface, labeled and bagged in plastic (each marked with site name and tree number) and sent to the laboratory. Sampling took place from April to May 2017.

Sample treatment

The fruit exocarp was separated manually; the pulp was separated from the seeds by pounding the seeds embedded in the pulp using a porcelain mortar. The seeds were washed, drained off, sun-dried for 72 h and dehulled using a mechanical dehuller (prototype CNRST/IRSAT, Ouagadougou, Burkina Faso, 1997) with wheels made of stainless steel. Kernels were powdered and a quantity of 200-300 g was packaged in plastic boxes and conserved at 4°C for analyses.

Biochemical analysis

The amino acids profile was determined with the PICOTAG method using High Performance Liquid Chromatography (Kristofferson, 2011). Minerals (Fe, Zn, Ca, K, Mg) were determined according to AOAC 975.03 (2005) using the Atomic Absorption Spectrophotometric Method (Thermo Scientific AA). Moisture, ash, proteins and fat content were determined according to international standard methods (ISO 20483 2013; ISO 659 2009; ISO 712 2009; ISO 2171 2007); carbohydrate was quantified according to Montreuil and Spik (1969).

Statistical analysis

All analyses were conducted in triplicate. Data were processed to derive descriptive statistic values (e.g., means, coefficient of variation and relative standard deviation). In addition, an analysis of variance (ANOVA) followed by Tukey test was carried out to determine statistical differences between populations with a confidence interval of 95%, using the XLSTAT software, version 2015.4.01. 22368. Finally, to visualise the spread of values for all tree sampled with regard to macronutrients as well as mineral content and essential amino acids, a Principal Component Analyses (PCA) was performed using the FactoMinR package with the RStudio software, version 1.1.463.

RESULTS

The amino acid profiles in *P. biglobosa* kernels from the three populations studied are presented in Table 1. Glutamic acid was found in highest concentrations, varying from 2.72 (for a tree in Louda) to 6.44% (for a tree in Nobéré). Methionine and cysteine were observed in low concentrations, respectively from 0.11 (for a tree in Louda and Péni) to 0.33% (for a tree in Louda and

Table 1. Amino acids profile of *Parkia biglobosa* kernels (g/100 g DM).

Site	Variable	asp	Glu	ser	gly	his	arg	thr	ala	pro	tyr	val	met	cys	ile	leu	phe	lys
Louda (n= 25) ^a	Minimum	1.17	2.72	0.73	0.69	0.43	0.91	0.39	0.73	0.78	0.32	0.53	0.11	0.37	0.43	1.01	0.59	1.23
	Maximum	3.02	6.02	1.64	1.53	0.93	1.91	0.88	1.48	1.70	0.60	1.20	0.33	1.44	0.93	2.35	1.26	3.17
	Mean	2.29 ^b	4.68 ^{**}	1.25 ^{**}	1.21 ^{**}	0.73 ^{**}	1.52 ^{**}	0.66 ^{**}	1.18 ^{**}	1.31 ^{**}	0.48 ^{**}	0.96 ^{**}	0.24 [*]	0.83 [*]	0.72 ^{**}	1.83 ^{**}	0.96 ^{**}	2.30 ^{**}
	CV%	18.66	16.16	15.57	15.27	16.13	15.64	15.17	14.37	15.87	17.49	15.50	18.98	34.64	15.06	16.19	15.33	17.52
Nobéré (n= 20)	Minimum	1.29	4.60	1.27	1.25	0.72	1.44	0.73	1.28	1.28	0.49	1.04	0.17	0.50	0.78	1.97	1.05	2.05
	Maximum	3.36	6.44	1.63	1.54	0.95	2.07	0.95	1.55	1.99	0.84	1.35	0.33	1.26	1.03	2.42	1.40	2.72
	Mean	2.43 [*]	5.57 [*]	1.43 [*]	1.38 [*]	0.83 [*]	1.74 [*]	0.84 [*]	1.41 [*]	1.60 [*]	0.63 [*]	1.19 [*]	0.25 [*]	0.76 [*]	0.90 [*]	2.22 [*]	1.23 [*]	2.47 [*]
	CV%	26.29	9.54	7.50	6.89	9.05	9.70	6.47	6.48	10.28	15.07	7.97	15.41	26.50	8.25	6.85	8.24	8.17
Péni (n= 20)	Minimum	1.50	3.11	0.98	0.80	0.49	1.13	0.48	0.98	0.90	0.38	0.70	0.11	0.21	0.54	1.34	0.81	1.41
	Maximum	2.64	5.50	1.48	1.30	0.77	1.76	0.73	1.54	1.48	0.61	1.10	0.23	0.83	0.85	2.09	1.18	2.20
	Mean	1.99 ^{**}	4.18 ^{***}	1.19 ^{**}	1.00 ^{***}	0.62 ^{***}	1.39 ^{***}	0.59 ^{***}	1.22 ^{**}	1.12 ^{**}	0.49 ^{**}	0.89 ^{***}	0.20 ^{**}	0.55 ^{**}	0.68 ^{**}	1.67 ^{***}	0.97 ^{**}	1.81 ^{***}
	CV%	14.06	15.62	12.29	14.70	11.68	13.16	12.70	12.56	15.73	13.44	12.84	15.63	28.52	12.54	13.86	11.73	12.71
All locations (n= 65)	Minimum	1.17	2.72	0.73	0.69	0.43	0.91	0.39	0.73	0.78	0.32	0.53	0.11	0.21	0.43	1.01	0.59	1.23
	Maximum	3.36	6.44	1.64	1.54	0.95	2.07	0.95	1.55	1.99	0.84	1.35	0.33	1.44	1.03	2.42	1.40	3.17
	Mean	2.24	4.80	1.29	1.20	0.73	1.55	0.69	1.26	1.34	0.53	1.01	0.23	0.72	0.76	1.90	1.05	2.20
	SD	0.62	0.89	0.20	0.22	0.13	0.26	0.14	0.19	0.28	0.12	0.19	0.05	0.26	0.14	0.34	0.18	0.43
	P-value	0.00	<10-3	<10-3	<10-3	<10-3	<10-3	<10-3	<10-3	<10-3	<10-3	<10-3	0.00	<10-3	<10-3	<10-3	<10-3	<10-3

^aNumber of trees sampled in each population. ^b values with different number of superscript stars in the same column are significantly different (P-value ≤0.05).

Nobéré) and from 0.21 (for a tree in Péni) to 1.44% (for a tree in Louda). The contents in valine and phenylalanine were equal in various samples from Nobéré and Louda.

The PCA analysis on the content of essential amino acids in *P. biglobosa* kernels, resulted in two main axes explaining 94% of the total variation; 85% was associated with axis 1 and 9% with axis 2. The PCA biplot (Figure 1) displays the variation among the three populations. Samples from Nobéré presented the highest average amounts in amino acids, while samples from Péni showed the lowest average. A significant difference was observed between the two locations for all amino acids. Péni presented the

lowest and Louda the highest statistically significant dispersion around the mean amino acid values.

The values of the mineral composition of *P. biglobosa* kernels are presented in Table 2. They are expressed in mg/100 g of dry matter of kernels. The iron (Fe) content of the samples varied from 4.15 mg/100 g (for a tree in Louda) to 18.48 mg/100 g (for a tree in Nobéré); potassium (K) content from 662.55 mg/100 g (for a tree in Péni) to 2801.69 mg/100 g (for a tree in Louda); magnesium (Mg) content from 178.19 mg/100 g (for a tree in Péni) to 1624.83 mg/100 g (for a tree in Nobéré); zinc (Zn) content varied from 2.58 mg/100 g (for a tree in Louda and Péni) to 4.86

mg/100 g (for a tree in Nobéré) and calcium (Ca) from 316.13 mg/100 g (for a tree in Péni) to 1731.41 mg/100 g (for a tree in Louda). By comparing values among the three locations, significant differences were observed for potassium and calcium (P-value < 0.0001). For iron there was no significant difference among the three locations (P-value = 0.38). For magnesium and zinc no differences were found between Louda and Péni.

The PCA analysis for minerals (Fe, K, Mg, Zn, Ca) of *P. biglobosa* kernels (Figure 2) shows how the individual trees in each site were plotted against the two main axes, which explained 86% of the total variation, with 58.2% attributed to axis

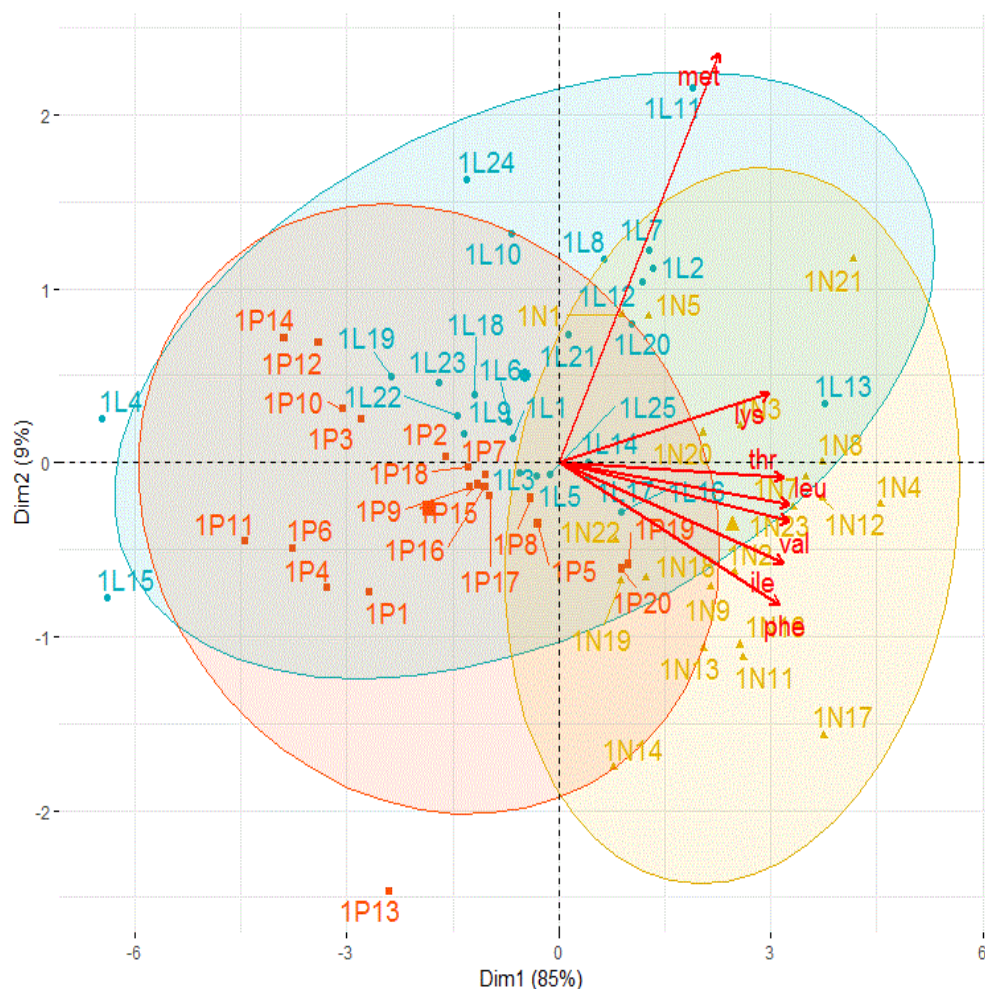


Figure 1. PCA biplot of the essential amino acids of *Parkia biglobosa* kernels. The different circles represent the three populations studied. Vectors show the relative weight of the variables examined (met, lys, thr, leu, ile, val, phe), which determines the spread of points (individual trees) on the biplot. The code associated to each point is the unique identifier of each individual tree.

1 and 27.8% to axis 2. Trees from Péné showed the lowest mineral content and samples from Nobéré the highest mean values; the highest dispersion around the mean in the value of different minerals was observed in Louda.

Mean values of proximate composition of *P. biglobosa* kernels across the three locations were obtained on a dry matter basis (Table 3). Over all samples, moisture varied from 4.49 to 7.56%; the tree with highest moisture content was observed in Louda and the one with lowest moisture content in Nobéré. The kernel samples had a content of carbohydrate, ashes and lipids varying from 10.85 to 19.81, from 4.18 to 5.85% and from 21.28 to 30.94% respectively, with the highest contents observed in Louda and the lowest in Péné; the sample with highest protein content was observed in Nobéré (43.91%) and the sample with lowest protein content in Péné (30.32%). Comparing the three locations, Péné had the highest

average moisture (6.78%) and fat (25.83%) content. Louda presented the highest average protein (39.60%), ashes (5.02%) and carbohydrate (17.13%) content. The comparison among sites revealed statistical differences ($P < 0.05$) for all nutrients except lipids (P -value of 0.25). More precisely, differences were significant for moisture, carbohydrates and ashes (P -value < 0.0001) and for proteins (P -value < 0.001). For what concerns the protein content, differences between Péné and Nobéré were not significant statistically. The PCA analysis for the proximate composition of *P. biglobosa* kernels (Figure 3) showed a spread of individual trees along the main axes, which explained 71.9% of the total variation, with 37.5% of variation associated to axis 1 and 34.4% to axis 2. The dispersion along axis 1 was mainly related to protein and lipid content, while the dispersal along axis 2 was mainly linked to variation in humidity, ash and carbohydrates. Louda and Nobéré seemed to be more similar between

Table 2. Mineral composition of *Parkia biglobosa* kernels (mg/100 g DM).

Site	Variable	Fe	K	Mg	Zn	Ca
Louda (n= 25) ^c	Minimum	4.15	906.77	186.40	2.58	449.64
	Maximum	13.78	2801.69	1619.45	4.85	1731.41
	Mean	7.83 ^{*d}	1458.69 ^{**}	475.14 ^{**}	3.54 ^{**}	820.88 ^{**}
	CV%	29.08	43.27	91.38	16.07	36.52
Nobéré (n= 20)	Minimum	4.51	961.66	209.80	3.16	466.52
	Maximum	18.48	2725.31	1624.83	4.86	1571.41
	Mean	7.35 [*]	1921.58 [*]	996.09 [*]	3.76 [*]	1112.21 [*]
	CV%	40.68	36.87	55.92	13.34	32.58
Péni (n= 20)	Minimum	4.87	662.55	178.19	2.58	316.13
	Maximum	13.30	2531.67	1472.11	4.69	1052.59
	Mean	7.31 [*]	1171.46 ^{***}	488.74 ^{**}	3.41 ^{**}	586.05 ^{***}
	CV%	27.66	41.62	69.31	14.59	34.85
All locations (n= 65)	Minimum	4.15	662.55	178.19	2.58	316.13
	Maximum	18.48	2801.69	1624.83	4.86	1731.41
	Mean	7.52	1512.74	634.05	3.57	838.26
	SD	2.40	682.69	499.07	0.53	357.43
	P-value	0.38	<10-3	<10-3	<10-2	<10-3

^cNumber of trees sampled in each population. ^dvalues with different number of superscript stars in the same column are significantly different (P-value ≤ 0.05).

themselves for most of the nutrients assessed, as revealed by both the ANOVA and PCA.

DISCUSSION

The concentrations of methionine and cysteine were higher than those found by Elemo et al. (2011) in dehulled and defatted seeds of *Parkia biglobosa* (cys: 0.1%; met: 0.06%). Glutamic acid and methionine were the amino acids found in highest and lowest concentrations, respectively, among all *P. biglobosa* samples. The same observations were made by Hassan and Umar (2005) in a study on African locust bean. Methionine and cysteine, sulfur containing amino acids, found in low concentrations in our samples, have been reported to be limited in legumes (Baudoin and Maquet 1999; Laurena et al., 1991). Cysteine is an important amino acid due to its positive effect on mineral absorption, particularly zinc (Mendoza, 2002; Sandstrom et al., 1989). Cysteine as well as arginine is functional amino acids. They play an important role in the regulation of various metabolic pathways (Guelzim, 2011).

The study reported a higher minerals content of *P. biglobosa* than that reported by Elemo et al. (2011): iron 9.3 mg/100 g, potassium 1101.5 mg/100 g magnesium and zinc 280.2 mg/100 g and 3.8 mg/100 g respectively, calcium 222.2 mg/100 g.

The high average moisture content in the samples from

Péni (6.78%) can be explained by the higher rainfall levels that characterize this site located in the most southern of the three locations investigated, in the South-Soudanian zone. The moisture and carbohydrate content of the present study were lower than those reported in previous studies (Koura et al., 2014; Elemo et al., 2011; Odeunmi et al., 2010; 8.6 to 11.21% for moisture and 41.10 to 48.5 for carbohydrates). The same authors showed amounts of proteins varying from 21.38 to 33.70%, of ashes varying from 3.51 to 5.01 and of fats varying from 15.48 to 22.56%. Our results contain higher values than those previously reported by these authors.

Results show that *P. biglobosa* kernels have a good nutrient content that can be improved during transformation in 'soubala'. *P. biglobosa* kernels are not consumed raw. The technological process by which they are transformed through fermentation allows an improvement of their nutritional quality, particularly the availability of proteins. The differences in nutrient composition of samples collected from different locations can be attributed to environmental factors, such as soil composition and weather variability, to genetic variability (Koura et al., 2014; Elemo et al., 2011; Odeunmi et al., 2010), or to a combination of these sets of factors. A previous study on *P. biglobosa* (Olujobi, 2012) showed that the location significantly affects the nutritional composition of this species, similarly to our study. The higher values for most nutrients found in our study versus previous studies (Koura et al., 2014; Elemo et al., 2011;

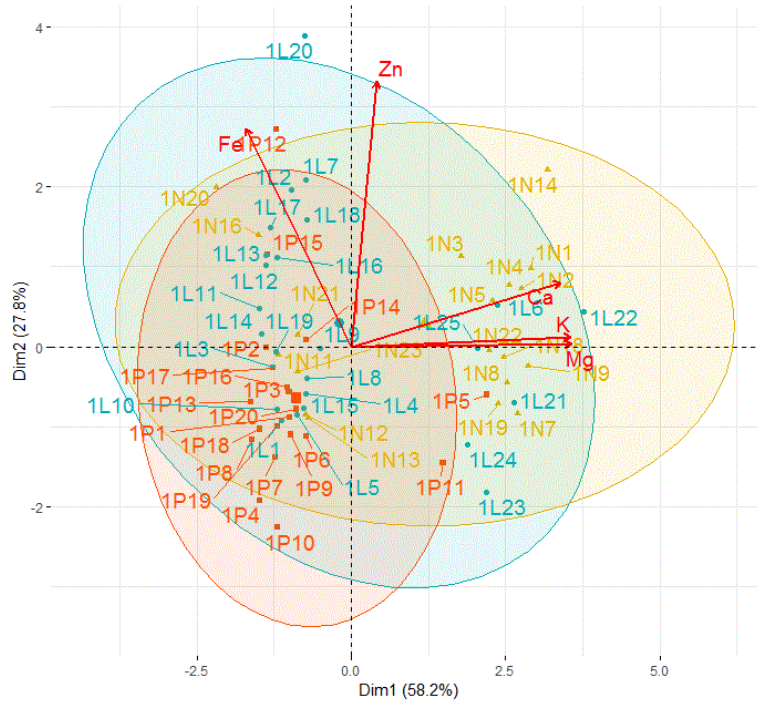


Figure 2. PCA biplot of the mineral composition of *P. biglobosa* kernels. The different circles represent the three populations studied. Vectors show the relative weight of the variables examined (Fe, Zn, Ca, K, Mg), which determines the spread of points (individual trees) on the biplot. The code associated to each point is the unique identifier of each individual tree.

Table 3. Proximate composition of *P. biglobosa* kernels.

Site	Variable	Humidity (%)	Carbohydrates (g/100 g DM)	Proteins (g/100 g DM)	Ashes (g/100 g DM)	Lipids (g/100 g DM)
Louda (n= 25) ^e	Minimum	4.56	13.09	34.60	4.45	21.85
	Maximum	7.56	19.81	43.02	5.80	30.77
	Mean	5.92 ^{**f}	17.13 [*]	39.60 [*]	5.02 [*]	25.23 [*]
	CV%	11.10	10.10	5.78	7.09	8.03
Nobéré (n= 20)	Minimum	4.49	14.35	32.69	4.42	21.64
	Maximum	5.84	18.28	43.91	5.36	30.03
	Mean	5.16 ^{***}	16.01 ^{**}	37.99 ^{**}	4.79 ^{**}	25.67 [*]
	CV%	7.94	7.28	7.72	5.43	9.69
Péni (n= 20)	Minimum	6.35	10.85	30.32	4.19	21.68
	Maximum	7.27	18.52	42.75	5.15	30.01
	Mean	6.78 [*]	14.97 ^{***}	38.13 ^{**}	4.55 ^{***}	25.83 [*]
	CV%	4.39	13.32	7.73	5.78	8.19
All locations (n= 65)	Minimum	4.49	10.85	30.32	4.19	21.64
	Maximum	7.56	19.81	43.91	5.80	30.03
	Mean	5.95	16.12	38.65	4.80	25.55
	SD	0.82	1.90	2.78	0.36	2.18
	P-value	<10-3	<10-3	<10-2	<10-3	0.25

^eNumber of trees sampled in each population. ^fvalues with different number of superscript stars in the same column are significantly different (P-value ≤0.05).

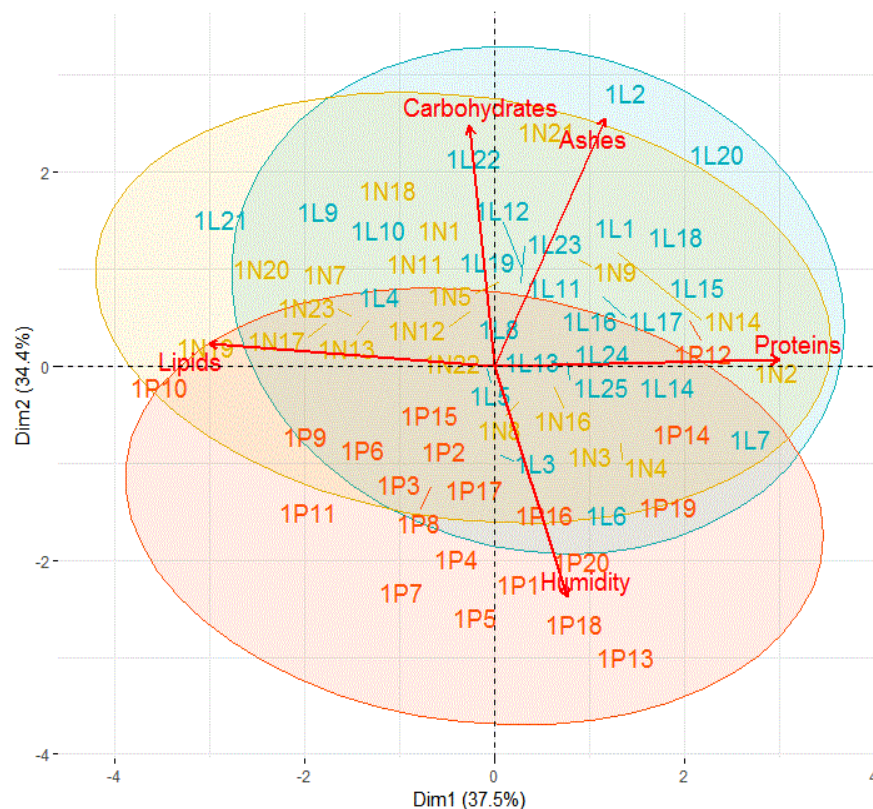


Figure 3. PCA biplot of the proximate composition of *Parkia biglobosa* kernels. The different circles represent the three populations studied. Vectors show the relative weight of the variables examined (moisture, ashes, lipids, proteins and carbohydrates), which determines the spread of points (tree individuals) on the biplot. The code associated to each point is the unique identifier of each individual tree.

Odeunmi et al., 2010) could be partly explained also by differences in experimental procedures used. For example, in these previous studies, the samples were dried at room temperature and dehulling was not done using mechanic methods (Koura et al., 2014).

Conclusion

The chemical composition of *P. biglobosa* kernels showed that they are a good source of macro- and micronutrients. They contain a fair amount of some amino acids that are known to be limited in legumes and they also contain high amounts of minerals. The nutritional composition of *P. biglobosa* kernels varied significantly according to the location where seeds were collected. Samples from Péni in the South-Soudanian zone showed overall lower nutritional value, while samples from Nobéré and Louda showed more variation. Additional studies would be required to increase understanding of the impact of environmental variables, mainly soil characteristics and genetic factors, on the nutritional composition of *P. biglobosa* kernels.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The study was supported by the Austrian Development Agency within the project on “Nutrition-sensitive forest restoration to enhance the capacity of rural communities in Burkina Faso to adapt to change” (2016-2019), led by Bioversity International (Italy). The project was co-financed by the CGIAR research programme on Forests, Trees and Agriculture (FTA) and Agriculture for Nutrition and Health (A4NH). The author appreciates the technicians from Centre National de Semences Forestières (CNSF) for their assistance in collection of the samples. Finally, Dr Abel Tankoano is thanked for his support in statistical analyses.

REFERENCES

Adeyeye EI (2006). Amino acids composition of fermented African

- locust bean (*Parkia biglobosa*) seeds. Journal of Applied and Environmental Sciences 2(2):154-158.
- AOAC (2005), official method analysis 975.03. Metals in Plants and Pets Food Atomic Absorption Spectrophotometric Method.
- Baudoin JP, Maquet A (1999). Improvement of protein and amino acid contents in seeds food legumes. A case study in Phaseolus. Biotechnologie Agronomie, Société et Environnement 3(4):220-224.
- Elemo GN, Elemo BO, Oladunmoye OO, Erukainure OL (2011). Comprehensive investigation into the nutritional composition of dehulled and defatted African locust bean seed (*Parkia biglobosa*). African Journal of Plant Science 5(5):291-295.
- Esenwah CN, Ikenebomeh MJ (2008). Processing effects on the nutritional and anti-nutritional contents of African locust bean (*Parkia biglobosa* Benth.) seeds. Pakistan Journal of Nutrition 7(2) :214-217.
- Guelzim N (2011). Regulation du métabolisme secondaire de l'arginine et de la cystéine par l'acide alpha-linolénique. Implication dans la physiopathologie du syndrome métabolique. PhD dissertation, AgroParisTech.
- Hassan LG, Umar KJ (2005). Protein and amino acids composition of African locust bean (*Parkia biglobosa*). Tropical and Subtropical Agroecosystems 5(1):45-50.
- Koura K, Peace IGO, Azokpota P, Ganglo JC, Hounhouigan DJ (2014). Caractérisation physique et composition chimique des graines de *Parkia biglobosa* (Jacq.) R. Br. En usage au Nord-Bénin. Journal of Applied Biosciences 75:6239-6249.
- Laurena AC, Rodriguez FM, Sabino NG, Zamora AF, Mendoza EMT (1991). Amino acid composition, relative nutritive value and in vitro protein digestibility of several Philippine indigenous legumes. Plant Foods for Human Nutrition 4:59-68.
- Mendoza C (2002). Effect of genetically modified low phytic acid plants on mineral absorption. International Journal of Food Science and Technology 37(7):759-767.
- Montreuil J, Spik G (1969). Microdosage de sucres : Méthodes colorimétriques de dosage des sucres totaux. Faculté des Sciences, Université de Lille France.
- Norme internationale ISO 20483 (2013). Céréales et légumineuses, 2^e éd., 14 p
- Norme internationale ISO 2171 (2007). Céréales, légumineuses et produits dérivés, 4^e éd., 11 p.
- Norme internationale ISO 659 (2009). Graines oléagineuses, 4^e éd., 13 p.
- Norme internationale ISO 712 (2009). Céréales et produits céréaliers, 4^e éd., 17 p.
- Nyadanu D, AduAmoah R, Obeng B, Kwarteng AO, Akromah R, Aboagye LM, Adu-Dapaah H (2016). Ethnobotany and analysis of food components of African locust bean (*Parkia biglobosa* (Jacq.) Benth.) in the transitional zone of Ghana: implications for domestication, conservation and breeding of improved varieties. Genetic Resources and Crop Evolution 63(6).
- Odebunmi EO, Oluwaniyi OO, Bashiru MO (2010). Comparative Proximate Analysis of Some Food Condiments. Journal of Applied Sciences Research 6(3):272-274.
- Olujobi OJ (2012). Comparative Evaluation of Nutritional Composition of African Locust Bean (*Parkia biglobosa*) Fruits from Two Locations. Nigerian Journal of Basic and Applied Science 20(3):195-198.
- Omafuvbe BO, Falade OS, Omuntogun BA, Adewusi SRA (2004). Chemical and biochemical changes in African locust bean (*Parkia biglobosa*) and Melon (*Citullus vulgaris*) seeds during fermentation to condiments. Pakistan Journal of Nutrition 3(3):140-145.
- Ouoba LI, Diawara B, Annan NT, Poll L, Jakobsen M (2005). Volatile compounds of soumbala, a fermented African Locust bean (*Parkia biglobosa*) food condiment. Journal of Applied Microbiology 99(99):1413-1421.
- Pelig-Ba KB (2009). Effect of ash, KOH and Millet on the fermentation of *Parkia biglobosa* seeds to form a condiment. Pakistan Journal of Nutrition 8(10):1548-1554.
- Sandstrom BM, Almgren A, Kivisto B, Cederblad A (1989). Effect of protein and protein source on zinc absorption in humans. Journal of Nutrition 119(1):48-53.
- Kristofferson TL (2011). Amino acid analysis of savannah tree seeds. Danish technological institute, 31p.
- Wang J, Fung DYC (1996). Alkaline-Fermented Foods: A Review with Emphasis on Pidan Fermentation. Critical Reviews in Microbiology 22(2):101-138.

Full Length Research Paper

Effect of the incorporation of graded levels of turmeric (*Curcuma longa*) on different qualities of stirred yoghurt

Eze Chinazom Martina^{1*}, Aremu Kehinde Oludayo¹, Nnamani Chidera Linda¹, Omeje Patience Chinasa¹, Omelagu Chizoba Ambrose² and Okonkwo Thomas Muoneme¹

¹Department of Food Science and Technology, University of Nigeria, Nsukka, Enugu State, Nigeria.

²Department of Food Science and Technology, University of Mkar, Gboko, Benue State, Nigeria.

Received 8 January, 2020; Accepted 2 April, 2020

There is an increasing trend in yoghurt consumption due to the health benefits from the gut bacteria present in yoghurt. However, there is need to evaluate other inexpensive nutrient sources such as spices (turmeric) which contain a lot of phytochemicals to make yoghurt more nutritious. Fresh turmeric rhizome was sorted, washed, peeled and milled. Ethanol was added to obtain turmeric extract. The turmeric extract was added to the yoghurt before (YTBF) and after fermentation (YTAF) at different ratios of yoghurt: Turmeric (95:5, 90:10, 85:15, 80:20, 75:25 and 100:0). Proximate composition and sensory characteristics of the blends were determined using standard procedures. Results obtained show that the addition of turmeric extract to the yoghurt had significant ($p < 0.05$) effect on the parameters analyzed. The protein, fat, ash and carbohydrate content of YTBF samples ranged from 2.70 - 3.98, 1.56 - 1.74, 0.20 - 0.38 and 7.69 - 8.25%, respectively while that of sampled YTAF ranged from 2.64 - 3.85, 1.53 - 1.69, 0.24 - 0.54 and 7.87 - 8.26%, respectively. From the sensory scores, sample with the lowest level of turmeric extract (YTBF1) (95:5) was most preferred and compared favorably with the control sample based on colour, taste and overall acceptability. The incorporation of turmeric extract in yoghurt improved the nutrient content of the yoghurt samples. Increased levels above 10% (90:10) led to a more intense colour and spicy taste which did not appeal to the panelists.

Key words: Yoghurt, turmeric, fermentation, proximate composition, sensory characteristics.

INTRODUCTION

Yoghurt is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*. In some countries, less traditional

microorganisms such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii ssp. lactis*, are sometimes mixed with the starter culture (McKinley, 2005). Yoghurt is valued for controlling the growth of bacteria and in

*Corresponding author. E-mail: chinazom.obodoechi@unn.edu.ng.



Plate 1. Turmeric rhizome.

curing of intestinal disease such as constipation, diarrhoea and dysentery, anti-carcinogenic effect and lowering of blood cholesterol (Kamruzzaman et al., 2002). Due to the aforementioned health benefits, there is an increasing trend for yoghurt consumption and is the fastest growing dairy sector in the market, in particular, standard yoghurt and yogurt drinks. Yoghurts come in a variety of textures (e.g. liquid, set and stirred curd), fat contents (e.g. regular fat, low-fat and fat-free) and flavours (e.g. natural, fruit, cereal, chocolate), and can be consumed as a snack or part of a meal, as a sweet or savoury food.

Yoghurt is generally considered as a safer product and its unique flavour, is so appealing that consideration is being given by nutritionists to incorporate inexpensive source of nutrients in order to make it an almost complete food (Boghra and Mathur, 2000). Nowadays, yoghurts are being sold with different flavours. For instance, ginger and herbs are added to the fresh milk before fermentation or served with sugar syrup. Various fruits, vegetables and spices e.g. turmeric are being incorporated into yoghurts to give them desirable flavours. Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant of the ginger family (Zingiberaceae) originated in tropical South Asia but is now widely cultivated in the tropical and subtropical regions of the world (Jurenka, 2009). It has a warm, bitter taste and is frequently used to flavour or colour curry powders, mustards, butters, and cheeses. It contains a yellow-coloured chemical substance called curcumin, which is often used to colour foods and cosmetics (Akande and Adegoke, 2018).

Curcumin is the main active ingredient in turmeric responsible for turmeric's numerous activities. It is known

to possess anti-oxidative (Cousins et al., 2007), anti-microbial (Cho et al., 2006) and anti-inflammatory properties as well as having radio-resistant and chemopreventive properties (Bar-sela et al., 2010).

Yoghurt is naturally produced from milk which contains a reasonable amount of live cultures mainly bacteria. Milk, from which yoghurt is made, contains a reasonable quantity of fat globules referred to as milkfat. Yoghurt therefore is prone to oxidation and can produce off-flavor. However, there are some spices that possess anti-oxidative, anti-microbial and anti-inflammatory properties which if incorporated in yoghurt, can help avert the off-flavour. However, it is most likely that the main reason that spices are being used is because, they help keep the foods free of unwanted microorganisms and thus contribute to health (Brul and Coote, 1999). However, spices such as turmeric are known to contain proteases and to have proteolytic activity (Nagarathnam et al., 2010).

Therefore, a yoghurt product with spice extract should serve to provide the combined health benefits from the spice plus those from the gut healthy bacteria present in the yoghurt. The objective of this study was to produce stirred yoghurt with graded levels of turmeric and evaluate the effect of turmeric in the yoghurt before and after fermentation on the physicochemical, microbiological and sensory characteristics of the yoghurt.

MATERIALS AND METHODS

Procurement of raw materials

The turmeric rhizome (Plate 1), skimmed powdered milk, starter

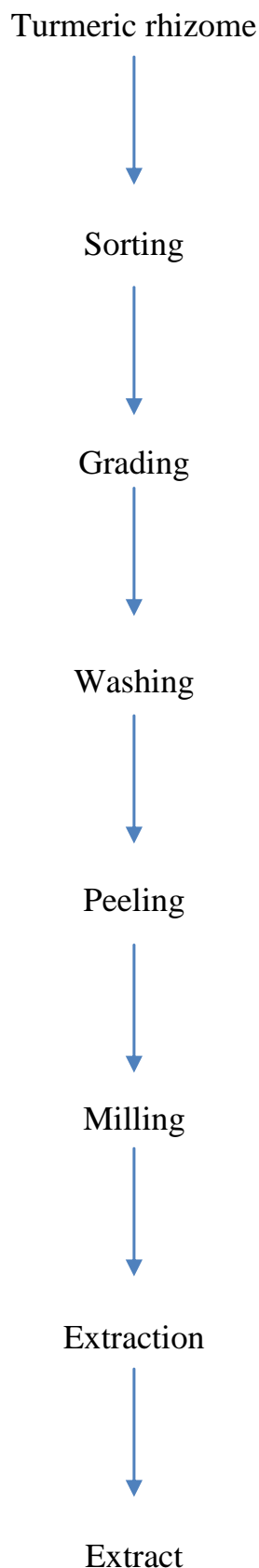


Figure 1. Preparation of turmeric extract

culture (Yoghurmet) and granulated sugar (Dangote Sugar Company) were purchased from Ogige main market in Nsukka local Government area of Enugu State, Nigeria.

Sample preparation

Preparation of turmeric extract

The turmeric was sorted, graded, washed thoroughly with water and the peel was separated from the flesh, milled and water was added for extraction as shown in Figure 1.

Preparation of ethanolic turmeric extract

180 g of ground spice was transferred to a 250ml conical flask. 200ml of 95% ethyl alcohol (ethanol) was added. The flask was covered, mixed, and stored overnight for 16 h at room temperature. The solution was filtered using a dry Whatman No. 1 filter paper. The ethyl alcohol was allowed to evaporate in a hot air oven at 110°C until a constant weight of the extract was obtained as shown in Plate 2A.

Preparation of aqueous turmeric extract

180 g of ground sample was weighed into a 500ml of conical flask. 200ml of water was added, covered and shaken vigorously. The flask was covered, mixed, and stored overnight for 16 h at room temperature. The solution was filtered using Whatman No. 1 dry filter paper, then dried in a hot air oven at 110°C until constant weight of extract was obtained as shown in Plate 2B.

Production of yoghurt

Yoghurt was produced as described by Lee and Lucey (2010) with slight modification. The milk mix was pasteurized at 80°C for 20 to 30 min to inactivate the pathogens in a Gallenkamp (220/240V, 50 Hz) water bath and homogenized at pasteurization temperature. The milk was cooled to inoculation temperature of 40 to 45°C and then inoculated with 2 to 3% starter culture (Yoghurmet consisting of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*). The yoghurt was fermented for 12 h at incubation temperature of 43 to 45 °C in a water bath after which it was homogenized and divided into six portions. Thereafter, six sample blends of 95:5, 90:10, 85:15, 80:20, 75:25, 100:0 (Table 1) indicating the ratio of Yoghurt to Turmeric, were formulated as shown in Figure 2 and 3.

Sample analysis

Proximate composition of turmeric and formulated yoghurt

The moisture, crude protein (N x 6.25), crude fat, crude ash and crude fibre contents were determined using standard Association of Official Analytical Chemists (AOAC, 2010).

Determination of total carbohydrate content

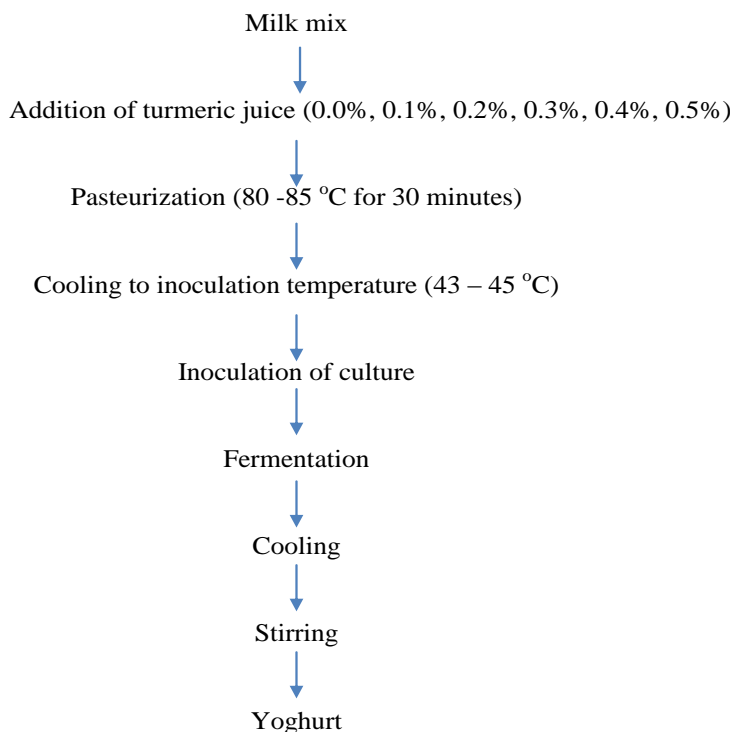
Total carbohydrate content was determined by difference (AOAC, 2005). This was simply carried out by subtracting the value of other food components (moisture, ash, fibre and protein) from 100 as shown in the Equation (1).

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

Table 1. Proportion of turmeric and yoghurt used in the formulation of yoghurt incorporated with turmeric.

Sample	Yoghurt (ml)	Turmeric (ml)
PY +TM (95:5)	95	5
PY +TM (90:10)	90	10
PY +TM (85:15)	85	15
PY +TM (80:20)	80	20
PY +TM (75:25)	75	25
PY +TM (100:0)	100	0

Turmeric was added before fermentation and after fermentation. PY = Plain yoghurt, TM = Turmeric extract.

**Figure 2.** Modified production of yoghurt with turmeric extract before fermentation.

Source: Lee and Lucey (2010)

Determination of ash content

The ash content of the freshly prepared yoghurt and turmeric samples was determined according to the standards of AOAC (2010). A preheated and cooled crucible was weighed (W_1) and 2 g of each of the samples was weighed into two preheated cooled crucibles (W_2). The samples were charred on a Bunsen flame inside a fume cupboard. The charred sample in the crucible was then transferred into a preheated muffle furnace at 550°C for 2 h until a white or light grey ash was obtained (W_3). It was then cooled in a dessicator, weighed and documented. The ash content of the samples was calculated using Equation 2

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (2)$$

Where W_1 = Weight of empty crucible; W_2 = Weight of crucible + Weight of sample before ashing; W_3 = Weight of crucible + Weight of the sample after ashing

Determination of moisture content

The moisture content of the samples was determined according to the standard method of Association of official Analytical Chemist (AOAC, 2010). The crucibles were washed thoroughly and dried in the oven at 100°C for 1 h. The hot dried crucibles were cooled and weighed and value noted down (W_1). The samples (2 g) each were weighed into the crucibles (W_2) and dried at 110°C until a constant weight (W_3) was obtained. The moisture content of the samples was calculated as given in Equation (3).

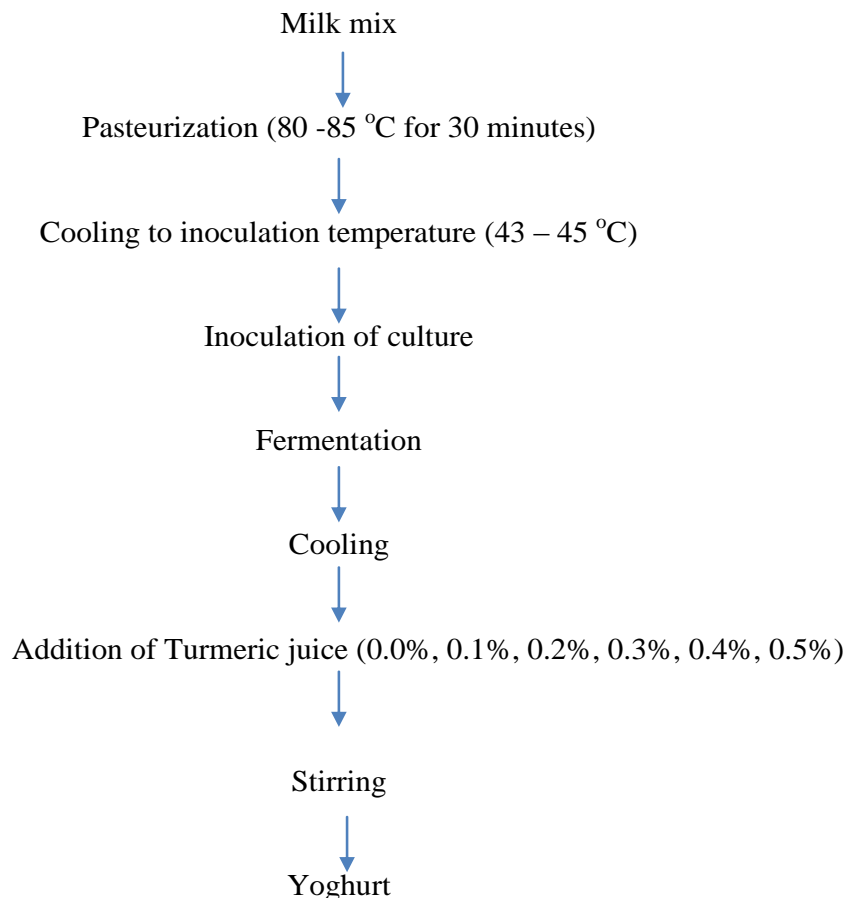


Figure 3. Modified production of yoghurt with turmeric extract after fermentation".

Source: Lee and Lucey (2010)

$$\% \text{ Moisture Content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad (3)$$

Where $W_1 = \text{Initial weight of empty crucible}$;
 $W_2 = \text{Weight of crucible} + \text{Weight of sample before drying}$; $W_3 =$
 $\text{Weight of crucible} + \text{Weight of the sample after drying}$

Determination of fat content

The fat content of the yoghurt samples was determined using standard AOAC (2010) method. A Soxhlet extractor with a reflux condenser and a 500 ml round bottom flask was fixed. The yoghurt sample (2 g) was weighed into a labeled thimble and petroleum ether (300 ml) was filled into the round bottom flask. The extraction thimble was sealed with cotton wool. The Soxhlet apparatus was allowed to reflux for about 6 h. The thimble was removed with care and the petroleum ether was collected on the top and drained into a container for reuse. As soon as the flask was free of ether, it was removed and dried at 70°C for 1 h in an oven. It was cooled in desiccators and then weighed. The fat content of the samples was calculated using Equation (4).

$$\% \text{ fat content} = \frac{\text{Weight of fat}}{\text{Weight of the sample}} \times 100 \quad (4)$$

Determination of crude protein

The protein content of the samples was determined according to the standard methods of AOAC (2010) using Kjeldahl's method.

Digestion of the sample

The yoghurt sample (5 ml) was weighed into Kjeldahl digestion flask, and 1 tablet of Kjeldahl catalyst was added. Twenty-five milliliters (25 ml) of concentrated H_2SO_4 was added with few boiling chips. The flask with its content was heated in a fume chamber until a clear solution was obtained. The solution was cooled to room temperature after which it was transferred into a 250 ml volumetric flask and made up to a known level with distilled water.

Distillation

The distillation unit was cleaned and the apparatus set up. A 100 ml conical flask (receiving flask) containing 5 ml of 3% Boric acid was placed under the condenser and 2 drops of methyl red indicator was added. A digest of 5 ml was pipetted into the apparatus through a small funnel and washed down with distilled water. This was followed by the addition of 5 ml of 60% sodium hydroxide solution (NaOH). Heat was applied to the digestion flask until 100 ml of distillate (ammonium sulphate) was collected in the receiving flask.

Titration

The solution in the receiving flask was titrated with about 0.04 M HCl to get pink colour. The same procedure was carried out on the blank. The percentage Nitrogen was evaluated as given in Equation 5.

$$\% \text{ Nitrogen} = \frac{V_s - V_b \times N_{\text{acid}}}{W} \times 0.0401 \times 100 \quad (5)$$

Where V_s = Volume(ml) of the acid required to titrate the sample;
 V_b = Volume(ml) of the required base to titrate the blank; N_{acid} = Normality of acid and W = Weight of sample (g)

The percentage crude protein of the samples was determined using Equation 6 as shown below.

$$\% \text{ Crude protein} = \% \text{ N} \times 6.25 \text{ (Conversion Factor)} \quad (6)$$

Micronutrient analysis

Determination of vitamin B₁₂ (Cyanocobalamin)

The fat content of the yoghurt samples was determined using the method described by AOAC (2010) was used. 1 g of the sample was weighed into a flat-bottom flask and 50 ml of 0.1 N HCl was added to it. The flask was thoroughly swirled and allowed to stand for 2 h. The mixture was then filtered and 10 ml aliquot taken in a test tube for spectrophotometric reading. Then 0.5 ml, 0.2 % alpha-alpha dipyrindyl were added to the sample which was read within 10 min in a spectrophotometer at 550 nm.

Determination of calcium content

Calcium content of the samples was determined by the Ethylene diamine tetra acetic acid (EDTA) complexometric titration of AOAC (1990) as described by Hussain et al. (2010). 20ml of sample was taken in a conical flask and 2 to 3 pellets of KOH were added. After shaking the solution 1 g of Patton and reeder indicator (calcon 3-carboxylic acid) was added and the sample was titrated against 0.01M EDTA solution until a colour change from wine red to blue appeared. The volume of EDTA is the equivalent volume of calcium in the sample.

Determination of phosphorus content

Phosphorus in the sample was determined by Molybdate method as described by Onwuka (2005). Hydroquinone was used as a reducing agent. A mixture of 1.0 sodium sulphate (Na₂SO₄), 1.0 ml hydroquinone and 0.5 ml of the mineral digest was agitated and allowed to stand for 30 min. The blue colour developed was quantified using a colorimeter at 660 nm against a standard. The Phosphorus in the sample was calculated using the following Equation 7.

$$\text{Phosphorus} = \frac{\text{Absorbance of test} \times \text{Dilution Factor}}{W \times 5} \quad (7)$$

Where W = Weight of the sample.

Determination of vitamin C (ascorbic acid) content

Vitamin C content was determined according to the method

described by Onwuka (2005). Five grams (5 g) of the sample and 2.5 ml of 20% metaphosphoric acid (as a stabilizing agent) was diluted with distilled water and weighed into a 100ml volumetric flask. Ten milliliters (10 ml) of the solution was mixed with 2.5ml acetone and homogenized. The absorbance reading was obtained using an Ultra-violet (UV) spectrophotometer to ascertain the Vitamin C content at 264 nm wavelength. Vitamin C content of the samples was calculated using the Equation 8. The calibration curve was constructed by plotting the concentration against the corresponding absorbance. The molar absorptivity was found using the Beer-Lambert's law.

$$\text{Vitamin C} = \frac{\text{Absorbance} \times \text{dilution factor}}{\text{Slope (from Standard curve)}} \quad (8)$$

Determination of vitamin B₂ (Riboflavin) content

AOAC (2005) standard method was used. A 2 g sample of the yoghurt was placed in a conical flask and 50 ml of 0.2 N HCl added. The solution was boiled for 1 h and cooled. The pH was adjusted to 6.0 using Sodium Hydroxide (NaOH). Also 1 N HCl was added to the solution of the sample to lower the pH to 4.5. The solution was filtered into 100 ml volumetric flask and made up to the required volume with distilled water. In order to remove interference, two tubes were taken and labelled 1 and 2. Ten millilitres (10 ml) of water was added to tube 1. Another 10 ml of filtrate and 1 ml of Riboflavin standard were added to test tube 2. Then 1 ml of glacial acetic acid was added to each tube and mixed 0.5 ml of 3% KMnO₄ solution was added to each tube. The test tube was allowed to stand for 2 min after which 0.5 ml 3% H₂SO₄ was added and solution mixed well. The fluorimeter was adjusted to an excitation wavelength of 470 nm and emission wavelength of 525 nm. The fluorimeter was adjusted to zero deflection against 0.1 N H₂SO₄ and 100 against tube 2 (standard). The fluorescence of tube 1 was added to both tubes and the fluorescence measured within 10 s.

Riboflavin (Vitamin B₂) was calculated as shown in Equation 9:

$$\text{Riboflavin (mg / g)} = \frac{Y}{Y-X} \times \frac{1}{W} \quad (9)$$

Where W = Weight of sample; X = Reading of sample - Blank reading; Y = Reading of sample + standard (tube 2) - reading of sample - standard blank.

Determination of vitamin B₆ (pyridoxal phosphate) content

The method described by AOAC (2010) was used. 1 g of each sample was weighed separately into a 100 ml conical flask and extracted with 10 ml 0.1 M HCl with vigorous shaking for 10 min. The sample was then filtered through Whatman No. 1 filter paper. The filtrate was then made up to 10 ml with distilled water. 5 ml of the slightly acidic filtrate was treated with 1 ml 0.40% Ferric Chloride. The optical density of the resultant brown solution was measured in a spectrophotometer at 450nm. The absorbance obtained from the sample extract was converted to pyridoxine concentration by means of a calibration curve generated using different standard concentrations. Vitamin B₆ was calculated as shown in Equation 10:

$$\text{Vitamin B}_6 \text{ (mg / 100 g)} = \frac{\text{Absorbance of sample} \times \text{Conc. of standard}}{\text{Absorbance of Standard} \times \text{Sample size}} \quad (10)$$

Determination of vitamin B₃ (Niacin) content

This was done using Pearson (1976) spectrophotometric method.

Table 2. Proximate analysis on turmeric extract of water and ethanol extraction.

Constituent	Water extraction (%)	Ethanol extraction (%)
Moisture	80.5	50.2
Protein	2.5	7.0
Fat	4.2	10.8
Ash	3.0	1.5
Carbohydrate	15.8	30.5

A 2 g portion of yoghurt sample was weighed into a conical flask and 20 ml of 0.5 M NaOH added. The contents of the flask were stirred with a magnetic stirrer for 30 min. The resulting solution was filtered into a clean container and 5 ml was transferred into a test tube. Four milliliter (4 ml) of 0.1 N KCl and 0.1 N NH₄Cl solutions were added into the extract and allowed to stand for yellow colour development. The absorbance was measured at 261 nm. A standard and blank solution was also prepared.

Nicotinic acid (Niacin) was calculated as given in Equation 11:

$$\text{Niacin (mg / g)} = \frac{\text{Absorbance of test sample} \times \text{Conc. of standard (5 mg/dl)}}{\text{Absorbance of standard}} \quad (11)$$

Physicochemical analyses of stirred yoghurt samples

Determination of pH

A standard pH meter (model 20 pH Conductivity Meter, Denver Instrument, United Nations Inventory Database), was standardized using buffer solutions of pH 4.0 and 9.0. The pH electrode was dipped into the yoghurt and after a few minutes of equilibration, the pH of the yoghurt sample was taken (AOAC, 2010).

Determination of apparent viscosity

The viscosity of yoghurt samples was determined by using Ostwald viscometer according to AOAC (2010). 20 g of each of the samples was taken and made Newtonian by dissolving in 50 ml of water to obtain the density of each sample. Water was sucked into the viscometer and time taken to fall back on its own after sucking to the mark was noted. The process was repeated for the yoghurt samples. The apparent viscosity was calculated in Centipoise (cP) using Equation 12.

$$\text{Apparent viscosity (cP)} = \frac{n_2 \times \rho_1 \times t_1}{\rho_2 \times t_2} \quad (12)$$

Where n_2 = Viscosity of water (0.89); ρ_1 = Density of sample; t_1 = time taken for the sample to fall back on its own (seconds); ρ_2 = Density of water (1g / cm³); t_2 = time taken for water to fall back on its own (2.5 s).

Determination of total titratable acidity

The total titratable acidity was determined using the method of AOAC (2010). The sample (5 ml) at 25°C was measured into a flask and diluted to twice its volume with distilled water. Phenolphthalein indicator (2 ml) was added to each yoghurt sample and titrated with 0.1 M NaOH to the first permanent pink colour. The total titratable

acidity was calculated as the percentage lactic acid by weight using Equation 13:

$$\text{Titratable acidity (\%)} = \frac{\text{Quantity of yoghurt sample}}{\text{Quantity of NaOH (ml)} \times 0.009 \times 100} \quad (13)$$

Microbial analysis of yoghurt samples

Microbiological analysis was carried out on the yoghurt samples. A serial dilution of the sample was done. The sample was placed at ambient temperature. Total viable count (TVC) and mould count was determined by pour plate method on nutrient agar and Saboroud Dextrose Agar (SDA) respectively as described by Prescott et al. (2005).

Sensory evaluation

The sensory evaluation was carried out according to Ihekoronye and Ngoddy (1985) using a 20-man semi-trained panelist consisting of students and lecturers of Food Science and Technology Department, University of Nigeria Nsukka. The panelists were instructed to indicate their preference of the samples using a nine-point Hedonic scale (where 9 signifies *extremely like* and 1 signifies *extremely dislike*) for each characteristic such as colour, flavour, mouth feel, taste after taste, consistency and overall acceptability being determined.

Data analysis and experimental design

The experiment was one in triplicates. Data obtained were subjected to analysis of variance (ANOVA) using split-plot in completely randomized design according to the methods of Gomez and Gomez (1985). Least significant difference was used to compare the treatment means and significance difference was used to compare the treatment means and significance was accepted at $p < 0.05$.

RESULTS

Comparison of the extract of aqueous and ethanolic extraction

Tables 2 and 3 shows selected chemical components and characteristics of turmeric extracted with ethanol and water (as shown in Plate 2A and B). Turmeric extracted with ethanol had higher chemical composition and

Table 3. Micronutrient analysis on turmeric extract of water and ethanol extraction (As shown in Plate 2).

Constituent	Water extraction	Ethanol extraction
Vitamin B ₂	19 mg	39 mg
Vitamin B ₁₂	2.10 μ g	5.10 μ g
Vitamin B ₆	0.80 mg	1.80 mg
Vitamin B ₃ (μ g/ml)	ND	0.233 mg
Vitamin C	4.5 mg	25.9 mg
Phosphorus	65.9 mg	268 mg
Calcium	3.10 mg	183 mg

**Plate 2.** (A) Turmeric extracts using ethanol; (B) Turmeric extracts using water.

characteristics than the turmeric extracted with water. This could be attributed to the fact that curcumin (C₂₁H₂₀O₅), the major bioactive compound in turmeric, is highly soluble in ethanol, acetone and dimethylsulfoxide (Remadevi et al., 2007). It can then be inferred that turmeric is an oil-soluble, hydrophobic pigment which is practically insoluble in water (Tonnesen, 2002). Hence turmeric extracted with ethanol was used for the production.

Proximate composition of yoghurt graded with different levels of turmeric

Effect of turmeric extract on the moisture and protein contents of the stirred yoghurt

Table 4 shows that the moisture content values of plain yoghurt sample (without turmeric) were found to be 8.55±0.01%. Generally, there were significant (p<0.05) differences between the moisture content of the stirred yoghurt at different levels of turmeric incorporated. The moisture contents of samples ranged from 8.55±0.01 to 87.29±0.01%. Sample YTB5 (75:25) yoghurt to turmeric

ratio before fermentation had the highest moisture content while the control Yoghurt (100:0) had the lowest moisture content. There was significant (p<0.05) difference in the protein content of the stirred yoghurt formulated with different amount of turmeric (Table 4). The values ranged from 2.64±0.05 to 4.13±0.01%. Sample YTB5 (75:25) yoghurt to turmeric ratio before fermentation had the lowest protein content while the plain yoghurt had the highest protein content. The effect of the different amount of turmeric also shows significant (p<0.05) differences at different levels. This is evident that protein content decreased with increase in the amount of turmeric added due to the fact that turmeric contains low protein content.

Effect of turmeric extract on the ash and fat contents of the stirred yoghurt

Data presented in Table 4 illustrated that there were significant (p<0.05) difference in the ash content of the stirred yoghurt at different levels of turmeric incorporated into the product. The ash content values were within the range of 0.20±0.01 to 1.81±0.01%. Yoghurt sample

Table 4. Proximate composition (%) of yoghurt graded with different levels of turmeric.

Sample (ml)	Moisture	Protein	Ash	Fat	Carbohydrate
YTA1(95:5)	86.05 ^d ±0.07	3.98 ^b ±0.13	0.38 ^d ±0.07	1.70 ^c ±0.06	7.89 ^a ±0.15
YTA2(90:10)	86.30 ^c ±0.03	3.84 ^c ±0.02	0.27 ^f ±0.03	1.63 ^e ±0.01	7.69 ^e ±0.10
YTA3(85:15)	86.98 ^b ±0.06	3.10 ^e ±0.00	0.25 ^g ±0.01	1.61 ^f ±0.00	8.06 ^e ±0.07
YTA4(80:20)	87.18 ^a ±0.08	2.85 ^f ±0.01	0.21 ^h ±0.02	1.56 ^g ±0.01	8.17 ^c ±0.13
YTA5(75:25)	87.29 ^a ±0.01	2.70 ^h ±0.01	0.20 ^h ±0.01	1.74 ^b ±0.01	8.25 ^b ±0.09
YTB1(95:5)	85.87 ^d ±0.05	3.85 ^c ±0.08	0.54 ^b ±0.01	1.69 ^c ±0.00	7.87 ⁱ ±0.03
YTB2(90:10)	85.81 ^d ±0.01	3.61 ^c ±0.01	0.50 ^c ±0.00	1.65 ^d ±0.01	7.96 ^g ±0.01
YTB3(85:15)	86.24 ^c ±0.03	3.09 ^d ±0.03	0.33 ^e ±0.01	1.57 ^g ±0.03	8.03 ^f ±0.03
YTB4(80:20)	87.28 ^a ±0.01	2.78 ^e ±0.01	0.27 ^f ±0.03	1.57 ^g ±0.00	8.12 ^d ±0.07
YTB5(75:25)	87.33 ^a ±0.08	2.64 ^f ±0.05	0.24 ^g ±0.03	1.53 ^h ±0.02	8.26 ^a ±0.04
Yoghurt	8.55 ^e ±0.01	4.13 ^a ±0.01	1.81 ^a ±0.01	1.81 ^a ±0.02	7.82 ⁱ ±0.01

Values are mean ± standard deviation of triplicate readings. Means on the same column with different superscripts are significant ($p < 0.05$) different. YTA=yoghurt with turmeric after fermentation; YTB=yoghurt with turmeric before fermentation.

YTA5(75:25), that is, yoghurt samples with 0.5% turmeric extract after fermentation, had the lowest ash content while Yoghurt (100:0), the control sample, had the highest fat content. Notably, the ash contents of the yoghurt samples before and after fermentation decreased with increasing levels of turmeric extracts in the formulation. The effect of turmeric extracts incorporated on fat contents of the yoghurt samples was studied at different levels (0.1, 0.2, 0.3, 0.4 and 0.5%). The results revealed that significant ($p < 0.05$) difference between the fat contents of the stirred yoghurt with different amount of turmeric existed (Table 4). The fat contents were within the range of 1.53±0.02 to 1.81±0.02%. Sample YTB5 (75:25) had the lowest fat content while Yoghurt (100:0) had the highest fat content.

Effect of turmeric extract on the carbohydrate content of the stirred yoghurt

Table 4 showed that there was significant ($p < 0.05$) difference between the carbohydrate contents of the stirred yoghurt samples and the different amount of turmeric extracted added (Table 4). The carbohydrate content ranged from 7.82±0.01 to 8.26±0.04%. The obtained results showed that sample YTB5(75:25) before fermentation had the highest carbohydrate content while the plain yoghurt (100:0), that is, the control sample, had the lowest carbohydrate content. In general, the effect of the different amount of turmeric also showed significant ($p < 0.05$) differences at different levels.

Effect of turmeric extract on micronutrient contents of the stirred yoghurt

Effect of different amount of turmeric on vitamin B₃ and vitamin B₁₂ content of the stirred yoghurt: Table 5 showed that there was significant ($p < 0.05$) difference in

vitamin B₃ content of the stirred yoghurt with different amount of turmeric (Table 5). The samples ranged from 0.12±0.00 to 1.72±0.01 mg. Sample YTA5(75:25) before fermentation had the highest vitamin B₃ content while sample YTB1(95:5) had the lowest vitamin B₃ content. Generally, the yoghurt samples formulated with turmeric before and after fermentation (YTB and YTA) have higher vitamin B₃ content than the plain yoghurt (YOGHURT). There was a significant ($p < 0.05$) difference in the vitamin B₁₂ content of the stirred yoghurt with different amount of turmeric (Table 5). The samples ranged from 8.42±0.04 to 34.30±0.01µg. Sample YTA5(75:25) yoghurt to turmeric ratio before fermentation had the highest vitamin B₁₂ content while plain yoghurt had the lowest Vitamin B₁₂ content.

Effect of turmeric extract on vitamin C and vitamin B₂ of the stirred yoghurt:

There was a significant ($p < 0.05$) difference between vitamin C content of the stirred yoghurt and different levels of turmeric incorporated (Table 5). The samples ranged from 42.5±0.06 to 66.15±0.10 mg. Sample YTA1 (95:5) had the highest vitamin C content while the control sample (YOGHURT) had the lowest vitamin C content. The effect of the different amount of turmeric also shows significant ($p < 0.05$) differences at different levels. Vitamin C content decreased with increase in the amount of turmeric. There was significant ($p < 0.05$) difference in the vitamin B₂ content of the stirred yoghurt with different amount of turmeric (Table 5). The samples ranged from 6.30±0.01 to 14.40±0.02 mg. Sample YTB5 (75:25) had the highest vitamin B₂ content while plain yoghurt had the lowest vitamin B₂ content.

Effect of turmeric extract on vitamin B₆, calcium and phosphorus of the stirred yoghurt: There was significant ($p < 0.05$) difference in the vitamin B₆ content of

Table 5. Micro-nutrients of yoghurt graded with different level of turmeric.

Sample (ml)	VitaminB ₃ (mg/100 g)	VitaminB ₁₂ (µg/100 g)	Vitamin C (mg/100 g)	Vitamin B ₂ (mg/100 g)	Vitamin B ₆ (mg/100 g)	Calcium (mg/100 g)	Phosphorous (mg/100 g)
YTA1(95:5)	1.01 ^e ±0.06	9.77 ^g ±0.00	66.15 ^a ±0.10	7.25 ^j ±0.05	0.23 ^c ±0.01	92.70 ^g ±0.01	87.29 ^a ±0.00
YTA2(90:10)	1.09 ^d ±0.01	10.52 ^h ±0.01	60.12 ^b ±0.07	7.42 ⁱ ±0.02	0.21 ^d ±0.01	93.20 ^f ±0.01	79.78 ^c ±0.00
YTA3(85:15)	1.12 ^c ±0.06	24.26 ^a ±0.01	58.26 ^h ±0.10	8.01 ^h ±0.00	0.20 ^a ±0.01	99.24 ^e ±0.03	71.45 ^e ±0.01
YTA4(80:20)	1.42 ^b ±0.03	25.15 ^a ±0.02	57.21 ^e ±0.07	9.24 ^d ±0.01	0.18 ^e ±0.01	105.35 ^b ±0.03	67.64 ^g ±0.01
YTA5(75:25)	1.72 ^a ±0.01	34.30 ^a ±0.01	56.44 ^f ±0.07	9.77 ^c ±0.02	0.16 ^f ±0.01	110.09 ^a ±0.01	61.04 ⁱ ±0.02
YTB1(95:5)	0.12 ^j ±0.00	9.99 ⁱ ±0.03	59.42 ^c ±0.04	8.45 ^g ±0.01	0.60 ^f ±0.01	87.32 ^j ±0.03	84.73 ^b ±0.03
YTB2(90:10)	0.22 ⁱ ±0.03	15.10 ^g ±0.03	49.30 ^h ±0.01	8.80 ^f ±0.01	0.40 ^b ±0.01	88.10 ^c ±0.00	78.60 ^d ±0.01
YTB3(85:15)	0.40 ^h ±0.01	24.53 ^e ±0.00	48.50 ^h ±0.01	9.01 ^e ±0.02	0.30 ^e ±0.01	89.24 ⁱ ±0.02	70.90 ^f ±0.02
YTB4(80:20)	0.50 ^g ±0.01	26.20 ^c ±0.01	46.51 ⁱ ±0.06	10.24 ^b ±0.05	0.20 ^g ±0.01	100.12 ^h ±0.01	66.42 ^j ±0.00
YTB5(75:25)	0.63 ^f ±0.02	27.20 ^b ±0.00	44.21 ^j ±0.07	14.40 ^a ±0.02	0.12 ^g ±0.01	100.42 ^d ±0.01	60.53 ^h ±0.03
YOGHURT	0.21 ⁱ ±0.01	8.42 ^k ±0.04	42.50 ^h ±0.06	6.30 ^f ±0.01	0.13 ^g ±0.01	85.90 ^c ±0.01	25.60 ^j ±0.01

Values are mean ± standard deviation of triplicate readings. Means on the same column with different superscripts are significant ($p < 0.05$) different. YTA=yoghurt with turmeric after fermentation, YTB=yoghurt with turmeric before fermentation.

Table 6. Functional properties of the effect of stirred yoghurt.

Sample	pH	Viscosity(cP)
YTA1(95:5)	4.72 ^e ± 0.02	118.89 ^f ± 0.23
YTA2(90:10)	4.75 ^d ± 0.05	114.87 ^g ± 0.17
YTA3(85:15)	4.78 ^c ± 0.03	101.01 ^d ± 0.10
YTA4(80:20)	5.00 ^b ± 0.08	96.18 ^a ± 0.25
YTA5(75:25)	5.21 ^a ± 0.07	90.94 ^e ± 0.29
YTB1(95:5)	4.68 ^g ± 0.02	115.94 ^{de} ± 0.10
YTB2(90:10)	4.71 ^f ± 0.02	110.03 ^a ± 0.21
YTB3(85:15)	4.72 ^e ± 0.06	100.96 ^d ± 0.17
YTB4(80:20)	4.75 ^d ± 0.03	95.94 ^b ± 0.21
YTB5(75:25)	4.79 ^c ± 0.00	90.92 ^e ± 0.17
YOGHURT(100:0)	4.70 ^f ± 0.01	120.65 ^d ± 0.20

Values are mean ± standard deviation of triplicate readings. Means on the same column with different superscripts are significant ($p < 0.05$) different. YTA=yoghurt with turmeric after fermentation, YTB=yoghurt with turmeric before fermentation.

the stirred yoghurt with different amount of turmeric (Table 5). The vitamin B₆ content of the samples were within the range of 0.12±0.01 to 0.60±0.01 mg. Sample YTB1 (95:5) had the highest vitamin B₆ content while plain had the lowest vitamin B₆ content. The values of Calcium in the samples ranged from 85.90±0.01 to 110.09±0.01mg. The calcium content of the stirred yoghurt showed significant ($p < 0.05$) difference at different levels of turmeric extract incorporated (Table 5). It was observed that sample YTA5 (75:25) had the highest calcium content as compared to sample yoghurt (100:0) which was found to have the lowest calcium content. This equally shows that yoghurt itself contains high amount of calcium which is necessary in young and adult for good bone and teeth development. Based on data presented in Table 5, there were significant ($p < 0.05$) difference in the phosphorus content of the stirred

yoghurt at different levels of turmeric extracts added. The phosphorus in the yoghurt samples ranged from 25.60±0.01 to 87.29±0.00 mg. Sample YTA1 (95:5, that is, yoghurt to turmeric ratio before fermentation) had the highest phosphorus content while the control sample (Yoghurt) had the lowest phosphorus content. It was noted that for all samples in which turmeric extract was added, the phosphorus content decreased with increased level of turmeric before and after fermentation.

Effect of turmeric extraction on the physicochemical properties of the stirred yoghurt

Effect of turmeric extract on the pH and viscosity of the stirred yoghurt: Table 6 shows the pH and viscosity of the yoghurt samples in which turmeric extract was

Table 7. Microbial count (cfu/ml) of the stirred yoghurt.

Sample	Mould count (cfu/ml)	Total viable count (cfu/ml)	Lactic acid bacteria (LAB)count (cfu/ml)	Coliform count (cfu/ml)
YTA1(95:5)	5.0x10 ¹	2.0 x10 ⁵	2.1 x10 ⁵	1.0 x10 ¹
YTA2(90:10)	4.0x10 ¹	2.0 x10 ⁵	2.0 x10 ⁵	0.5 x10 ¹
YTA3(85:15)	ND	1.9 x10 ⁵	1.9 x10 ⁵	ND
YTA4(80:20)	ND	1.8 x10 ⁵	1.5 x10 ⁵	ND
YTA5(75:25)	ND	1.7 x10 ⁵	10.0 x10 ⁵	ND
YTB1(95:5)	2.1x10 ¹	1.9 x10 ⁵	2.0 x10 ⁵	1.0 x10 ¹
YTB2(90:10)	0.1x10 ¹	1.7 x10 ⁵	1.8 x10 ⁵	0.4 x10 ¹
YTB3(85:15)	ND	1.6 x10 ⁵	1.5 x10 ⁵	ND
YTB4(80:20)	ND	1.4 x10 ⁵	1.3 x10 ⁵	ND
YTB5(75:25)	ND	1.2x10 ⁵	1.1x10 ⁵	ND
Yoghurt (100:0)	3.0x10 ¹	2.2 x10 ⁵	2.3x10 ⁵	1.4 x10 ¹

Values are mean \pm standard deviation of triplicate readings. Means on the same column with different superscripts are significant ($p < 0.05$) different. YTA=yoghurt with turmeric after fermentation, YTB=yoghurt with turmeric before fermentation, ND = Not Detected.

incorporated and the pH and viscosity of yoghurt sample without turmeric (that is, the control). The values for pH ranged from 4.68 ± 0.02 to 5.21 ± 0.07 (Table 6). Within the yoghurt, the pH increased with increase in the amount of turmeric added. This indicates that the addition of turmeric significantly increased the pH of the stirred yoghurt. Data presented in Table 6 also showed that all samples except sample YTB1 (95:5) had pH values significantly higher than that of the plain yoghurt. Considerable increase in pH of the yoghurt samples was also observed when pH of the samples before and after fermentation was compared. The values for viscosity ranged from 90.92 ± 0.17 to 120.65 ± 0.20 cP. Within the yoghurt, the viscosity decreased with increase in the amount of turmeric added.

Effect of turmeric extract on the microbial characteristics of the stirred yoghurt: Table 7 shows the total viable count, lactic acid bacteria, mould and coliform counts of the formulated stirred yoghurt. The mould count ranged from 3.0×10^1 cfu/ml in the plain yoghurt to a non-detectable (ND) amount in the sample YTB5(75:25) where turmeric extract was added before fermentation. The plain yoghurt, that is, Yoghurt (100:0) had the highest mould count while sample YTB5(75:25) had the lowest mould count. The total viable count (TVC) was within the range of 1.2×10^5 to 2.2×10^5 cfu/ml. The plain yoghurt was found to have the highest TVC (2.2×10^5 cfu/ml) while sample YTB5 (75:25) gave the lowest total viable count. Also, the Coliform Count of the yoghurt samples ranged from 0.4×10^1 to 1.4×10^1 cfu/ml. The lactic acid bacteria (LAB) Count of the samples ranged from 1.0×10^5 to 2.1×10^5 cfu / ml. Generally, total viable count (TVC), mould count, and coliform count decreased with increase in the amount of

turmeric added this could be attributed to anti-oxidant properties of turmeric extract.

Effect of different amount of turmeric on the sensory scores for stirred yoghurts: There were significant ($p < 0.05$) differences in colour, taste aftertaste, consistency, firmness and overall acceptability (Table 8). The plain yoghurt was most appealing (8.23 ± 1.45) as having the highest score while sample YTA5 (75:25) had the lowest score (5.43 ± 0.91). Data obtained also revealed that there was a decrease in the acceptability of colour as the level of turmeric added increased (Table 8).

This could be attributed to high intense colour in the samples due to the effect of curcumin, a colouring agent in the turmeric extract. The sample YTB1 (95:5) scored the highest for taste (8.87 ± 0.49), while the sample YTA5 (75:25) within the stirred yoghurt group had the lowest score (5.23 ± 1.07). The plain yoghurt scored (7.23 ± 1.48) for taste. There was a decrease in the overall acceptability of taste as higher amount of turmeric was incorporated. This could be traceable to a very characteristic spicy taste of turmeric in the samples thus changing the samples' taste from sweet to somewhat bitter taste. The sample YTA1(95:5) scored the highest in consistency (8.45 ± 1.26) and the sample YTA5 (75:25) had the lowest in consistency (5.21 ± 1.57). The panelists rated sample YTB1 (95:5) highest (8.43 ± 0.98) while sample YTB5(75:25) had the lowest score (5.21 ± 0.26) for firmness. This was so evident that the higher amount of turmeric reduced the consistency and firmness of the stirred yoghurt. The plain yoghurt (100:0) sample had the highest score in the overall acceptability (8.86 ± 0.84) while sample YTB5 (75:25) had the lowest score (5.67 ± 1.00). Samples YTB5 (75:25) and YTB3 (85:15) had the lowest (5.24 ± 0.50) and highest score

Table 8. Sensory scores of the formulated stirred yoghurt.

Sample	Colour	Taste	Aftertaste	Consistency	Firmness	Overall acceptability
YTA1(95:5)	7.40 ^c ± 1.26	8.80 ^b ± 0.49	7.24 ^c ± 0.50	8.45 ^a ± 1.26	7.98 ^c ± 0.44	8.21 ± 0.80
YTA2(90:10)	7.01 ^d ± 1.04	7.52 ^d ± 1.91	7.02 ^d ± 1.91	7.42 ^d ± 1.35	7.44 ^d ± 0.38	7.77 ± 1.84
YTA3(85:15)	6.21 ^h ± 0.77	6.97 ^f ± 1.14	7.23 ^c ± 1.63	6.41 ^g ± 0.99	6.43 ^g ± 0.09	7.03 ± 1.10
YTA4(80:20)	6.01 ⁱ ± 0.85	6.42 ^g ± 1.05	6.15 ^f ± 1.28	5.98 ^g ± 1.39	5.22 ^j ± 0.52	6.67 ± 1.24
YTA5(75:25)	5.43 ^k ± 0.91	5.23 ^j ± 1.07	5.90 ^g ± 1.33	5.21 ^j ± 1.57	5.53 ^g ± 0.37	5.77 ⁱ ± 0.75
YTB1(95:5)	7.54 ^b ± 1.26	8.87 ^a ± 0.49	7.23 ^c ± 0.50	8.23 ^b ± 1.35	8.43 ^b ± 0.98	8.39 ^a ± 1.13
YTB2(90:10)	7.03 ^c ± 1.64	7.91 ^c ± 2.03	7.41 ^c ± 0.89	7.6 ^c ± 1.81	7.43 ^d ± 0.57	7.55 ^d ± 1.01
YTB3(85:15)	6.45 ^e ± 1.14	6.43 ^g ± 1.45	7.44 ^a ± 1.29	6.98 ^f ± 1.26	6.77 ^e ± 0.36	6.87 ^f ± 1.57
YTB4(80:20)	6.23 ^f ± 1.07	6.00 ^h ± 1.49	6.43 ^e ± 0.89	5.55 ^h ± 1.51	5.41 ⁱ ± 0.81	6.33 ^h ± 1.45
YTB5(75:25)	5.47 ^j ± 0.49	5.43 ⁱ ± 0.46	5.24 ^h ± 0.50	5.24 ^j ± 0.47	5.21 ^j ± 0.26	5.67 ^j ± 1.00
Yoghurt	8.23 ^e ± 1.45	7.23 ^e ± 1.48	7.41 ^b ± 1.63	7.13 ^e ± 0.28	7.21 ^e ± 0.51	8.86 ^a ± 0.84

respectively for after taste.

DISCUSSION

Effect of different levels of turmeric on the proximate composition of formulated stirred yoghurt

The significant ($p < 0.05$) increase in the moisture content of yoghurt samples formulated with graded levels of turmeric extracts when compared to the moisture content of the control sample (YOGHURT) could be traced to the reduction in the water holding capacity of milk by the extracts before fermentation. This observation was in agreement with the result obtained by Akande and Adegoke (2018) in which there was increase in moisture content of spiced yoghurt. According to Ammon et al. (1992), the marked increase in the moisture content of yoghurt formulated with spices could be attributed to the antibacterial mechanism exhibited by the spices involving formation of water in the electron transport system. For the yoghurt samples examined in this research, the protein contents of those samples with turmeric extracts (before and after fermentation) were lower than that of the plain yoghurt. This significant ($p < 0.05$) decrease could be traced to the presence of proteolytic enzymes (proteases) in the turmeric extract incorporated in the yoghurt samples which degrade proteins into peptides and amino acids. This assertion was in agreement with Nagarathnam et al. (2010). Altogether, the protein contents of the yoghurt samples did not exceed the commercial yoghurts' recommended range (11-18%) of proteins prescribed by the National Yoghurt Association. Yoghurt, like ice cream, is a milk-and-water-based dairy product which is poor in fibre level (Cheeseman and Lean, 2000). Result showed that ash content decreased with increase in the amount of turmeric added. This is attributed to low ash content of the turmeric extract. The ash contents of the turmeric-containing yoghurt samples before and after fermentation were low compared to the

plain yoghurt sample (without turmeric extract). This finding was in agreement with Akande and Adegoke (2018). According to U.S. Food and Drug Association, low fat yoghurt must contain 0.5 to 2% fat while regular yoghurt must be no less than 3.25% fat (Food Source Information – Colorado, 2018). The yoghurt samples examined in this study had low fat contents. This was attributed to the low fat content of the skimmed milk which was used as a major ingredient for yoghurts. The carbohydrate content of the yoghurt samples containing turmeric extracts increased with increase in the amount of turmeric added when compared to the carbohydrate content of plain yoghurt. This increase could be traced to carbohydrate present in the turmeric (Table 2).

Effect of different levels of turmeric on the physicochemical properties of the stirred yoghurt

The incorporation of turmeric extract in yoghurt before and after fermentation showed significant ($p < 0.05$) increase in pH leading to decrease in acidity. This observation could be attributed to the alkaline nature of turmeric itself. Decrease in viscosity was observed in the viscosity of stirred yoghurt formulated with turmeric extract as compared to the value obtained for the plain yoghurt sample. This observation correlates with high moisture content of the yoghurt samples in which turmeric extracts were incorporated. Thus, the higher the moisture content, the less viscous the samples become (and vice-versa).

Effect of different levels of turmeric on the micronutrient composition of the stirred yoghurt

The incorporation of turmeric extract (before and after fermentation) at different levels in the stirred yoghurt samples showed significant ($p < 0.05$) improvement in the vitamin B₂, B₃, and B₁₂. However, vitamins C and B₆ contents of each of the stirred yoghurt samples



Plate 3. Formulated Yoghurt samples.

formulated with turmeric extracts (Plate 3) significantly decreased with increase in the levels of turmeric. The significant increase ($p < 0.05$) in vitamins B₂, B₃, and B₁₂ could be attributed to the starter culture used in the stirred yoghurt samples. Lactic acid producing bacteria have been reported to produce or utilize B-group vitamins to meet their nutritional requirement during fermentation (Snell, 1993). The turmeric extract incorporated in the stirred yoghurts have been discovered to be rich in B-group vitamins especially vitamins B₂, B₃, and B₁₂ and some mineral elements (Table 2). So, the contributions from turmeric extract in the stirred yoghurt and starter culture during fermentation could be categorically pointed to as the factors leading to the significant ($p < 0.05$) improvement of vitamins B₂, B₃, and B₁₂ in the stirred yoghurt samples. Calvince et al. (2019) reported that fermentation caused marked increase in niacin (vitamin B₃) of milk. This is consistent with the result of Gu and Li (2016).

Capozzi et al. (2012) explained that B-group vitamins are present in a number of foods but are easily destroyed or removed during food processing and that succinctly explains why their deficiencies are commonly found a large population. Vitamins C and B₆ decreased with increasing concentration of turmeric in the stirred yoghurt samples. These vitamins are heat-labile and can be destroyed or removed during pasteurization (80 to 85°C) and inoculation (40 to 45°C). Moreso, vitamins C and B₆ are vital nutritional requirements for lactic acid bacteria

(LAB). The more the lactic acid bacteria present in the sample, the less the amount of vitamins C and B₆ turnout.

From the mineral analysis of the samples, there were significant ($p < 0.05$) improvement in the Calcium (Ca) and Phosphorus (P) of the stirred yoghurt samples wherein turmeric were incorporated. The aforementioned deductions corroborate with the reports of Hale et al. (2010), Ihemeje et al. (2015) and Mbaeyi and Anyanwu (2010). The results agreed with the assertion of Gray (2007) in which the author reported that yoghurt is a good dairy product and a source of indispensable minerals required for human metabolism and cells' functionality.

Effect of different levels of turmeric on the Microbial qualities of the stirred yoghurt

The total viable count, coliform count, Lactic Acid Bacteria (LAB) count and mould count of the stirred yoghurt samples formulated with turmeric extracts were compared to study the effect of addition of turmeric extract before fermentation and after fermentation, with the plain yoghurt sample. When compared with the plain yoghurt sample, the mould count of the yoghurt-turmeric samples before fermentation decreased from 2.1×10^1 to 0.1×10^1 then became undetectable as the levels of turmeric increased. Similar trend was observed after fermentation where the mould count decreased from 5×10^1 to 4×10^1 then became not detectable (ND) at 0.3, 0.4

and 0.5% of turmeric extract incorporated. This could be traced to the antimicrobial effect of turmeric extract on the samples. This finding was in line with the report by Akande and Adegoke (2018) on production, microbiological and quality evaluation of low-fat spiced yoghurts with low glycemic loads. The total viable count of the samples decreased far more before fermentation (1.9×10^5 to 1.2×10^5) than what it was after fermentation (2.0×10^5 to 1.5×10^5). Fermentation increased the amount of viable microorganisms in the samples. The total viable count levels are very much below the acceptable range (0.0 - 8.7 cfu) according to National Yoghurt Association (NYA, 2000). Similar trend as the mould count was obtained for the coliform count of the samples. Upon the incorporation of turmeric extract, the samples' coliforms decreased to a non-detectable level before and after fermentation. It has been suggested that yoghurt should contain abundant and viable organisms of starter origin or above 1.0×10^4 cfu/ml of the starter culture organisms (FAO and WHO, 2003) and, whichever format is adopted, there is a general agreement that yoghurt should contain live bacteria unless specifically designated as pasteurized or heat treated (Tamime and Robinson, 2007). Thus the values were consistent with the standard. Generally, total viable count and lactic acid bacteria count decreased with increase in concentration and this could be attributed to the conditions of fermentation which did not favour the rapid growth of microorganisms.

Effect of different levels of turmeric on the sensory characteristics of the stirred yoghurt

There were significant ($p < 0.05$) differences in colour, taste, aftertaste, consistency, firmness and overall acceptability (Table 7). The sample YTB1 (95:5) scored the highest for colour (8.24 ± 0.01), while the sample YTA5 (75:25) within the stirred yoghurt group, had the lowest score (5.43 ± 0.01). The plain yoghurt scored (7.93 ± 0.01). There was a decrease in the acceptability of colour as higher amount of turmeric added this is attributed to high intense of colour in the sample due to the effect of curcumin a colouring agent in the turmeric extract. The sample YTB1 (95:5) scored the highest for taste (8.87 ± 0.01), while the sample YTA5 (75:25) within the stirred yoghurt group had the lowest score (5.63 ± 0.01). The plain yoghurt scored (7.23 ± 0.01) for taste. There was a decrease in the acceptability of taste as higher amount of turmeric was added. This could be attributed to high intense of the spice taste of turmeric impacting a bitter taste in the sample. The plain yoghurt scored the highest in consistency (8.13 ± 0.01) and firmness (8.21 ± 0.01), and the sample YTA5 (75:25) had the lowest in consistency (5.28 ± 0.01) and firmness (5.53 ± 0.01). This was evident in that, higher amount of turmeric reduces the consistency and firmness of the stirred yoghurt. The sample YTB1 (95:5) has the highest

score in the overall acceptability of the whole samples.

Conclusion

This study shows that turmeric extracted with ethanol have higher nutrient composition than turmeric extracted with water. The different concentrations of turmeric affected the nutritional composition of the yoghurt. pH increased with increase in concentration of the turmeric due to the alkalinity of the turmeric. The use of turmeric affected the colour of the yoghurt, changing it from white to yellowish-orange due to the curcumin in the turmeric. Although the protein and carbohydrate contents of the yoghurt samples formulated decreased with increasing concentrations of turmeric, the minerals (calcium and phosphorus) and vitamins (B_2 , B_3 , and B_{12}) improved significantly ($p < 0.05$) between the range of 100:0 to 75:25 before and after fermentation. There was a decrease in microbial load of the yoghurt as the concentration of turmeric increases due to the fact that turmeric possesses antimicrobial ability thus increasing the keeping quality of the yoghurt. In terms of overall acceptability, the stirred yoghurt sample with 0.1% turmeric (that is, YTB1 (95:5) was most preferred sample. Therefore, the yoghurt: turmeric concentration of 90:10 and less should be used to achieve the taste effect and colour of the stirred yoghurt.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors would like to appreciate Prof. T.M. Okonkwo of the Department of Food Science and Technology, University of Nigeria Nsukka for his support and encouragement.

REFERENCES

- Ammon HP, Anazodo M, Safayh H, Dhawan BN, Srimal RC (1992). Curcumin: A potent inhibitor of Leukotriene B₄ formation in rat peritoneal polymorphonuclear neutrophils (PMNL). *Planta Med* 58:226.
- Akande AA, Adegoke GO (2018). Production, Microbiological and Quality Evaluation of Low-fat Yoghurts with Low Glycemic Loads. *African Journal of Food, Agriculture, Nutrition and Development* 18(2):13287-13303.
- AOAC (2005). Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists. Washington DC, USA.
- AOAC (2010). Official Methods of Analysis. Association of Official Analytical Chemists. 18th Ed. Gaithersburg, Maryland, USA.
- Bar-sela G, Epelbaum R, Schaffer M (2010). Curcumin as an anti-cancer agent: review of the gap between basic and clinical applications. *Current Medicinal Chemistry* 17:190-197.
- Boghra VR, Mathur ON (2000). Physico-chemical status of major milk constituents and minerals at various stages of shrikhand preparation.

- Journal of Food Science and Technology 37:111-115.
- Brul S, Coote P (1999). Preservative agents in foods: mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology* 50(1-2):1-17.
- Calvince A, Arnold O, Samuel I, Julius M (2019). Effect of Lactic Acid Bacteria Starter Cultures on Vitamins and Oligosaccharide Composition of ilk extracted from Three Common Bean (*Phaseolous vulgaris* L.) varieties. *Journal of Food Research Archives* 8:3.
- Capozzi V, Russo P, Dueñas MT, López P, Spano G (2012). Lactic Acid Bacteria producing B-group vitamins: A great potential for functional cereal products. *Applied Microbiological Technology* 96:1383-1394.
- Cheeseman GC, Lean MC (2000). Yoghurt Nutritional and Health Properties. *Journal of National Yoghurt Association* 3:35.
- Cho JY, Choi GJ, Lee SW, Lim HK, Jang KS, Lim CH, Cho KY, Kim JC (2006). *In vivo* antifungal activities against various plant pathogenic fungi of curcuminoids isolated from the rhizomes of *Curcuma longa*. *Plant Pathology Journal* 22:94-96.
- Cousins M, Adelberg J, Chen F, Rieck J (2007). Antioxidant capacity of fresh and dried rhizomes from four clones of turmeric (*Curcuma longa* L.) grown in vitro. *Industrial Crop Production* 25:129-135.
- FAO, WHO (2003). Codex Alimentarius: abridged version. Joint FAO/WHO Food Standards Programme: Codex Alimentarius Commission. Smith BL (Ed). Food and Agricultural Organization of the United Nations, Rome.
- Food Source Information- Colorado (2018). Yoghurt / Types of Yoghurts. Available at: www.fsi-colorado.edu/yoghurt.
- Gomez KA, Gomez AA (1985). *Statistical Procedures for Agricultural Research*, 2nd Edition. Wiley and Sons Publishers, New York, pp. 45-67.
- Gu Q, Li P (2016). Biosynthesis of vitamins by Probiotic Bacteria. In *Probiotics and Prebiotics in Humans and Health*, pp.136-148. China: INTECH: Open Science Open Mind.
- Gray C (2007). *Yoghurt and Your Health*. Star Base Publishers, Washington. pp. 6-8.
- Hussain JA, Bahader N, Rehman AL, Khan W, Ullah SZK (2010). Proximate And Nutrient Analysis of the Locally Manufactured Herbal Medicines and its Raw Material. *Journal of America Science* 6(5):91-96.
- Ihekoronye AI, Ngoddy PO (1985). *Integrated Food Science and Technology for the Tropics*. First edition Macmillan Publishers, pp. 383.
- Ihemeje A, Nwachukwu CN, Obi KC, Ekwe CC (2015). Production and Quality Evaluation of flavoured Yoghurts using Carrot, Pineapple and Spiced Yoghurts using Ginger and Pepper fruit. *African Journal of Food Science* 9(3):163.
- Jurenka JS (2009). Anti-inflammatory Properties of Curcumin, a Major Constituent of *Curcuma longa*: A Review of Preclinical and clinical research. *Alternative Medicine Reviews* 14(2):141-153.
- Kamruzzaman M, Islam MN, Raman MM, Parvin S, Rahman MF (2002). Evaporation Rate of Moisture from Dahi (Yoghurt) during Storage at Refrigerated Condition. *Pakistan Journal of Nutrition* 1:209-211.
- Lee WJ, Lucey JA (2010). Formation and physical properties of yoghurt. *Asian-Australian Journal of Animal Science* 23(9):1127-1136
- Mbaeyi IE, Anyanwu LN (2010). Production and Evaluation of Yoghurt flavoured with Solar-dried Bush Mango (*Irvingiagabonensis*) Pulp. *Journal of Tropical Agriculture, Food, Environment and Extension* 9(2):137-146.
- McKinley MC (2005). The Nutrition and Health Benefits of Yoghurt – Review. *Society of Dairy Technology* 58:1-12.
- Nagarathnam R, Rengasamy A, Balasubramanian R (2010). Purification and Properties of Cysteine Protease from Rhizomes of *Curcuma longa* (Linn). *Journal of the Science of Food and Agriculture* 90(1):97-105.
- National Yoghurt Association, NYA (2000). *Yoghurt Varieties*. Available at <http://about.yogurt.com/index>.
- Onwuka GJ (2005). *Food Analysis and Instrumentation Theory and Practice*. Naphthali Prints, Lagos, Nigeria, pp. 64-76.
- Pearson DA (1976). *Chemical Analysis of Foods*. 7th Ed. Churchill Livingstone, New York, pp. 218-336.
- Prescott LM, Harley JP, Klein OA (2005). *Microbial Nutrition: Types of Media*. Microbiology 6th edition. McGraw Hill Publishers, New York, pp. 93-105.
- Remadevi R, Surendra E, Kimura T (2007). Turmeric in Traditional Medicine. In: *Turmeric: the genus Curcuma*, Ravindran PN, Nirmal BN, Sivaraman K Ed. CRC press: Boca Raton, London New York. pp. 409-436.
- Snell EE (1993). From Bacterial Nutrition to Enzyme Structure: A Personal Odyssey. *Annual Review of Biochemistry* 62:1-27.
- Tamime AY and Robinson RK (2007). Yoghurt: Technology and Biochemistry. *Journal of Food Protection* 43:939-977.
- Tonnesen HH (2002). Alginate in Drug Delivery Systems. *Drug Development and Industrial Pharmacy* 28(6):621-630.

Related Journals:

