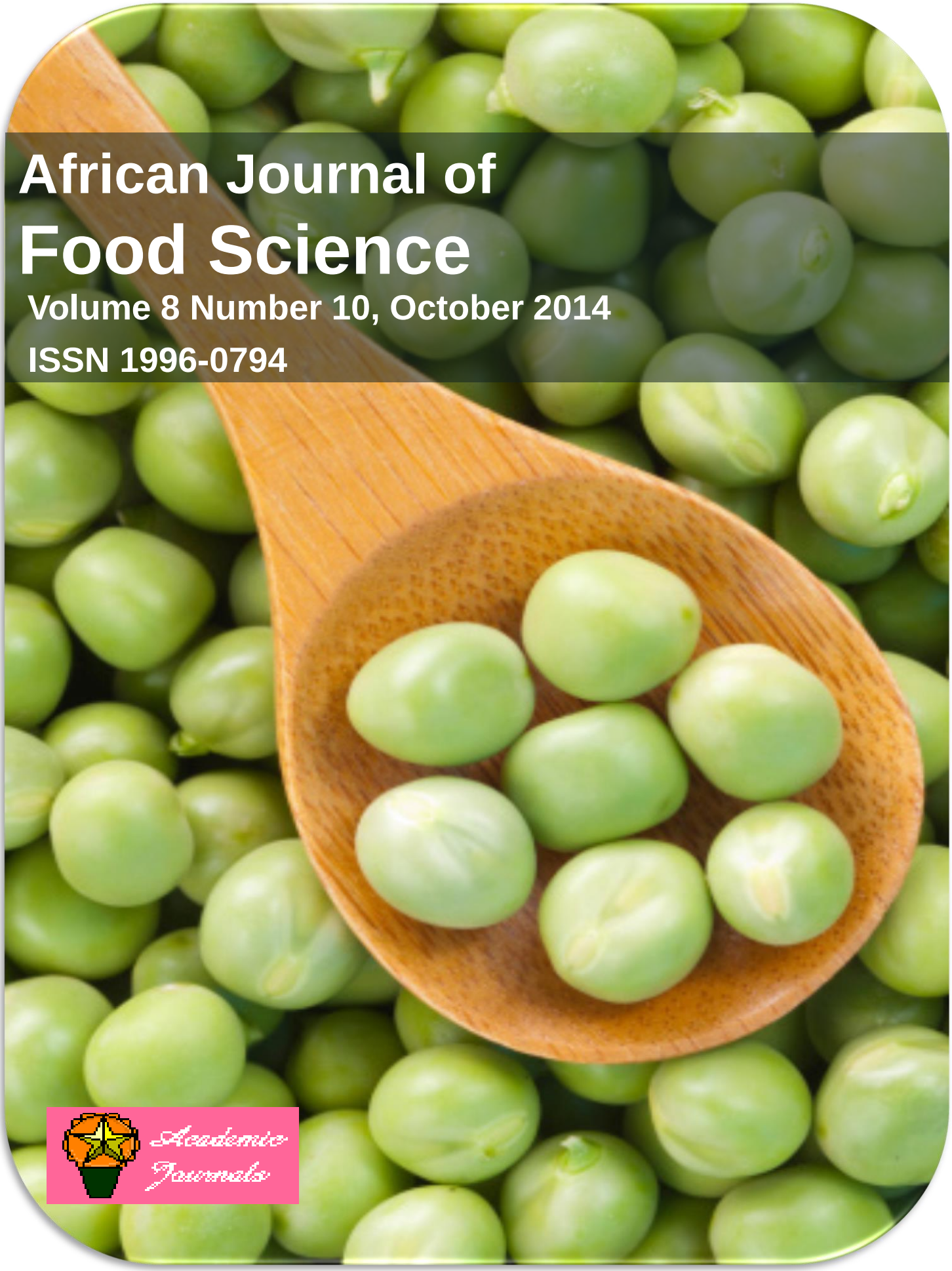

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Physico-chemical and pasting properties of starch from three plantain cultivars grown in Nigeria

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Starch was extracted from three different plantain (*Musa paradisiaca*) cultivars: agbagba, cadaba and French horn, and the physico-chemical and pasting properties of the starch were investigated. The percentage starch yield ranges from 6.67 (cadaba) to 15.00% (French horn). pH values fall between 4.46 to 5.50 with the highest pH occurring in cadaba (5.50). A higher moisture content of 13.15% was observed in French horn which was significantly different ($p \geq 0.05$) from 11.56% recorded in Agbagba. The swelling power showed no significant difference ($p \geq 0.05$) between the cultivars studied. However, Agbagba cultivar had higher starch solubility (7.49%) as compared to other cultivars. Although, a higher amylose (%) was observed in three cultivars (29.96 - 30.91%), it showed no significant difference ($p \geq 0.05$) which also affected the amylopectin. The peak viscosity and trough was higher in starches from French horn (163.17 and 135.00 RVU, respectively) with the lowest breakdown viscosity of 28.17 RVU. Pasting temperature was observed to be higher in Agbagba (94.75°C) with a corresponding peak time of 5.58 min; however there was no significant difference ($p \geq 0.05$) in peak time (which is an indication of cooking time) between the cultivars studied.

Key words: Plantain starch, cultivars, pasting properties, physico-chemical properties.

INTRODUCTION

Plantain (*Musa paradisiaca*), a member of the banana family, originating in Southeast Asia serves as a major starchy staple food with great economic value in many parts of the African Subcontinent. It provides a rich source of dietary energy and also a good source of carbohydrate (Adeniji et al., 2007). The efforts of research bodies like International Institute of Tropical Agriculture (IITA) has developed several cultivars of plantain that has led to availability of high yielding varieties of plantain, disease and pest-resistant, combined with good post-harvest qualities (FAO, 2005). Almost all the known edible-fruited cultivars arose from two diploid species, *Musa acuminata* and *Musa balbisiana* (Simmonds, 1996).

Nigeria is one of the largest plantain producing countries in the world (FAO, 2006). However, about 35 to 60% post- harvest losses had been reported and attributed to lack of storage facilities and inappropriate

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technologies for food processing. Technological improvement in methods for long term preservation could thus be a way of attending to this problem (Anon, 1991). Unripe plantain can be peeled, diced and dried to make chips. Since unripe plantain contain large amounts of starch (over 80% of dry weight), their processing into flour and starch is of interest as a possible resource for food and/or other industrial purposes. Starch contributes greatly to the textural properties of many foods and is widely used in food and industrial applications as thickener, stabilizer and gelling agent (Marriot et al., 1981).

The starch in unripe plantain consists of mainly amylose and amylopectin and this is replaced by sucrose, fructose and glucose during ripening. The relative proportion of amylose and amylopectin and the organization within solid granules determine the physicochemical and functional properties of starch, as well as the susceptibility for physical (for example, gelatinization) and chemical modifications (for example, hydrolysis). Da mota et al. (2000) and Bihaderis et al. (1993) reported that the type of the starch used is a critical factor in the modification of food texture rather than the amount of starch used.

Pasting properties is an important index in determining the quality of starch. Plantain starches have been reported to have potentials for commercial utilization due to its pasting properties and physiochemical properties (Saifullah et al., 2009; Odenigbo et al., 2013). Knowledge on these starch and physiochemical properties of various cultivars of plantain in Nigeria could enhance the potential commercial utilization of plantain starch in food and pharmaceutical industry. The objective of this study was therefore to produce plantain starch from different plantain cultivars; as well as investigate their physicochemical and pasting properties.

MATERIALS AND METHODS

Three bunches of unripe plantain cultivars: Agbagba, Cadaba and French horn was bought from Rivers State Agriculture Development Programme farm (ADP) Rumuodumaya, Port Harcourt, Nigeria and transported to the Department of Food Science and Technology Laboratory for processing.

Extraction of starch

Starch was extracted from the different plantain cultivars by a modification of the method of Kim et al. (1995). The fruits were weighed (6 kg), washed, peeled and the pulp diced into 5-6 cm cubes for easy disintegration during maceration. They were then macerated at low speed in a Waring blender (McConnellsburg, PA) with water (1:4 w/v) for about 2 min. The slurry was then filtered through a muslin cloth.

The filtrate was allowed to stand for 2 h for starch to settle and the supernatant was discarded. The sediment (starch) was put in a jute bag and pressed to remove water. The starch was dispersed on aluminum foil and oven dried in an air oven (DHG 9140A) at 55°C for 12 h. Dried starch samples were milled, sieved, weighed and their weights noted, packaged and sealed in polythene bag prior to analyses. The starch yield was calculated using the formular below:

$$\text{Starch yield (\%)} = \frac{W1}{W2} \times 100$$

Where W1 is the weight of starch extracted in kg from a known weight (W2) of the plantain fingers. W1 = weight of dried starch; W2 = weight of plantain.

Physico-chemical properties of starch

The pH, moisture content and ash content of starch from the three plantain cultivars were determined using the method of AOAC (1990). The percentage solubility and swelling power were determined according to the method described by Svarovsky (1987); and the water binding capacity of starch was done using the method of Medcalf and Gille (1965).

Determination of percentage amylase and amylopectin

The amylose content of starch extracted from the three plantain cultivars was determined using the iodine colorimetric method reported by Zakpaa et al. (2010). A standard curve for amylose was prepared using different concentrations ranging from 0 - 70 mg of pure amylase. These were weighed into separate 100 ml volumetric flasks and 1 ml of ethanol, 10 ml of distilled water and 2 ml of 10% NaOH were added. The flask with their contents was heated in water bath until a clear solution was obtained. The samples were cooled and diluted to mark with distilled water. Five (5 ml) of the solution was measured into a 500 ml volumetric flask and 100 ml of distilled water added and immediately acidified with drops of 6 M Hcl. The contents were mixed before 5 ml of iodine solution was added and made up to mark with distilled water. The absorbance of each standard was measured using a spectrophotometer (UV - visible model 754, China) at a wavelength of 640 nm. 100 mg of plantain starch was weighed into 100 ml volumetric flask and the above procedures repeated. The concentration of amylose was calculated from the standard curve. The percentage amylopectin was calculated from the amylose obtained.

Pasting properties

The pasting properties of the flours were characterized by using Rapid Visco Analyzer (RVA) (model 3c, Newport scientific PTV Ltd, Sydney) as described by Delcour et al. (2000) and Sanni et al. (2006). 2.5 g of each sample was accurately weighed into a weighing vessel, 25 ml of distilled water was dispensed into a new canister. Samples were transferred into the water surface of the canister, after which the paddle was placed into the canister. The blade was vigorously joggled up and down through the sample ten times or more until no flour lumps remained either on the water surface or on the paddle. The paddle was placed into the canister and both were inserted firmly into the paddle coupling, so that the paddle is properly centred. The measurement cycle was initiated by depressing the motor tower of the instrument. The test was then allowed to process and terminated automatically.

RESULTS AND DISCUSSION

The result for the physico-chemical properties are as presented in Table 1. Values for moisture content ranged

Table 1. Physico-chemical properties of starch from three different cultivars of plantain.

Parameter	French horn	Cadaba	Agbagba
Moisture content (%)	11.56 ± 0.05[b]	12.86 ± 0.05[a]	13.15 ± 0.020[a]
Ash (%)	0.05 ± 0.03[a]	0.29 ± 0.09[b]	0.45 ± 0.02[ab]
Swelling power (%)	9.48 ± 0.42[a]	10.76 ± 0.72[a]	10.10 ± 0.69[a]
Solubility (%)	7.49 ± 1.70[a]	3.55 ± 0.04[b]	5.02 ± 0.76[ab]
WBC (%)	65.50 ± 1.70[a]	54.40 ± 0.14[b]	62.90 ± 0.28[a]
Amylase (%)	29.96 ± 0.14[a]	30.66 ± 0.28[a]	30.91 ± 0.21[a]
Amylopectin (%)	70.04 ± 0.14[a]	69.34 ± 0.28[a]	69.09 ± 0.21[a]
pH	4.46 ± 0.09[a]	5.50 ± 0.14[a]	4.70 ± 0.56[a]
Starch yield (%)	15.00 ± 0.01[a]	6.67 ± 0.02[c]	13.30 ± 0.04[b]

Mean ± SD of duplicate determinations. Values in the same row with different superscripts differ significantly ($p < 0.05$). WBC; water binding capacity.

from 11.56% in agbagba to 13.15% in French horn. The values obtained for moisture content falls within the range of 10 to 13.5% reported by Onwueme (1982). Agbagba starch with the least moisture content of 11.56% gave the highest value for ash (0.5%). The result for pH falls between 4.46 to 5.50. Onwueme (1982) also reported pH values of 4.47 - 5.50 for a good starch quality. Swelling power ranged from 9.48 (Agbagba) to 10.76% in Cadaba. These values were comparable with the observation of Zakpaa et al. (2010) who reported swelling power of 10.28% for giant horn plantain. Safo-Kantanka and Acquistucci (1996) reported that the swelling power of a starch based food is an indication of the strength of the hydrogen bonding between the granules. Eke-Ejiofor and Owuno (2012) also reported the findings of Richard et al. (1991) which further described swelling power as a factor of the ratio of amylose to amylopectin, the characteristics of each fraction in terms of molecular weight/distribution, degree/length of branching and conformation. Swinkels (1985) reported that a good quality starch has a low solubility and a higher swelling power. There was no significant difference ($P \geq 0.05$) in swelling power values for all the starches. The solubility of cadaba starch (3.55%) was significantly lower than the other two cultivars. Solubility according to Hari et al. (1989) reflects the extent of intermolecular cross bonding within the granule. Values for solubility for French horn was 5.02% and Agbagba (7.49%), the value for French horn is close to the value of 5.28% reported by Zakpaa et al, (2010) for giant horn plantain starch.

The water binding capacity (WBC) of the plantain starches ranged from 54.40 - 65.50%. Water absorption capacity is the ability of flour particles to entrap large amount of water such that exudation is prevented. Niba et al. (2001) described water absorption capacity as an important processing parameter that has implications for viscosity, while Wooten and Bamnuaruchi (1978) describes it as important in determining the quality and texture of some food products because it stabilizes them against effects such as syneresis which sometimes occur during retorting and freezing. The WBC value of 54.40% obtained for cadaba was very close to the value of 54.07% obtained for giant horn by Zakpaa et al. (2010).

Amylose levels of the starches ranged from 29.96 - 30.91% with cadaba and French horn significantly different from agbagba ($P \geq 0.05$). Michael (1990) reported that amylose level in starches range between 21 to 30%. The values obtained in the study falls within the range given by Michael (1990). Result for amylopectin showed Agbagba was significantly higher with a value of 70.04%. Values for starch yield ranged from 6.67% in cadaba to 15% in French horn.

The result of pasting characteristics is as shown in Figures 1, 2 and 3. Peak viscosity which is the maximum viscosity developed during or soon after the heating process is lower in agbagba starch (133.50 RVU) and higher in French horn (163.17 RVU). Peak viscosity is a measure of the ability of starch to form a paste on cooking (Adewole et al., 2012).

Adewole et al. (2012) reported a value of 166.00 RVU for plantain starch which was comparable to values obtained for French horn (163.17 RVU) and Cadaba (161.17 RVU). This high viscosity showed that the starches formed a paste on cooking with a corresponding pasting temperature of 86.05 and 85.95°C for French horn and Cadaba, respectively. Peak viscosity is indicative of the strength of pastes, which are formed from gelatinization during processing in food application. It also reflects the extent of granule swelling (Liang and King, 2003).

Trough values ranged from 85.08 to 135.00 RVU. French horn gave the highest trough (hold) value. These values were lower than 141.75RVU reported by Adewole et al. (2012) which could be as a result of varietal or environmental differences. The holding strength is the ability of granules to remain undisrupted when the starch

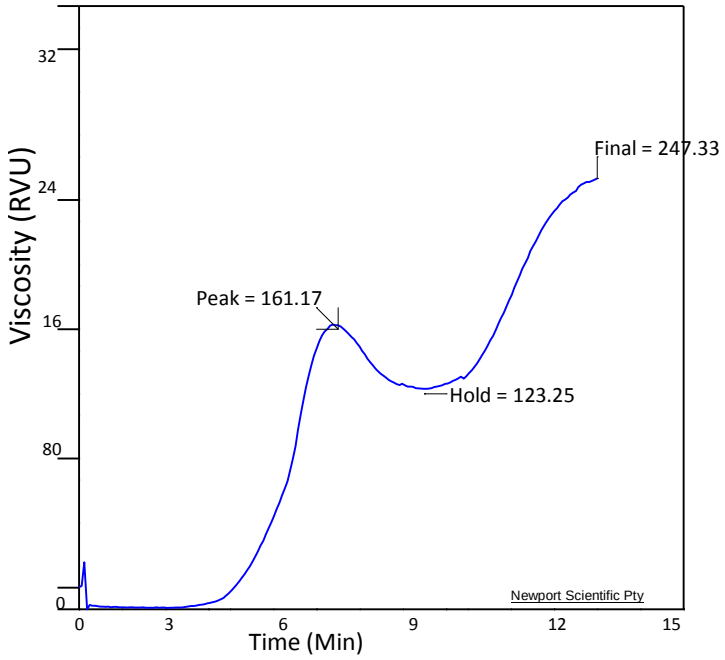

Figure 1. Pasting curve of cadaba plantain starch.

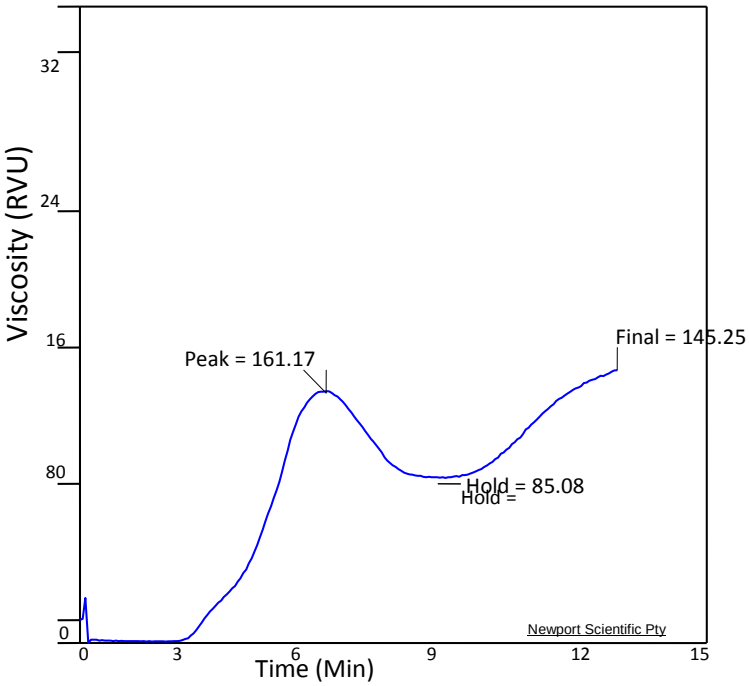

Figure 2. Pasting curve for agbagba plantain starch.

is subjected to a period of constant high temperature and mechanical shear stress, this hold period is often accompanied by a breakdown in viscosity. The breakdown was highest in Agbagba (48.42RVU) and least in French horn (28.17RUV). Adewole et al. (2012) reported values of 24.45 RVU for plantain and 29.42 RVU for banana

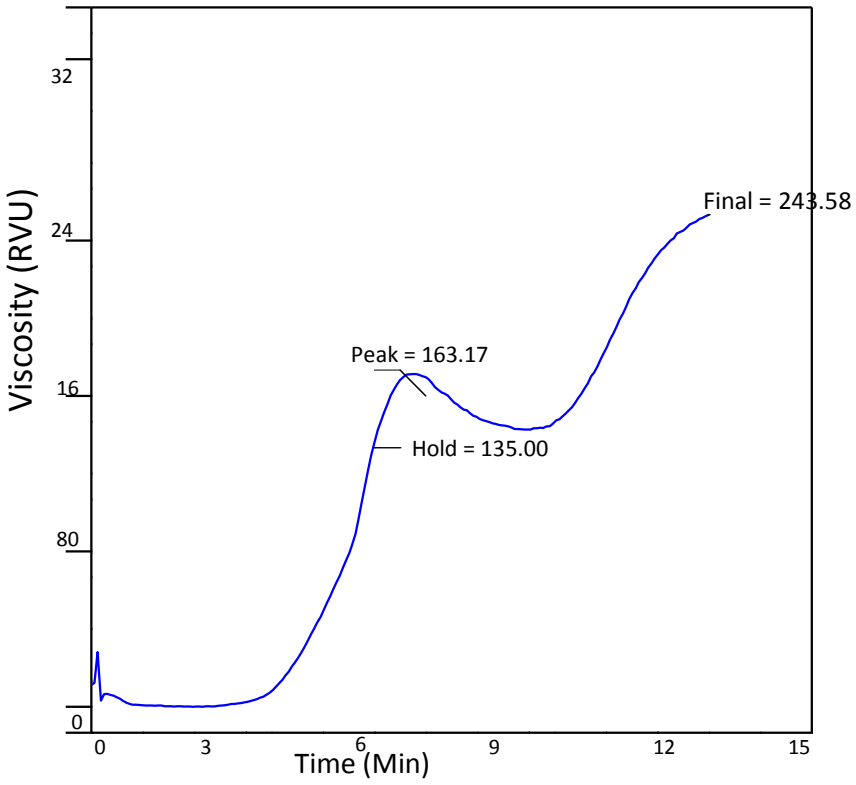

Figure 3. Pasting curve of french horn plantain starch.

starches.

The ability of starch to withstand shear thinning or breakdown in viscosity (that is high breakdown value) is of high industrial significance in starches (I.I.T.A, 2011), thus all the starches obtained in this study will be useful in industrial food applications. The final viscosity ranged from 145.25 RVU in Agbagba to 247.33 RVU in cadaba. These values are lower than the value of 298.67 RVU reported for plantain starch by Adewole et al. (2012). Values for setback viscosity ranged from 60.17 RVU in Agbagba to 124.08 RVU in Cadaba. Final viscosity is an important parameter in predicting and defining the final and textural quality of foods (Kramer and Twigg, 1970). High set back viscosity is associated with a cohesive paste, while a low setback viscosity is indicative of a non-cohesive paste (IITA, 2011). This shows that starches from cadaba and French horn will produce a more cohesive paste, these values correlate with values for final viscosity which was also higher in cadaba and French horn indicating that the two starches will be more useful industrially as compared to Agbagba. Bihaderis et al. (1993) and Brooks and Schiltach (1999) reported the use of starches with high viscosity value as tablet binders in pharmaceutical companies. The time to attain peak viscosity ranged from 5.35 min in French horn to 5.68 min in cadaba.

Conclusion

The starches extracted from the cultivars possess good physico-chemical and pasting properties and could be utilized in plantain based food and pharmaceutical products. Starch from French horn cultivar had a better yield and lower value of breakdown viscosity indicating a more stable paste formation.

Conflict of Interests

The author(s) did not declare any conflict of interests.

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Antimicrobial activity of lactic acid bacteria isolated from fermented milk products

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The bacteriocins produced by lactic acid bacteria (LAB) which acted as antimicrobial substances or preservative component on fermented food product (yoghurt, kunu and fufu) was investigated against selected indicator food spoilage causing bacteria and antibiotics. The agar-well diffusion assay was employed to investigate the antagonistic activity against indicator organisms (*Staphylococcus aureus*, *Shigella* spp., *Salmonella typhi* and *Escherichia coli*). *Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus delbrueckii* sub *bulgaricus, Lactobacillus amylophilus, Lactococcus lactis* and *Leuconostoc mesenteroides* were isolated from yoghurt, fufu and kunu. *L. lactis* showed the largest inhibition zone against *S. typhi* and *S. aureus* as 19 and 14 mm, respectively, while *L. amylophilus* showed antimicrobial activity against all the indicator organisms. Isolates from fufu generally showed more inhibition on *S. aureus*, *S. typhi* and *E. coli,* while *L. plantarum* showed no inhibitory zone against the indicator organisms. All the isolates were susceptible to ciprofloxacin, but resistant to ampiclox, zinnacef and amoxacillin, while variable susceptibility/resistance to pefloxacin, gentamycin rocephin, streptomycin, septrin and erythromycin was observed. LAB showed inhibitory properties against the food spoilage and pathogenic organisms tested, and have been reported to produce various antimicrobial compounds including organic acid, hydrogen peroxide, di-acetyl and bacteriocin. This study is important in risk assessment related to fermented food products, as well as the potential use of these LAB isolates in pharmaceutical industries.

Key words: Lactic acid bacteria, fermented foods, yoghurt, 'kunu', 'fufu.

INTRODUCTION

Lactic acid bacteria (LAB) are widely distributed in nature. They are typically involved in a large number of the spontaneous food fermentation, and they have been extensively studied for their use in the production of indigenous fermented foods (Holzapfel et al., 1995). Members of LAB produce bacteriocins and bacteriocins-like substances which may inhibit growth of spoilage and pathogenic microorganisms (Klaenhammer, 1988). Lactic acid fermentation of cereal-based foods and mammary glands derivatives (milk) is a traditional technology in Africa and has long been used in the processing of different foods (Mensah, 1997; Oyewole, 1997). Furthermore, LAB contributes to the enhancement of the organoleptic attributes of foods, as well as to their

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preservation and microbial safety (Caplice and Fitzgerald, 1999;Calderon et al., 2001).

The preservative action of starter culture in food and beverage systems is due to a range of antimicrobial metabolites produced during the fermentation process, which include many organic acids such as lactic, acetic and propionic acids which provide an acidic environment unfavorable for the growth of many pathogenic and spoilage microorganisms (Caplice and Fitzgerald, 1999; Rattanachaikunsopon and Phumkhachorn, 2010). These acids compounds generally exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH and inhibiting a variety of metabolic functions (Ross et al., 2002). Others are low-molecular-mass (LMM) compounds such as hydrogen peroxide, carbon dioxide, diacetyl (2,3-butanedione), uncharacterized compounds, and high-molecular-mass compounds like bacteriocins (Jay, 1982; Piard and Desmazeaud, 1992). The antimicrobial-producing LAB may be used as protective cultures to improve the microbial safety of foods and also play an important role in the preservation of fermented foods, which is usually achieved by inhibition of contaminating spoilage bacteria such as *Pseudomonas* and foodborne pathogens such as *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes* (Buckenhuskes, 1993; Brinkten et al., 1994; Olasupo et al., 1995). LAB have the ability to trap mycotoxins, some might be used to reduce the availability and toxicity of toxins in the gastrointestinal tract of humans and animals, and might therefore be used as a probiotic agent (Dalié et al., 2010).

In Nigeria, yoghurt, fufu and Kunu-zaki are part of most common lactic acid fermented products popular with consumers. Consumption of these fermented foods has many advantages including enhanced nutritional value, digestibility, therapeutic benefits and safety against pathogens (Oranusi et al., 2003). Yoghurt is a smooth, fermented milk product that evolved empirically some centuries ago through the growth of thermophilic LAB such as *Streptococcus thermophilus and Lactobacillus bulgaricus* which ferment the lactose to produce lactic acid. It has a characteristic acidic taste possessing 0.95-1.5% and pH ranging from 3.7-4.2 with viable and abundant fermenting microorganisms (Adolfsson et al., 2004). Fufu is a fermented white paste made from cassava, it is ranked next to gari as an indigenous food of most Nigerians in the South. Fufu is made by steeping whole or cut peeled cassava roots in water to ferment for maximum of three days, during the steeping, fermentation decrease the pH, softens the roots and help to reduces the potentially toxic cyanogenic compound (Agbor-Egbe and Lape Mbome, 2006, Egwim et al., 2013).

Kunu is a product obtained from cereal grains such as sorghum (*Sorghum vulgare*), maize (*Zea mays*) and millet (*Pennisetum typhoideum*). It is a Nigerian drink made of germinated grain, mostly millet. These grains can be used singly or in combination. Sorghum and millet are the most common combinations used in the ratio of 1:2 (w/w). It is a non-alcoholic cereal beverage commonly consumed by the people of the northern Nigeria, though it is becoming popular in other parts of the country (Gaffer et al., 2002).

In Nigeria, cassava is processed into garri and fufu, pellets for compounding animal feeds, kpokpo garri and also into instant aromatized (fermented) flour (Oyewole, 1997). Fufu (variants of the name include foofoo, foufou, fufuo) is a staple food of the Asante, the Akyem, Bono and Fante peoples of the Akan ethnic group of Ghana and is eaten in Guinea (Guinean cuisine). It is made by boiling starchy food crops like cassava, yams or plantains and then pounding them into a dough-like consistency. In this context, the aim of this work was to identify the bacterial isolates from fermented sorghum (kunu), cassava (fufu) and milk product (yoghurt), and to determine the antimicrobial properties of the isolates against foodborne pathogens.

MATERIALS AND METHODS

Collection of samples

Three samples of yoghurt, fufu and kunu-zaki were bought from Akungba market in Akungba Akoko. The samples were carefully labeled. They were brought to the laboratory and processed within 6 h of collection.

Foodborne pathogens used

The food and human pathogenic organisms used include *Staphylococcus aureus*, *Shigella* spp., *Escherichia coli* and *Salmonella typhi*. They were obtained from Adekunle Ajasin University Microbiology Laboratory.

Analysis of samples

The pour plate method was used to isolate microorganisms producing antimicrobial activity from samples of fufu, kunu and yoghurt. Each sample was serially diluted using sterile distilled water as diluents (Speck, 1976). Nine milliliters of distilled water were put into test tubes. One milliliter of each of yoghurt, fufu and kunu-zaki sample were added into the first test tube and thoroughly mixed. Using a different sterile pipette, 1 ml from the first test tube was pipetted into the second test tube already containing 9 ml of distilled water. This procedure continued until the last dilution. Using the pour plate assay, 1 ml of the last dilution of each sample was pipetted into Petril dishes, which were subsequently filled with MRS agar. The plates were mixed and after solidification, they were incubated anaerobically at 37°C for 48 h.

After incubation, the representative colonies on the plates were sub-cultured on fresh MRS agar to obtain pure cultures of the isolates. The pure cultures were then transferred into MRS agar slants for biochemical identification.

Microbiological analysis

Isolation of bacteria

After incubation time, the different culture plates were examined for

microbial growth; subcultures were made to get discrete colonies, which were then stored at 40°C for further biochemical investigations in order to identify microorganisms. Bacterial isolates were characterized to generic level and were possible to the species level on the basis of their cultural features (shape, colour, edge, elevation) and morphological features such as motility, gram staining, cell arrangement and shape, and biochemical features.

Preparation of cell-free supernatant

The cell-free supernatants were prepared based on methods by Schillinger and Lucke (1989). The culture extract of the producer strain were obtained from 72 h culture grown on MRS broth. The cultures were then centrifuged at 4.3008 kg force for 15 min. The supernatant was used immediately.

Antibiotic susceptibility tests

A modified method of Bauer-Kirby disk diffusion assay was used for the study. The antibiotics discs were tested on sterile Muller Hilton Agar, which was put into sterile Petri dishes and waited for solidification. A suspension of the isolated organisms was spread over the agar plates, and incubated at 37°C for 1 h. A forceps was used to transfer each antibiotic disc on the plate and incubated for 24 h at 37°C. Ten antibiotics including Pefloxacin, Gentamycin, Ampiclox, Zinnacef, Amoxacillin, Rocephin, Ciprofloxacin, Streptomycin, Septrin and Erythromycin were tested. Each disc was firmly pressed to ensure complete contact with the agar surface. The discs were evenly distributed so that they were not too close to one another. In an inverted position, the plates were incubated at 37°C for 24 h. The results were expressed as inhibition zones around the disc. These were measured, each antibiotic disc was grouped as susceptible or resistance by comparing the measured diameter with the standard given in the manufacturer's instruction.

***In vitro* inhibition test with indicator organisms**

The antimicrobial activity of the isolated LAB (cell free filtrate) against *E. coli*, *Shigella* sp., *Salmonela* sp. and *S. aureus* was performed using the well diffusion assay. Elimination of inhibitory substances like hydrogen peroxide, organic acids and bacteriophages to ensure that the inhibition was caused by only bacteriocin was performed. This was carried out by using MRS containing 0.2% glucose and preparation of cell free supernatant at pH 6.5. The pathogenic indicator bacteria were incubated in Muller Hinton broth at appropriate temperature for 24 h.

Petri dishes containing 20 ml of Muller Hinton agar were prepared previously, another broth was prepared and incubated for 4 h and inoculated with 0.1 ml of 4 h broth culture of pathogenic bacteria. Once solidified, the dishes were stored for 2 h in a refrigerator. Three wells were made and filled with 2 ml of cell-free filtrate. Incubation of the Petri dishes was done at 37°C for 24 h. The antimicrobial activity was determined by measuring the zone inhibited around the wells (Zhennai, 2000).

Identification of bacterial isolates

Identification of isolates was based on cultural, morphological and biochemical characteristics following standard methods.

Gram staining

The method described by Carpenter (1977) and Thomas (1973) were used. Smears of the isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with a gentle running tap water. The slides were flooded with dilute Gram's iodine solution. This was washed off with water and the smears were decolorized with 95% alcohol until the blue colour no more dripped out (about 30 s). The smears were then counter stained with saffranin solution for about 10 s. Finally, the slides were washed with tap water; air dried and observed under oil immersion objectives.

Biochemical tests

Catalase test

This test was performed to demonstrate which of the isolates could produce the enzyme catalase that release oxygen from hydrogen peroxide. This test is usually used as an aid to differentiate *Staphylococci* from *Streptococci* and to differentiate other catalase positive organism from catalase negative. The method employed here was that described by Speck (1976). The catalase production was indicated by the prompt effervescence of oxygen due to the fact that the enzyme aids in the conversion of hydrogen peroxide into water and oxygen bubbles (in the form of effervescence). Effervescence of gas as a white froth indicates a catalase positive reaction while the absence of the effervescence showed negative reaction (AOAC, 2000).

Motility test

To demonstrate the ability of isolates to be motile, this indicating the possession of movement structures (flagellum), was investigated by using semi-solid medium. This medium was prepared and the test performed. The isolates were used to sub-inoculate the medium to a depth of about two-third of the medium and incubated overnight at 37°C. Motility of an organism was indicated by growth beyond the inoculation line, while growth confined to the line of inoculation was regarded as being non-motile.

Sugar fermentation

Each of the isolates was tested for their ability to ferment a given sugar with the production of acid and gas or acid only. Since most bacteria especially Gram-negative bacteria utilize different sugars as source of carbon and energy with the production of both acid and gas, or acid only the test is used as an aid in their differentiation. The growth medium used was peptone water and the method used was that described by Kirk et al. (1975). Peptone water was prepared in a conical flask and the indicators bromocresol purple was added. The mixture was dispensed into test tubes containing Durhams tubes. The tubes with their content were sterilized by autoclaving at 121°C for 15 min. One percent solution of the sugar was prepared and sterilized separately at 115°C for 10 min. This was then aseptically dispensed in 5 ml aliquot volume into the tubes containing the peptone water and indicator. The tubes were inoculated with young culture of the isolates and incubated at 37°C. Acid and gas production or acid only were observed after about 24 h incubation. Acid production was indicated by the change of the medium from light green to yellow color, while gas production was indicated by the presence of gas in the Durham's tubes.

Coagulase test

Slide and tube method was used (Carpenter 1977). In slide test, a

Table 1. Lactic acid bacteria isolated from different food samples.

Fermented food	LAB
Yoghurt	*Lactobacillus plantarum* *Lactobacillus bulgaricus* *Lactococcus lactis*
Fufu	*Lactobacillus delbrueckii* sub *bulgaricus* *Lactobacillus amylophilus* *Lactococcus lactis*
Kunu-Zaki	*Lactobacillus plantarum* *Lactococcus lactis* *Leuconostoc mesenteroides*

loop full of the isolate was mixed with human plasma and allowed to stand for some minutes. Particles indicating agglutination was used as indication of coagulase reaction. In the tube method, plasma was added unto a culture of the isolate in peptone water in bijou bottles. The bottles were incubated at 37°C for 24 h. A clumping/agglutination of the plasma were used to indicate presence of coagulase.

Citrate utilization

In actual practice, Koser's citrate medium containing 'citric acid' serves as the exclusive carbon source. It detects the ability of an organism to use citrate as the sole source of carbon and nitrogen. Evidently, the ability as well as the efficacy for the 'citrate utilization' (the prevailing substrate) is adequately indicated by the production of measurable turbidity in the medium. In carrying out this test, the isolate was stabbed into test tube containing the citrate medium from a 24 h culture. Simmon Citrate Agar was prepared and dispensed into each clean test tube, sterilized, and allowed to solidify in a slanted position. An inoculum from the broth culture was picked with an inoculating loop and incubated unto the surface of the slanted Citrate Agar prepared in each test tube. It was then incubated at 37°C for 5 days and examined. Those that changed color from green (original color of prepared medium) to blue or yellow were considered positive, while those that retained the green color were negative.

Starch hydrolysis test

A medium containing starch was used. After inoculation and overnight incubation, iodine reagent was added to detect the presence of starch. Iodine reagent complexes with starch to form a blue-black color in the culture medium. An inoculation from a pure culture is streaked on a sterile plate of starch agar. The inoculated plates were incubated at 35-37°C for 24 h. Iodine reagent was then added to flood the growth. Clear halos surrounding colonies is indicative of their ability to digest the starch in the medium due to the presence of alpha-amylase.

Production of hydrogen sulphide (H_2S)

There are several sulphur containing amino acids like cystein, methionine that may decompose certain organisms to yield hydrogen sulphide gas among the products of microbial degradation. Lead acetate was duly incorporated into the culture media which eventually turned into either black or brown due to the formation of lead sulphide (AOAC, 2000).

Spore stain

The malachite green staining method was used. The staining was carried out as described by Carpenter (1977). Smears of the pure isolates were made on grease-free glass slide and heat fixed. The slides were flooded with 5% v/v malachite green solution. The slides were flamed in such a way that the stain steamed but did not boil. The slides were then allowed to stand for 5 min. The stain was then washed out in running tap water. The smears were counter-stained with saffranin for 30 s. It was stained with saffranin, blotted dried and examined under the oil immersion objective. The spores stained green while vegetative cells stained red.

RESULTS

The results show the LAB isolated from different fermented dairy products: yoghurt and the fermented foods (fufu and kunu). Six LAB were isolated from the samples, *Lactobacillus plantarum, L. bulgaricus* and *Lactobacillus lactis* were obtained from yoghurt*, Lactobacillus delbrueckii* sub *bulgaricus, Lactobacillus amylophilus* and *L. lactis* were obtained from fufu, while *L. plantarum, L. lactis* and *Leuconostoc mesenteroides* were isolated from kunu (Table 1). *L. plantarum* was obtained from yoghurt and kunu, while yoghurt, fufu and kunu showed presence of *L. lactis.*

The morphological and biochemical characteristics of the bacterial isolates are shown in Table 2. The antibiotic resistance patterns of the isolates are presented in Table 3. All the isolates were susceptible to ciprofloxacin, but resistant to ampiclox, zinnacef and amoxacillin, while variable susceptibility/resistance to pefloxacin, gentamycin rocephin, streptomycin, septrin and erythromycin was observed. Table 4 shows inhibitory properties of the metabolites of the selected LAB against the target foodborne pathogenic organism (*S. aureus, Shigella* spp., *Salmonella* typhi and *E. coli*). The metabolites possess antagonistic activities against all the organisms used in this work.

L. plantarum isolates obtained from yoghurt and kunu was observed to be inactive against any of the indicator organisms. *L. lactis* showed the largest inhibition zone against *S. typhi* and *S. aureus* as 19 and 14 mm respectively, while *L. amylophilus* showed antimicrobial activity against all the indicator organisms. Isolates from fufu generally showed more inhibition on *S. aureus*, *S. typhi* and *E. coli*.

DISCUSSION

In this study, six LAB were isolated from traditionally fermented and diary food product: yoghurt, fufu and kunu obtain from Akungba. The results of the present

Table 2. Morphological and biochemical characteristics of the bacterial isolates.

Shape	Pigmentation	Surface	Margin	Elevation	Gram stain	Cell morphology	Motility	Catalase	Starch	Manitol	Fructose	Glucose	Sucrose	Lactose	Identified organisms
Circular	Creamy	Rough	Entire	Convex	+	Rod	-	-	-	+	+	+	+	+	*Lactobacillus plantarum*
Circular	Whitish	Shiny	Entire	Convex	+	Long rod	-	-	-	+	+	+	-	-	*Lactobacillus bulgaricus*
Circular	Creamy	Rough	Entire	Flat	+	Cocci	-	-	-	+	+	+	-	-	*Lactococcus lactis*
Circular	Whitish	Shiny	Entire	Flat	+	Rod	-	-	-	+	+	+	-	+	*Lactobacillus delbrueckii sub bulgaricus*
Circular	Creamy	Smooth	Entire	Flat	+	Short rod	-	-	-	-	+	+	-	-	*Lactobacillus amylophilus*
Circular	Creamy	Smooth	Entire	Convex	+	Cocci	-	-	-	-	+	+	-	-	*Lactococcus lactis*
Circular	Creamy	Rough	Entire	Flat	+	Rod	-	-	-	+	+	+	-	-	*Lactobacillus plantarum*
Circular	Creamy	Smooth	Entire	Flat	+	Cocci	-	-	-	-	+	+	+	-	*Lactococcus lactis*
Circular	Whitish	Shiny	Entire	Flat	+	Cocci	-	-	-	+	+	+	+	+	*Leuconostoc mesenteroides*

-: Negative, +: positive.

Table 3. Antibiotic susceptibility discs test

Lactic acid bacteria	Inhibition zone (mm)									
	PEF	GEN	APX	ZNF	AMX	RCP	CPX	STP	SEP	ERT
Lactobacillus bulgaricus	S	R	R	R	R	R	S	R	R	R
Lactobacillus plantarum	R	S	R	R	R	S	S	R	S	R
Lactococcus lactis	S	R	R	R	R	S	S	S	R	R
Lactobacillus delbrueckii sub bulgaricus	R	R	R	R	R	R	S	R	R	R
Lactobacillus amylophilus	S	R	R	R	R	R	S	R	S	R
Lactococcus lactis	R	R	R	R	R	S	S	S	S	R
Lactobacillus plantarum	S	S	R	R	R	S	S	R	R	R
Lactococcus lactis	S	R	R	R	R	R	S	S	R	S
Leuconostoc mesenteroides	R	R	R	R	R	R	S	R	S	R

R- Resistance (no value), S- Susceptible (value ranges from 4-32 mm); PEF- Pefloxacin, GEN- Gentamycin, APX- Ampiclox, ZNF- Zinnacef, AMX- Amoxacillin, RCP- Rocephin, CPX- Ciprofloxacin, STP- Streptomycin, SEP- Septrin, ERT- Erythromycin.

study is in accordance with the report of Odunfa and Adeyele (1985), who revealed that members of LAB could be detected in a variety of habitats including fermented foods. Alli et al. (2010) reported the presence of *L. acidophilus, L. bulgaricus, Lactobacillus cremoris, L. fermentum,*

Table 4. Inhibition of indicator organisms by LAB.

Sample	Bacteria isolated	Test organisms (mm)			
		Staphylococcus aureus	***Shigella spp***	***Salmonella typhi***	***Escherichia coli***
Yoghurt	*Lactobacillus bulgaricus*	NI	NI	9	10
	Lactobacillus plantarum	NI	NI	NI	NI
	Lactococcus lactis	8	6	7	NI
Fufu	*Lactobacillus delbrueckii sub bulgaricus*	8	NI	12	10
	Lactobacillus amylophilus	8	4	8	7
	Lactococcus lactis	14	NI	19	11
Kunu-Zaki	*Lactobacillus plantarum*	NI	NI	NI	NI
	Lactococcus lactis	9	7	NI	6
	Leuconostoc mesenteroides	8	NI	8	NI

Value shows the mean number of triplicate well zone (mm); NI- No zone of inhibition.

L. lactis, Lactococcus mesenteroides, Micrococcus acidophilus, Rhodosporium spp., *Saccharomyces bayanus, Saccharomyces cerevisiae, Streptococcus lactis* and *Streptococcus thermophilus* in yoghurt samples, out of which only *L. bulgaricus, L. plantarum* and *L. lactis* were obtained in this study.

The identification carried out for representative LAB strains from the fermented food products demonstrated the dominance of *L. amylophilus*, *L. bulgaricus, L. delbrueckii, L. lactis* and *L. mesenteriode*. These identified *Lactobacillus* species were in accordance with those earlier identified from similar fermented food products (Halm et al., 1993; Wakil et al., 2004).

Lactobacilli grow best in highly nutritive substrates. They use the nutrients in the substrate for their own metabolism and cell growth and multiply in food (from one million per millilitre to one billion per millilitre). They are present in the fermented food not only as viable cells and non-colony forming units, but also with the primary and secondary metabolites produced during the fermentation process (Robinson, 1991). The MRS medium used was selective for the isolation of *Lactobacillus* species since they are extremely fastidious. Lindquist (1998) reported that a medium that would support their growth must contain a fermentable carbohydrate and many growth factors. Gilliland and Speck (1977) had earlier reported that *Lactobacilli* showed stronger antibacterial properties against Gram-positive bacteria (*S. aureus* and *Clostridium perfringens*) than Gram-negative bacteria (*E. coli* and *Salmonella typhimurium*). *L. lactis* from yoghurt, fufu and kunu inhibited *S. aureus* in the order of 8, 14 and 9 mm with respect to diameter of inhibition zone. *L. bulgaricus*, *L.* lactis and *L. amylophilus* also inhibited *E. coli* in order of 10, 7 and 6 mm. Among the lactobacilli, there has been great interest in *L. plantarum*, due to the potential applications of the microorganisms as a starter bacterium for a variety of fermented foods. The bacteriocin produced from *L. plantarum* has been found to be inhibitory towards closely related LAB, particularly the mesophilic and thermophilic lactobacilli. In this study, *L. plantarum* produced no inhibition zone against the tested microorganisms.

Several studies have shown that pathogens such as enterotoxigenic *E. coli*, *Shigella flexneri*, *S. typhimurium* and *B. cereus* are adversely affected when present in traditional fermented foods (Kingamkono et al., 1995; Kunene et al., 2000; Obadina et al., 2006). Some of the antimicrobial properties exhibited by these fermented foods may be as a result of the low pH of the food as well as metabolites produced by microorganisms such as LAB involved in the fermentation. The pathogens used in this study were sensitive to the LAB metabolites. Brooks et al. (1998) reported rapid development of resistance by *Staphylococcus* sp. to antimicrobial agents. However, the *S. aureus* used in this study was sensitive to six LAB used against it.

Antimicrobial activity of LAB may be due to decrease in pH, depletion of nutrients and production of antimicrobial compounds (Olsen et al., 1995), including bacteriocins (Parente and Ricciardi, 1999), and various organic acids such as lactic acids, acetic acid. Microbial food safety is an increasing public health concern worldwide and many Gram negative bacteria such as *E. coli*, *Klebsiella* sp. together with Gram positive bacteria such as *S. aureus* have been implicated in food borne diseases (Mead et al., 1999). The LAB isolated from yoghurt, fufu and *kunu-zaki* in this study probably produced different antimicrobial compounds, the quantity of which might vary with time. Collins et al. (1983) also noted the inhibition of *Psuedomonas fragi* and *S. aureus* against other microorganisms by hydrogen peroxide by some LAB strains which contribute to their inhibitory activity. Liasi et al. (2009) emphasized that research on antimicrobial substances produced by LAB, had led to their potential use as natural preservatives, which may be used to combat the growth of pathogenic microorganisms in the food industry.

Conclusion

The promising results of bacteriocins produced by LAB from fermented food underline its important role in improving food quality and increasing safety, by using it as starter/protective cultures to combat the growth of food-borne pathogens and spoilage microorganisms.

Conflict of Interests

The author(s) did not declare any conflict of interests.

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Kinetin-serine regulation of photosynthetic pigments and some antioxidant enzymes during dark induced senescence in spinach leaf discs

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Interesting observation of earlier investigation on regulation of leaf senescence in pigeon pea with serine prompted us to undertake studies on kinetin (Kn)-serine interaction in senescent leaf discs of *Spinacia oleracea* L., cv. S-23, over time, in the dark to determine their effectiveness individually, and in combination, on photosynthetic pigments, total soluble proteins, lipid peroxidation and activities of the senescence associated enzymes- protease, lipoxygenase (LOX), guaiacol peroxidase (POD) and superoxide dismutase (SOD). Serine and Kn each individually, and in combination, minimized loss in chlorophyll a and b in all days and carotenoids at day 6. Kinetin and serine retarded protein loss and also curtailed protease activity. POD activity increased in untreated leaf discs up to 6-day; SOD activity decreased in control as leaf discs senescence advanced. Senescence increased POD activity and promoted chlorophyll degradation. Serine along with Kn was able to minimize increases in POD and maintained slightly higher level of SOD activity in all days when compared with the control. Although, combined treatment of serine and Kn could lower malondialdehyde content slightly at day 2, LOX activity was reduced. Serine and Kn can scavenge ROS by regulating antioxidant enzymes during leaf senescence.

Key words: *Spinacia oleracea*, chlorophyll, lipid peroxidation, lipoxygenase, superoxide dismutase.

INTRODUCTION

When detached leaves are placed in dark several changes characterizing senescence such as loss in chloroplast pigments, proteins; and increase in lipid peroxidation and membrane permeability resulting in injury are triggered, all of which lead to decreased photosynthetic output (Nooden et al., 1997; Prochazkova et al., 2001). Among the plant growth regulators, ABA and ethylene accelerate senescence symptoms (Smart, 1994). Exogenous application of cytokinins inhibits degradation of chlorophyll and photosynthetic proteins (Richmond and Lang, 1957; Badenoch-Jones et al., 1996). Low light intensities, or dark, result in reduced expression of light-dependent genes and disappearance of photosynthetic proteins and chlorophyll (Wingler et al.,

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1998). Light is an important factor initiating, and/or modulating, senescence rate in attached and detached plant systems (Biswal and Biswal, 1984; Ono et al., 2001). Higher photon flux density may cause photo-xidative damage and induce leaf senescence (Prochazkova and Wilhelmova, 2004). Complete deprivation of light induces senescence in individual leaves of *Arabidopsis* ecotype Landsberg *erecta* but may inhibit leaf senescence when applied to the whole plant (Weaver and Amasino, 2001).

Regulation of many plants developmental processes by light are affected by plant growth regulators, and increased endogenous cytokinins may inhibit leaf sene-scence under certain conditions (Wingler et al., 1998). There is evidence that this could be mediated by an increment of antioxidant defenses (Synkova et al., 2004). Kinetin application delays senescence and is effective in minimizing chlorophyll and protein loss (Thimann, 1980; Mukherjee and Ponmeni, 2004; Mukherjee and Jakhar, 2009). Experiments with pea (*Pisum sativum* L. cv. Alaska) seedlings and oat (*Avena sativa* L. cv. Victory) leaf segments showed the mode of action of cytokinins, and the role of amino acids in regulation of senescence (Shibaoka and Thimann, 1970; Martin and Thimann, 1972a, b, 1973; Satler and Thimann, 1983; Veierskov et al., 1985).

Two most important markers of leaf senescence, viz. chlorophyll degradation and massive hydrolysis of protein occurring in the detached first leaves of *Avena* were found to be vigorously promoted by L-serine (Shibaoka and Thimann, 1970). Selecting various protein amino acids also showed that cysteine, threonine, alanine and glycine exhibited weaker action in promoting senescence whereas arginine did not favor the action of L-serine when both were present. Using three different concen-trations of kinetin viz. 0.05,0.5 and 5.0 ppm along with L-serine at 0.5, 5.0 and 50 X 10^{-3} M concentrations, Martin and Thimann (1972a) clearly indicated how serine could overcome the action of kinetin and promote senescence in oat leaves.

Along with the decline in chlorophyll and protein content, increment was observed in α-amino nitrogen content. That was the basis for further investigation to determine how leaf senescence was promoted by L-serine (Veierskov et al., 1985). Antagonism was established between kinetin and L-serine; and amino acids L-alanine, L-cysteine and others. However, investigations carried out in our laboratory with spinach (*Spinacia oleracea* L.) and pigeon pea (*Cajanus cajan* L. cv. UPAS-120) leaves, serine alone, and in presence of kinetin, minimized protein degradation and accumulation of free amino acids (Mukherjee and Jakhar, 2009; Mukherjee et al., 2011). There were less degradation of chlorophylls and carotenoids in leaf discs in the presence of serine, and also when serine and kinetin were both present. Spinach (*S. oleracea* L.) was used as a test plant because being a leafy vegetable, we wanted to find out whether serine can alter activities of some antioxidant enzymes and mem-brane damage. An experiment was undertaken to determine how antioxiodant enzymes and lipoxygenase behave in the presence of serine and kinetin to regulate senescence in spinach leaf discs as these aspects have not been investigated earlier.

MATERIALS AND METHODS

Leaf discs were cut using cork borer from senescent spinach leaves cv. S-23 (procured from CCS Haryana Agricultural University, Hisar, India). Leaf discs, 0.75 cm^2 area, were placed in Petri dishes (9 cm diameter). Sixty to 65 leaf discs were placed on moistened filter paper (Whatman No. 1) In Each Petri dish having 5 ml of test solution and maintained in complete darkness at 25 ± 2°C. Applied treatments were carried out as follows:

1) 0.38 µM kinetin (Kn),
2) 5 mM DL-serine,
3) 0.38 µM Kn + 5 mM DL-serine,
4) Phosphate buffer, pH 5.2 as control and
5) Double distilled water (DDW) as control.

For each treatment, leaf discs were maintained in 5 Petri dishes. Sampling and estimations were carried out at 0, 2, 4 and 6-day after placement in the Petri dishes to determine amount of chloroplast pigments, protein and MDA contents, and enzymatic activities of peroxidase, lipoxygenase and superoxide dismutase (SOD).

Estimation of chlorophylls and carotenoids

Leaf sample (200 mg) was ground in chilled 80% acetone (AR grade) with 20 mg of $CaCO_3$ and centrifuged at 3000 g for 5 min. Absorbance of the filtrate was recorded at 645 and 663 nm for chlorophylls and at 480 and 510 nm for carotenoids depending on respective peaks in their absorption spectra using a UV-Visible spectrophotometer (Specord-205, Analytic-Jena, Germany). Chlorophyll (Chl) amount was estimated with the formula of Arnon (1949). Carotenoid level was calculated by the method of Holden (1965).

Estimation of total soluble protein

Total soluble proteins were estimated according to the method described by Bradford (1976) using Coomassie Brilliant Blue G-250. Fifty milligrams of fresh leaf tissue (earlier stored in a freezer) was dropped in boiling 80% ethanol (EtOH) on a water bath for a minute. The tissue along with EtOH was cooled to room temperature and homogenized. The extract was centrifuged at 10,000 g for 5 min. The residue was re-extracted with 5% perchloric acid followed by centrifugation at 10,000 g for 5 min. 5 mL of 1N NaOH was added to the residue and maintained in warm water (40-50°C) with regular shaking for 30 min. The clear supernatant was used for further analysis.

Estimation of protease activity

The procedure for protease extraction was a slight modification of that described by Yomo and Varner (1973) and Ihnen (1976). Samples, 200 mg, were homogenized in 100 mM phosphate buffer (using equimolar KH_2PO_4 and Na_2HPO_4), pH 7.2, and the final volume

volume raised to 25 mL. Casein (1%) (Sigma, St. Louis, U.S.A.) was prepared by dissolving 1.0 g casein in 2.0 mL of 0.1 N NaOH and the final volume made to 100 mL with 100 mM phosphate buffer, pH 7.6. To 1 mL of casein, 1 mL of enzyme extract was added and incubated for 3 h at 37°C. The reaction mixture was pH 7.5. After incubation, 1 mL of 16% TCA was added to all reaction sets and centrifuged. The residue was discarded. Of the resultant 3 mL filtrate, 0.5 mL was used to estimate protease activity by the ninhydrin method (Yemm and Cocking, 1955) as modified by Reimerdes and Klostermeyer (1976).

Measurement of peroxidase (POD) activity

Total peroxidase activity was measured by the method of Maehly (1954). Plant material (0.1 g) was homogenized with ice cold distilled water and centrifuged in a Remi centrifuge at 6000 g for 10 min. The supernatant was used as the enzyme source and final volume of the extract raised to 10 mL with ice cold double distilled water. The reaction set was prepared by mixing 2 mL each of enzyme source; phosphate buffer (pH 7.0); guaiacol (20 mM), and H_2O_2 (10 mM) in sequence. A blank set was prepared by mixing 2 mL of enzyme source; 2 mL of phosphate buffer (pH 7.0) and 4 mL of double distilled water. Blank, and reaction sets were kept undisturbed at room temperature exactly for 10 min, then the absorbance was recorded in a spectrophotometer at 420 nm. Protein was estimated from the same extract following the procedure of Bradford (1976).

Measurement of lipoxygenase (LOX) activity

The LOX activity was estimated according to Doderer et al. (1992). Two hundred milligrams of leaf sample was homogenized in ice cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 µM EDTA in a pre-chilled pestle and mortar. The homogenate was transferred to tubes and centrifuged at 4°C in a refrigerated centrifuge (Compufuge, CPR-24, Remi, New Delhi, India) for 15 min at 15000 g. The supernatant was transferred to 30 mL test tubes for use as the enzyme extract. 5 mL of distilled water with 50 µL Tween 20 was added to 35 µL linolenic acid (substrate), pH 8.5-8.6. The final pH was adjusted to 9 by adding 0.2 M NaOH drop by drop until the linoleic acid dissolved completely. The pH was adjusted to 6.5 by adding 0.2 M HCl. To this solution, 0.1 M phosphate buffer, pH 6.5, was added and the final volume of substrate raised to 100 mL with the same buffer. Blank set was prepared in a cuvette with 2.95 mL of substrate solution. In the reaction set, 0.05 mL of the enzyme extract was added to the cuvette containing 2.95 mL of the substrate solution at zero time. Absorbance was noted at 234 nm for every minute upto 5 min. The amount of protein was estimated by the method of Bradford (1976).

Measurement of superoxide dismutase (SOD) activity

Fifty milligrams of fresh leaf tissue was crushed in 2 mL of 0.1 M EDTA- phosphate buffer, pH 7.8, containing K_2HPO_4 and EDTA and the final volume raised to 100 mL with double distilled water (DDW). This was centrifuged at 15000 g and the resultant supernatant used as crude extract. The reaction mixture was prepared by adding 0.1 mL of crude extract followed by 0.9 mL of DDW, 0.5 mL of 300 mM Na_2CO_3 (pH 10.2), 0.5 mL of 378 µM p-nitrobluetetrazolium chloride (NBT), 0.5 mL of 78 mM L-methionine and 0.5 mL of 7.8 µM riboflavin. The final reaction mixture was 3 mL. The reaction was carried out in test tubes at 25°C for 15 min under 100 µmol photon $m^{-2}s^{-1}$ PFD from fluorescent lamps. The initial rate of reaction, measured by the difference in increase in absorbance at 560 nm in the presence, and absence, of extract was proportional to the amount of enzyme. The unit of SOD activity was obtained as that amount of enzyme which under the experimental conditions caused a 50% inhibition of the reaction observed in the absence of enzyme (Giannopolitis and Ries, 1977).

Measurement of lipid peroxidation

The level of lipid peroxidation in samples was measured by estimating the malondialdehyde (MDA) present (Heath and Packer, 1968). Leaf samples (0.2 g) were homogenized in 3 mL of 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 15000 g for 15 min. To 1.0 mL aliquot of the supernatant, 2.0 mL of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) was added. The mixture was heated at 95°C for 30 min in a water bath and then cooled in an ice bath. After centrifugation at 10000 g (Remi) for 10 min the absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption of each sample at 600 nm was recorded and subtracted from the absorbance recorded at 532 nm.

Statistical analysis

A mean of three readings was taken in every replication. In biochemical estimation, three aliquots were used for each replication. Statistical analysis was done using Statistical Packages for Social Sciences (SPSS) version 16.0. One-way ANOVA was used to test whether there was a significant difference in various estimations.

RESULTS

As the senescence of leaf discs progressed over a period of 6-day, the reduction in total chlorophyll and carotenoids was 45 and 47.24%, respectively (Table 1). Application of serine alone and in combination with Kn (0.38 µM), reduced the loss as compared to the control. The Chla : Chl b ratio increased as leaf discs senesced in the control (Table 1). Serine application minimized loss of total Chl and carotenoids by about 4.5 and 7% respectively. The combined action of Kn and serine at a highest rate reduced pigment loss. Total soluble protein content and total crude protease activity over 6 days in control and treated leaf discs varied due to treatment (Tables 2 and 3). In untreated control (buffer) and treated leaf discs, protein content gradually declined and protease activity increased from 0 to 6 days. For crude protease activity, a sharp increase was noticed not only in the control leaf discs but also in the sets having serine and kinetin when applied alone and in combination (Table 2). However, the degree of rise was lower with Kn and serine as compared to the control. Combined treatment of serine + Kn was most effective in minimizing protein degradation and reducing protease activity.

Peroxidase (POD) and superoxide dismutase (SOD) activities varied due to treatments. The POD activity gradually increased over 6 days regardless of treatment; the magnitude of increments between 2-4 and 4-6 day stages were greater in the control. Application of serine and kinetin reduced POD activity.

Table 1. Interaction effect of day and treatment on the Chl. a, Chl. b, total Chl., and carotenoids (mg g^{-1} dry wt) in excised leaf discs of *Spinacea oleracea* L. incubated in the dark.

Day	X Treatment	Chl.a	Chl.b	Total Chl.	Carotenoid
0	None	1.251^{a}	0.382^{a}	1.633^{a}	0.477^{a}
	DDW	0.533^{cd}	0.196^{bcd}	0.729^{d}	0.257^{b}
	Phosphate buffer	0.597^{bc}	0.208^{bc}	0.805^{bcd}	0.235^{bcd}
2	Serine	0.627^{b}	0.212^{bc}	0.839^{bc}	0.243^{bcd}
	Kinetin	0.629^{b}	0.221^{bc}	0.850^{bc}	0.249^{bcd}
	Serine + Kinetin	0.631^{b}	0.231^{b}	0.862^{b}	0.252^{bc}
	DDW	0.423^{ef}	0.149^{ef}	0.572^{ef}	0.237^{bcd}
	Phosphate buffer	0.453^{de}	0.179^{cde}	0.632^{e}	0.220^{cd}
4	Serine	0.565^{bc}	0.193^{bcd}	0.758^{d}	0.225^{bcd}
	Kinetin	0.579^{bc}	0.199^{bcd}	0.778^{cd}	0.236^{bcd}
	Serine + kinetin	0.590^{bc}	0.201^{bcd}	0.791^{bcd}	0.216^{d}
	DDW	0.310^{e}	0.075^{e}	0.385^{h}	0.120^{g}
	Phosphate buffer	0.330^{e}	0.113^{f}	0.443^{gh}	0.124^{fg}
6	Serine	0.360^{fg}	0.139^{ef}	0.499^{fg}	0.145^{efg}
	Kinetin	0.379^{efg}	0.148^{ef}	0.527^{f}	0.249^{ef}
	Serine + kinetin	0.393^{efg}	0.160^{de}	0.553^{f}	0.171^{e}

Means followed by different letters are significantly different at 0.05% level, using DMRT. *Each value indicates mean of three replicates.

Table 2. Interaction effect of day and treatment on protein (mg mg^{-100} dry wt), protease (µM lysine equivalent mg^{-100} dry. Wt. h^{-1}) and peroxidase activity (mg mg^{-100} dry wt) in excised leaf discs of *Spinacea oleracea* L. incubated in the dark.

Day	X Treatment	Protein	Protease	Peroxidase
0	None	18.76^{a}	4.36^{a}	2.08^{a}
	DDW	15.23^{d}	22.46^{h}	3.12d^{efg}
	Phosphate buffer	15.98^{cd}	21.36^{hi}	3.09^{efg}
2	Serine	16.99^{bc}	19.30^{ij}	3.00^{efg}
	Kinetin	17.16^{abc}	18.00^{j}	2.86^{fg}
	Serine + kinetin	17.96^{ab}	17.46^{j}	2.67^{gh}
	DDW	9.36^{h}	46.76^{d}	3.89^{c}
4	Phosphate buffer	9.98^{gh}	42.46^{e}	3.69^{cde}
	Serine	11.36^{fg}	36.41^{f}	3.40^{cdefg}
	Kinetin	12.64^{ef}	35.40^{f}	3.10^{efg}
	Serine + kinetin	13.46^{e}	32.47^{g}	2.76^{gh}
	DDW	2.39^{i}	66.36^{a}	4.93^{a}
	Phosphate buffer	3.46^{ij}	60.36^{b}	4.61^{ab}
6	Serine	3.97^{ij}	58.30^{b}	4.01^{bc}
	Kinetin	4.56^{i}	54.31^{c}	3.86^{cd}
	Serine + Kinetin	4.76^{i}	47.19^{d}	3.56^{cdef}

Means followed by different letters are significantly different at 0.05% level, using DMRT. *Each value indicates mean of three replicates.

The order of effectiveness of treatments in lowering POD activity was serine + kinetin > kinetin>serine. SOD activity increased during first two days in control and treated discs. From day 2 to day 6, a decrease in SOD occurred. Effectiveness of serine and kinetin individually, and together, occurred at day 2 and 4 in leaf discs.

Changes in MDA content due to lipid peroxidation and lipoxygenase (LOX) activity rose on specific days irrespective of treatment (Table 3). The MDA increment from day 2 to 4 was higher than LOX activity in the control. The MDA value decreased slightly due to treatment of serine and kinetin only up to day 2, but not thereafter. Both serine and kinetin arrested the rise in LOX activity. The additive effect of these compounds was greater than each alone.

DISCUSSION

Presented data have indicated that very rapid degradation of chlorophylls and carotenoids in leaf discs held in water control can be effectively reduced even by phosphate buffer (Table 1). Further, effectiveness of serine and Kn in lowering total Chl. was more evident when they were applied together (Table 1). Relatively greater retention of Chl a, Chl b and carotenoids have been revealed in spinach leaf discs also by the

Table 3. Interaction effect of day and treatment on Superoxide dismutase (SOD) (units min^{-1} mg^{-1} protein), MDA content (nmol g^{-1} dry wt) and Lipoxygenase (LOX) activity (µmol min^{-1} mg^{-1}) in excised leaf discs of *Spinacea oleracea* L. incubated in the dark.

Day	X Treatment	SOD	MDA content	LOX
0	None	4.23^{bc}	0.00367^{d}	0.167^{i}
	DDW	4.68^{ab}	0.01296^{c}	0.296^{fg}
	Phosphate buffer	4.70^{ab}	0.01310^{c}	0.270^{gh}
2	Serine	4.86^{ab}	0.01260^{c}	0.206^{hi}
	Kinetin	4.97^{a}	0.01036^{cd}	0.189^{i}
	Serine + Kinetin	5.21^{a}	0.00987^{cd}	0.200^{hi}
	DDW	3.31^{e}	0.03476^{b}	0.379^{e}
	Phosphate buffer	3.32^{e}	0.03400^{b}	0.356^{ef}
4	Serine	3.59^{de}	0.03390^{b}	0.340^{ef}
	Kinetin	3.76^{cde}	0.03390^{b}	0.300^{fg}
	Serine + Kinetin	3.98^{cd}	0.03381^{b}	0.204^{hi}
	DDW	1.07^{f}	0.05237^{a}	1.067^{a}
	Phosphate buffer	1.17^{f}	0.05210^{a}	0.949^{b}
6	Serine	1.19^{f}	0.05160^{a}	0.720^{c}
	Kinetin	1.26^{f}	0.05150^{a}	0.624^{d}
	Serine + Kinetin	1.28^{f}	0.05103^{a}	0.480^{hi}

Means followed by different letters are significantly different at 0.05 % level, using DMRT. * Each value indicates mean of three replicates.

application of both Kn and serine like earlier studies in pigeon pea (Mukherjee and Ponmeni, 2000) and in spinach leaves (Mukherjee and Jakhar, 2009). The retention in the amount of chlorophylls by Kn ttreatment has been also observed by other workers (Paranjothy and Wareing, 1971; Hukmani and Tripathi, 1994).

Alteration in POD activity increased in untreated leaf discs. In treated discs POD activity increased to a lesser extent as compared to the control. The combined treatment reduced the activity of POD. Grover and Sinha (1985) reported increase in POD activity as leaf senescence continued in detached pigeon pea and chickpea leaves. Further, a decline in POD activity in leaves occurred after flowering; and control leaves had lower activity of this enzyme than leaves from deflowered plants at all stages (Grover et al., 1985). When leaves were induced to senesce by detaching and incubation in water, POD activity increased. The POD activity increased during senescence of detached leaves or leaf discs (Parish, 1968; Jakhar and Mukherjee, 2006). However, Srivastava et al. (1983) found no difference in POD activity between young and mature leaves of barley (Hordeum vulgare L.). In *Festuca aurundinaceae* Schreb leaves the increment in POD activity was responsible for cessation of growth (MacAdam et al., 1992). Activity of guaiacol-dependent POD increased during the entire ontogeny of bean cotyledons (Wilhelmova, 1998). Serine and Kn could not completely prevent senescence-related increase in protease and POD in complete darkness. During an investigation on chlorophyll metabolism on chlorophyll metabolism in tobacco leaves, Kato and Shimizu (1985) concluded that peroxidase could also degrade chlorophyll with a number of phenolics including guaiacol and p-coumaric acid. Moreover, this process of chlorophyll bleaching was inhibited by peroxidase inhibitors.

The MDA concentration, and specific activity of POD, tended to increase and SOD decreased as senescence progressed. Serine individually and in combination with Kn, produced slightly higher SOD activity during senescence. Decrease in reactive oxygen species (ROS) may be due to the increase in SOD activity in serine and Kn treated plants; which are an intrinsic part of plant senescence and inhibit the process of oxidative deterioration (Thompson et al., 1987). Antioxidative enzymes SOD, CAT and POD are the main protective enzymes engaged in removal of free radicals and activated oxygen species (Blokhina et al., 2003; Devi and Prasad, 2005). A decrease in SOD activity occurring during senescence (Droillard and Paulin, 1990) can be taken as a supporting marker for the progress of senescence. Huang and Liu (2002) reported that stress decreases SOD activity which can be mitigated by exogenously applied cytokinin.

Although a sharp increase was noticed in MDA content during 0 to 6-day in leaf discs in dark, effect of serine and kinetin was very little; that too was visible only at 2-day. There were no changes in leaf discs in the presence of serine and kinetin. An increment in LOX activity occurred in leaf discs over six days. Serine and Kn lowered the activity considerably; further reduction was brought about by a combination of serine and Kn. Similarly, treating intact pea leaves with either cytokinin or α-tocopherol resulted in lower LOX activity relative to controls (Leshem, 1988). The enzyme is responsible for lipid peroxidation by forming lipid hydroperoxides and superoxide radicals. The LOX increased in controls over 6 days. The elevated LOX activity is a common feature of senescent plant organs (Grossman and Lesham, 1983; Thompson et al., 1987) and flower petals (Rouet- Mayer et al., 1992).

However, reports are also available where a decline in LOX activity has also been reported in detached wheat and rye leaves (Kar and Feierabend, 1984) and soybean cotyledons (Peterman and Siedow, 1985). Since lipid peroxidation is mediated by ROS (Arora et al., 2007), kinetin may either be directly scavenging ROS and decreasing lipid peroxidation, or modulating activity of antioxidant enzymes.

Kinetin and serine delayed senescence in cut leaf discs of spinach by maintaining protein content and lowering protease and POD activity, keeping a higher activity of SOD enzyme and reducing oxidative stress such as lipid peroxidation and LOX activity.

Conflict of Interests

The author(s) did not declare any conflict of interests.

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Impact of cooking time on the nutritional profile of sesame milk

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The effect of cooking time on nutritional characteristics of sesame milk was determined. Sesame milk was cooked at the temperature of 100°C for various duration (0, 15, 30 and 45 min), to produce samples marked as A, B, C and D. The sesame milk products were subjected to physico-chemical and sensory analyses using standard analytical methods. The moisture, protein, crude fat and energy contents decreased significantly (p≤0.05) with increase of cooking time from 89.30 to 87.32%, 2.5 to 2.3%, 5.5 to 4.0% and 54.81 to 49.55 kcal/g respectively; while ash, fiber and carbohydrate contents increased significantly (p≤0.05). Total solids and pH varied from 7.95 to 10.90 and 6.57 to 6.83%, respectively. Calcium was highest (273.44 mg/100 g) followed by phosphorus (196.2 mg/100 g), magnesium (173.5 mg/100 g) and potassium (95.58 mg/100 g) in milk cooked for 45 min when compared with lower values observed at 0, 15 and 30 min. The vitamins (thiamine and riboflavin) significantly reduced in sesame milk after boiling for 45 min. This accounted for a post-boiling decrease of about 76.2 and 64.0% in vitamins B_1 and B_2, respectively. Duration of cooking was observed to affect the phytate and oxalate concentrations significantly (p≤0.05) in sesame milk with a maximum reduction observed after 45 min. Mean sensory scores for colour and flavour ranged from 6.75 to 7.29 and 7.28 to 7.52, respectively. Sesame milk cooked at 100°C for 30 min (sample C) gave the highest acceptability score of 8.06, followed by samples B, A and D in that order. It was evident that there were varying degrees of changes that occurred in each of the chemical composition of the sesame milk with respect to the different periods of cooking. Processing at 100°C for 30 min gave the product with appreciable nutritional and sensory qualities with tolerable concentration of anti nutrients, and is therefore, recommended for sesame milk processors.

Key words: Chemical composition, cooking, sensory analysis, sesame milk.

INTRODUCTION

Oil seeds are agricultural species which contain substantial quantities of fixed oils with appreciable quantities of protein and mineral elements. Oil seeds are the second most valuable commodity in the world trade (USDA, 2005). In some developing countries where the supply of animal protein is inadequate to meet the rapid

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population growth, there is increase in research to utilize some of the protein-rich oil seeds in bridging the gap on demand. In Nigeria, there is a wide varieties of oil crops in various parts of the country ranging from largely known and highly utilized ones like soya bean, palm kernel and groundnut, to underutilized ones like walnut, locust bean, African oil bean and sesame seed.

Sesame seed (*Sesamum indicum* L., synonymous with *Sesamum orientale* L.), also known as sesamum, gingelly, sim sim, benniseed and til is probably the most ancient oilseed known and used by humans as a food source (Gharbia-Abau et al., 2000). It has been cultivated for centuries. Sesame seed contains about 25% crude proteins and 50% fat (Makinde and Akinoso, 2013). Much attention has been directed towards exploring the utilization sesame seed for new food uses seed due to its nutritional (Morris, 2002), functional (Oshodi et al., 1999) and health properties (Kapadia et al., 2002). Sesame protein has high biological value and it is cheaper as compared to animal protein sources e.g., meat and fish in the tropics. Sesame is also a good source of minerals especially calcium, potassium, magnesium, iron, zinc and copper and vitamins; thiamine, riboflavin and niacin (Biswas et al., 2001) with appreciable content of lignans, which in turn contains sesamin and sesamol. In addition, sesame are known to contain anti nutritional factors (majorly phytate and oxalate); but it is devoid of anti tryptic compounds.

The nutritional potentials of sesame can be explored to solve the protein gap problem among the low-income earners in Nigeria. Sesame seeds can be utilized in imitated dairy products, which could be used for infant and adults with lactose intolerance as well as for vegetarian or others who like to consume dairy products free of cholesterol. Additionally, the production of sesame-based dairy products can overcome the problems that limit consumption of soy-based dairy products such as flavour and flatulence.

Cooking is a common practise of food processing to prevent spoilage and enhance the keeping quality. The cooking process also gives the characteristics associated with edible food, which are generated through an intricate series of physical and chemical changes that occur when foods are heated.

However, cooking time is a key property of food processing which in most cases is not adhered to. Heat applied during cooking has no instant effect on the food being cooked; it takes time for heat to make the desired effect that is required of it on food generally (Orhevba, 2011). Cooking had some adverse effects on foods such as denaturation of protein, coagulation of lipids, breaking down of starch, leaching of mineral elements, development of melanoidins and cooked flavour, and destruction of essential amino acids and vitamins.

As a result, time is indeed a very important factor to be considered when processing food and indeed sesame milk to obtain its optimum benefit for man. Hence, the effect of cooking time on the nutritional parameters of sesame milk is of great importance to analyse.

MATERIALS AND METHODS

Source of material

The white variety of sesame seeds used for the preparation of the sesame milk were collected from National Cereal Research Institute (NCRI), Badegi, Nigeria and transported to the laboratory in an airtight polythene bag and stored under cool dry storage (4°C) condition until needed.

Preparation of sesame milk

The sesame seeds were cleaned to remove extraneous materials. The sesame seeds were dehulled by soaking in water (1: 5 w/v) for 4 h at 29 ± 2°C according to the method reported by Mohamed et al. (2007). The ruptured seed coats were then removed by rubbing with palms and washed with water. Dehulled sesame seed and tap water were weighed (1:4) to give the desired sesame seed percentage.

Sesame seed was transferred to the blender vessel and a small portion of the weighed water was added to facilitate the progress of mixing/grinding process. The blender was operated at highest speed for 10 min. After finishing the grinding process, the remaining quantity of water was added and mixed thoroughly. The resulted sesame dispersion was homogenized (in portions of 300 g) for 5 min using laboratory homogenizer.

The homogenized sesame milk base was squeezed through cheesecloth to separate coarse particles. The resulted milky solution was weighed and readjusted to its original weight (before filtration) by adding tap water.

The cooking of the extracted sesame milk

Sesame milk was cooked according to the method described by Orhevba (2011). A sample (500 mL) of the extracted sesame milk was boiled in a beaker to determine the temperature at which sesame milk boils. With the use of a thermometer, the boiling point (bp) of the sesame milk was measured once it began to boil. It was discovered that sesame milk boils at 100°C at ambient atmospheric conditions. Sesame milk at its boiling point (100°C) was collected as the control (sample A).

At 15 min from the time the sesame milk began to boil, sample B was collected from the boiling sesame milk, cooled and preserved in a sterile plastic bottle. The boiling sesame milk was allowed to cook for another 15 min, bringing the time to 30 min and then another sample C was collected, cooled and kept in sterile plastic bottle. The last sample of the experiment, sample D was collected after another 15 min of cooking from 30 min; bringing the total time of cooking to 45 min. The samples A, B, C and D were subjected to chemical analyses.

Chemical analysis

Proximate composition

The samples were analysed for moisture, crude protein (N × 6.25), fat, fiber and ash following standard procedures (AOAC, 2005). Total carbohydrates were calculated by difference. The sample energy value was estimated (kcal/g) by multiplying the percentages of crude protein, crude lipid and carbohydrate with the recommended factors (2.44, 8.37 and 3.57, respectively) as proposed by

by Martin and Coolidge (1978).

pH

The pH was determined using a digital pH meter (model: 3505, England).

Total solids

This was determined gravimetrically using the method described by Bradley (2003). A measured weight of each test sample was put in a previously weighed evaporating dish and evaporated to dryness over a steam bath. It was then dried in an oven at 105°C for an hour. It was cooled in desiccators and then reweighed by difference. The dry weight of the sample was obtained and expressed as a percentage of the sample weight.

Determination of mineral content

Analysis of potassium content of the samples was carried out using flame photometry, while phosphorus was determined by the phosphovanado-molybdate (yellow) method (AOAC, 2005). The other elemental contents (Ca, Mg, Fe, Se, Zn and Mn) were determined, after wet digestion of sample ash with an Atomic Absorption Spectrophotometer (AAS, Hitachi Z6100, Tokyo, Japan). All the determinations were carried out in triplicates.

Determination of vitamin content

Thiamine (vitamin B_1) and riboflavin (vitamin B_2) were determined by using spectrophotometric method AOAC (1990). Thiamine content was determined by weighing 0.5 g of the sample and adding 30 mL dichloroethane and 30 mL of 30% HCl (ratio1:1). Then 50 mL ammonium hydroxide solution was added. The solution was then filtered using Whatman No1 filter paper. Then the absorbance was read on a spectrophotometer (Spectronic 20 model) at 415 nm. Riboflavin content was determined by weighing 1 g of the sample and adding 50 mL of 50% methanol and 50 mL of 17% sodium carbonate. This is the extraction. Then, the absorbance was read on a spectrophotometer at a wavelength of 415 nm.

Determination of anti nutrient

The phytate content was determined by the method of Maga (1982). Two grams of each finely ground sample was soaked in 20 mL of 0.2 N HCl and filtered. After filtration, 0.5 mL of the filtrate was mixed with 1 mL ferric ammonium sulphate solution in a test tube, boiled for 30 min in a water bath, cooled in ice for 15 min and centrifuged at 3000 rpm for 15 min.

One millilitre of the supernatant was mixed with 1.5 mL of 2,2-pyridine solution and the absorbance measured in a spectrophotometer at 519 nm. The concentration of phytic acid was obtained by extrapolation from a standard curve using standard phytic acid solution.

The titration method described by Day and Underwood (1986) was used to determine the oxalate content. To 1 g of the ground powder, 75 mL of 15 N H_2SO_4 was added. The solution was carefully stirred intermittently with a magnetic stirrer for 1 h and filtered using Whatman No 1 filter paper. Filtrate (25 mL) was then collected and titrated against 0.1 N KMnO4 solution till a faint pink colour appeared that persisted for 30 s. The concentration of oxalate in each sample was obtained from the calculation:

1 mL 0.1 permanganate = 0.006303 g oxalate

Sensory evaluation

A 15 member sensory panellist comprising of students and staff of the Department of Food Science and Technology, Bowen University were used to assess sensory attributes. Panellists were asked to evaluate the samples for colour, flavour, taste, mouth feel and overall acceptability using a 5-point Hedonic Scale (5 = like extremely and 1 = dislike extremely) as described by Onwuka (2005).

Statistical analysis

Determinations were carried out in triplicates and the error reported as standard deviation from the mean. Analysis of variance (ANOVA) was performed and the least significant differences were calculated with the SPSS software for window release 16.00; SPSS Inc., Chicago IL, USA. Significance was accepted at $p \leq 0.05$ level.

RESULTS

Table 1 shows the physico-chemical properties for the sesame milk samples. Moisture, protein, fat and energy contents decreased significantly ($p \leq 0.05$) with increase in cooking time from 89.30 to 87.32%, 2.5 to 2.3%, 5.5 to 4.0% and 54.81 to 49.55 kcal/g in samples A to D respectively. In contrast, ash, fiber and carbohydrate increased significantly ($p \leq 0.05$) with increase in cooking time. The pH of the sesame milk samples also increased slightly but significantly ($p \leq 0.05$) with increase in cooking time from 6.57 in sample A to 6.83 in sample D. The total solids in the milk ranged from 7.95 to 10.90% with the sample A having the least value.

Table 2 shows the mineral and vitamin contents of the sesame milk samples. There was significant increase ($p \leq 0.05$) between the control and the samples cooked at 100°C for 15, 30 and 45 min. The concentrations of calcium and magnesium in all of the milk samples ranged from 262.05 to 273.44 and 163.50 to 173.45 mg/100 g, respectively. The values of potassium increased from 90.53 mg/100g in sample A to 95.58 mg/100g in sample D. Likewise the values of phosphorus increased from 189.78 to 196.20 mg/100 g in the sesame milk as it was cooked at 100°C. The remaining micro mineral elements under consideration were observed to increase in value in the following order: iron increased from 2.04 to 2.34 mg/100g, zinc increased from 2.31 to 2.89 mg/100g, selenium increased from 0.45 to 0.58 mg/100g and manganese increased from 1.22 to 1.36 mg/100g.

Water soluble vitamins B_1 (thiamine) and B_2 (riboflavin) were analyzed and were found present in all milk samples. The mean value determined for vitamin B_1 and vitamin B_2 in milk samples at 100°C was 0.42 and 0.25 mg/100 g, respectively. On boiling for 45 min, the mean values for vitamins in these milk samples showed a net decrease of 76.2 and 64.0% for vitamins B_1 and B_2, respectively.

Table 3 shows the anti nutrient composition of the sesame milk samples. At 45 min, the concentration of

Table 1. Physico-chemical properties of sesame milk samples.

Parameter	A	B	C	D
Moisture (%)	89.30^{d}±0.02	88.50^{c}±0.10	88.10^{b}±0.07	87.32^{a}±0.07
Ash (%)	1.08^{a}±0.06	1.32^{b}±0.02	1.47^{c}±0.10	1.54^{d}±0.07
Protein (%)	2.53^{d}±0.01	2.44^{c}±0.01	2.35^{b}±0.01	2.28^{a}±0.01
Fat (%)	5.50^{d}±0.02	4.62^{c}±0.05	4.19^{b}±0.02	3.95^{a} ±0.01
Fibre (%)	0.86^{a}±0.03	1.10^{b}±0.05	1.41^{c}±0.03	1.85^{d}±0.06
Carbohydrate (%)	0.73^{a}±0.01	2.02^{b}±0.01	2.48^{c}±0.01	3.06^{d}±0.06
Energy (kcal/g)	54.81^{d}±1.14	51.83^{c}±0.98	49.66^{ab}±0.72	49.55^{a}±1.12
Total solids (%)	7.95^{a}±0.06	8.41^{b}±0.04	9.20^{c}±0.04	10.90^{d}±0.06
pH	6.57^{a}±0.06	6.64^{b}±0.04	6.77^{c}±0.04	6.83^{d}±0.06

Values are means ± standard deviation of triplicate determinations; means with different superscripts within the same row are significantly different (p≤0.05); key: A = Control (Cooking at100°C for 0min); B = Cooking at 100°C for 15 min; C = Cooking at 100°C for 30 min; D = Cooking at 100°C for 45 min.

Table 2. Mineral and vitamin compositions of sesame milk (Mg/100 g).

Parameter	A	B	C	D
Calcium	262.05^{a}±1.12	267.27^{b}±1.80	270.20^{c}±2.07	273.44^{d}±1.11
Phosphorus	189.78^{a}±1.06	192.05^{b}±1.02	194.07^{c}±2.10	196.20^{d}±0.97
Potassium	90.53^{a}±0.01	92.54^{b}±0.15	93.56^{c}±0.81	95.58^{d}±0.12
Magnesium	163.50^{a}±0.02	167.40^{b}±0.05	170.43^{c}±0.02	173.45^{d} ±0.01
Iron	2.04^{a}±0.03	2.11^{b}±0.05	2.21^{c}±0.03	2.34^{d} ±0.06
Selenium	0.45^{a}±0.01	0.49^{ab}±0.01	1.54^{c} ±0.01	1.58^{d}±0.02
Zinc	2.31^{a}±0.06	2.71^{b}±0.04	2.82^{c}±0.04	2.89^{d}±0.05
Manganese	1.22^{a}±0.02	1.28^{b}±0.03	1.32^{c}±0.04	1.36^{d}±0.04
Thiamine	0.42^{d}±0.01	0.37^{c}±0.01	0.34^{b}±0.02	0.30^{a}±0.02
Riboflavin	0.25^{d}±0.04	0.22^{c}±0.03	0.19^{b}±0.01	0.16^{a}±0.05

Values are means ± standard deviation of triplicate determinations; means with different superscripts within the same row are significantly different (p≤0.05).

Table 3. Anti nutrient composition of sesame milk (Mg/100g).

Parameter	A	B	C	D
Oxalate	45.26^{d}±1.02	37.23^{c}±0.47	30.47^{b}±1.07	28.15^{a}±0.05
Phytate	26.50^{d}±0.09	16.63^{c}±0.02	15.25^{b}±0.40	13.36^{a} ±0.13

Values are means ± standard deviation of triplicate determinations; means with different superscripts within the same row are significantly different (p≤0.05).

phytate and oxalate in sesame milk was lowest and significantly different (p≤0.05) from the samples cooked for 0, 15 and 30 min. Cooking for 45 min at 100°C is required to reach 50.42 and 62.20% inactivation of phytate and oxalate respectively in sesame milk.

Table 4 shows the mean sensory scores of sesame milk cooked at various time. The scores for colour increased significantly (p≤0.05) from 6.73 in sample A to 7.12 and 7.29 in samples B and C respectively while sample D had least value. Similarly, the score for taste, flavour, mouth feel and overall acceptability increased significantly (p≤0.05) from sample A to sample B and C, respectively, with a decrease in sample D only. Cooking at 100°C for 30 min (sample C) gave the highest acceptability score of 8.10, followed by samples B, A and D in that order.

DISCUSSION

The decrease in moisture content as cooking time increase was as a result of evaporation of water during

Table 4. Mean sensory scores of sesame milk.

Parameter	A	B	C	D
Colour	6.73^{b}±0.02	7.12^{c}±0.10	7.29^{d}±0.07	5.98^{a}±0.07
Flavour	7.28^{b}±0.06	7.35^{c}±0.02	7.52^{d}±0.10	6.82^{a}±0.02
Taste	7.41^{b}±1.12	7.57^{c}±0.07	7.68^{d}±1.01	6.56^{a}±0.06
Mouth feel	7.35^{b}±0.08	7.48^{c}±0.05	7.63^{d}±1.12	6.44^{a}±0.03
Overall acceptability	7.67^{b}±0.07	7.83^{c}±0.45	8.06^{d}±0.73	6.66^{a}±1.02

Values are means ± standard deviation of triplicate determinations; means with different superscripts within the same row are significantly different ($p \leq 0.05$).

cooking. However, the observed high moisture content of sesame milk could affect the stability and safety with respect to microbial growth; hence the products require cold storage. Results are within the range reported for soymilk cooked under the similar condition (Akintunde and Souley, 2009). Apparently, the differences observed in protein values as a result of different heating period could be related to the denaturation. Proteins form different 3-dimensional structures, by the folding and subsequent bonding of the amino acid strands. Generally, the bonds which link the folded amino acid strands together (mostly hydrogen bonds), are much weaker than the strong peptide bonds forming the strands. During cooking, the heat causes the proteins to vibrate more vigorously which results in the breakage of the weak hydrogen bonds holding the amino acid strands in place. Consequently, the protein unravels to re-take its initial form of amino acid strands. Similarly, cooking process could reduce protein content due to the fact that protein can be involved in the Maillard reactions together with reducing sugars. Among the proteins, lysine is the most implicated in this reaction and its diminution in time became a nutritional indicator of the effects of heat treatments on food (Florea, 2001).

The significant decrease in fat content as sesame milk was cooked at its boiling temperature for 15 to 45 min as compared to sesame milk at its boiling point could be as a result of higher exposure times which introduce changes to lipids in such ways that affect their nutritional value. Some of these changes include lipid oxidation in the presence of oxygen and the removal of the film (rich in protein and fat) at the top of the milk during cooking. The remarkable reduction in the percentage fat agrees with the findings of Orhevba (2011). Significantly, higher ash, fiber and carbohydrate values observed with increase in cooking time may be attributed to concentration effects on sesame milk by loss of water. The significant ($p \leq 0.05$) increase in crude fiber with increase in cooking time is a positive nutritional development. Though crude fibre does not contribute nutrients to the body, it adds bulk to food thus facilitating bowel movements (peristalsis) and preventing many gastrointestinal diseases in man (Gordon, 1999). The increase in ash reflected in significant increase in mineral concentrations. The observed increase in carbohydrate content is in agreement with Yau-Chun et al. (2011) who also reported similar increase in soy milk produced by blanching and grinding of soybeans with hot water. Carbohydrates provide heat and energy for all forms of body activity. Deficiency of carbohydrate causes the body to divert proteins and body fat to produce needed energy, thus leading to depletion of body tissues (Gordon, 1999). The decrease in energy value observed with increase in cooking time could be attributed to decrease in fat content of the milk samples as fat contains about twice the food energy values of protein and carbohydrate.

The fact that all the pH values are below neutral (7.0) is an indication that microbial growth will not be easily encouraged in the sesame milk samples. The pH values of sesame milk samples were comparable to the pH of soymilk (6.60) as reported by Onweluzo and Owo (2005). Differences in total solids between the milk samples could be related to effect of heat treatment.

There was positive increase in the elemental concentrations from the control to sample D. Minerals are essential nutrients, without which the body cannot function correctly. Heating itself does not affect mineral levels but are usually leached if cooked in boiling water. Minerals tend to have higher heat stability and are less affected by cooking methods which involve heating foods for longer periods of time. However, the higher mineral concentrations observed as sesame milk was cooked at its boiling point for 0-30 min are the result of the decrease in anti-nutrients particularly phytate as the major elements such as phosphorus, calcium and potassium are parts of the molecular structure of phytic acid and phytin (Duhan et al., 2002).

The only factor that could account for the observed significant decrease in thiamine and riboflavin as the cooking time increased was the heat applied as these vitamins are thermo labile in nature. Milk boiling is a common processing practice around the globe to reduce the level of natural microbial pathogens found in milk, however, this practice has deleterious effect on the contents of B-vitamins (Asadullah et al., 2010). Due to their tendency to disperse in water, water-soluble vitamins in particular are immensely affected by cooking processes. Considering the effect of cooking on the reten-

tion of these water-soluble vitamins, it was observed that riboflavin is more stable than thiamine. Similar observation was reported by Fanelli et al. (1985). They further reported that such stability can only be ensured if light is excluded during processing and cooking of food as riboflavin undergoes photo degradation in the wavelength range of 400-550 nm.

The anti nutritional compositions of milk samples were reduced to innocuous level. The physical removal of sesame hull during initial dehulling was associated with decreased phytate and oxalate contents (Makinde and Akinoso, 2013) and much more is expected during the cooking of resultant sesame milk. In general, cooking time was reported to have pronounced effects on the anti-nutritional factors present in natural foods (Kaack, 1994).

Cooking has a substantial impact on the final sensorial characteristics of many different foods. The observed reduction in the colour score of sample D as compared to other milk samples could be due to Maillard browning reactions, which usually occur between reducing sugars and amino groups in proteins and amino acids. Such reactions were encouraged by increase in concentration of reactants (Alais and Linden, 1991). Similarly, the decrease in flavour score of sample D could be due to derived off-flavours due to concentration effects. The concentration also made the sesame milk thicker and heavier, thus adversely affecting the taste and the mouth feel. However, sesame milk cooked at 100°C for 30 min (sample C) gave the highest acceptability score.

Conclusions

Sesame milk offers array of nutrients which are essentials to normal body functions. In this study, cooking within the time limit (15-45 min) had a significant effect on sesame milk composition and sensory properties. Results indicated that the appropriate heat treatment for sesame milk processing was cooking at 100°C for 30 min.

Conflict of Interests

The author(s) did not declare any conflict of interests.

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Full Length Research Paper

Effect of some extrusion parameters on the nutrient composition and quality of a snack developed from cocoyam (*Xanthosoma sagittifolium)* flour

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There is increase in the use of cocoyam tubers for flour production which can be use for food and industrial purposes in Nigeria. Cocoyam (*Xanthosoma sagittifolium)* flour was cooked and extruded in a single screw extruder. A second order central composite response surface design was used in designing the experiment which generated 20 runs on selected process parameters including feed moisture (22, 24 and 26%), screw speed (60, 70 and 80rpm) and barrel temperature (200, 220 and 240°C) on the functional and physical properties (density, expansion, water absorption index (WAI), water solubility index (WSI) and hardness) of the extrudates. Furthermore, the nutritional compositions of the snacks were also determined. Increase in feed moisture content results in higher density, lower expansion, higher WAI, lower WSI and higher hardness in the extrudates while increase in barrel temperature decreases the density, WAI and hardness but led to increase in expansion and WSI in the extrudates. The nutrient value for the extruded snacks were protein (3.76%), fibre (6.41%), carbohydrate (79.50%), energy (343.03 kj/100 g), Ca (238.50 mg/100 g), Mg (113.71 mg/100 g), K (283.77 mg/100 g), Na (97.66 mg/100 g), P(161.23 mg/100 g), vitamin C (1.03 mg/100 g) and niacin (0.78 mg/100 g). This study has established that a nutritious and acceptable snack can be produced from cocoyam flour using extrusion cooking.

Key words: Cocoyam flour, single screw extrusion, functional, physical properties, nutritional composition.

INTRODUCTION

Cocoyam, a member of the Araceae family is an ancient crop and is one of the minor staple root crops commonly grown in the forest zone of Nigeria and Ghana (Ekanem and Osuji, 2006). Cocoyam contribute significant portion of carbohydrate content of the diet in many regions in developing countries and provide edible starchy storage

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Table 1. Coded levels for the response surface design.

Variables	Level				
	-α	-1	0	+1	+α
Feed moisture (%), X_1	20.32	22	24	26	27.68
Screw speed (rpm), X_2	58.32	60	70	80	81.68
Temperature (°C), X_3	198.32	200	220	240	241.68

α =1.682.

corms or cormels. Although cocoyam is less important than other tropical root crops such as yam, cassava and sweet potato, they are still a major staple in some parts of the tropics and subtropics (Ojinnaka et al., 2009). The world's leading producer of cocoyam is Nigeria producing an estimate of 3.7 million metric tonnes annually (Baruwa and Oke, 2012). The main nutrient supplied by cocoyam is dietary energy provided by the carbohydrates. Cocoyam is a good source of Na, K, P, Mg and Ca and is fairly rich in carotene, ascorbic acid, thiamine, riboflavin and nicotinic acid. The leaves contain beta-carotene, iron and folic acid (Eka, 1998; FAO, 1990). The problem of inherent nutritional hazard such as presence of acridity factors, oxalate and perishability of the tubers call for elaborate processing prior to consumption, thereby improving handling, convenience, palatability, storability and nutritional safety (Iwuoha and Kalu, 1995). Most processing methods are known to reduce the level of oxalate in cocoyam such as boiling, roasting, frying in oil, milling and conversion into 'fufu', soup thickeners, flour for baking, chips, beverage powder, porridge and specialty food for gastro-intestinal disorders (Iwuoha and Kalu, 1995).

Extrusion cooking is one of the most versatile and well established food processes and is used worldwide for the production of expanded snack foods, pastes, modified starch, flat breads, meat and cheese analogues, ready to eat cereal foods and porridge (Li and Lee, 2000; Thymi et al., 2005). The main purpose of extrusion is to increase the variety of foods in the diet, by producing a range of products with different shapes, textures, colours and flavours from basic ingredients (Fellows, 1999). It has been reported that small variations in processing conditions affect process variables as well as product quality, which can vary considerably depending on the extruder type, screw configuration, feed moisture, temperature profile in the barrel session, screw speed and feed rate (Ding et al., 2005).

Response surface methodology (RSM) is a statistical method for determining and simultaneously solving multivariate equation. It uses an experimental design such as central composite rotatable design (CCRD) to fit a first or second order polynomial by least significant techniques. An equation is used to describe how the test variables affect the response and to determine inter-relationship among the test variables in the response (Sobukola, 2007). The main objective of this research was to determine the effect of extrusion parameters on the physical, functional and nutritional composition of snacks developed from cocoyam flour.

MATERIALS AND METHODS

Preparation of flours

Cocoyam tubers (*Xanthosoma sagitifolium)* were purchased in Abeokuta, Ogun State. The processing of the tubers to flour as described by Idowu et al. (1996) was employed. Cocoyam tubers were selected, cleaned, hand peeled, washed and sliced into chips of 3-4 mm thickness. The chips were steeped for 12 h, rewashed and sulphited in 0.1% potassium metabisulphite solution for 3 h. The sulphited chips were dried in cabinet dryer at 60°C for 24 h, milled in attrition mill, and sieved (< 600µm) into flour. The cocoyam flour was stored in high density polyethylene films until processed.

Before extrusion, cocoyam flour (500 g) was pre-conditioned according to each moisture content (Table 1) by calculated amounts of water being incorporated into each sample. After that, the samples were sealed in high density polyethylene films and kept at ambient temperature for 12 h to reach homogeneous equilibrium moisture distribution.

Extrusion process

The cocoyam flours were cooked and extruded using a single screw extruder (model 1993 DD85G, 201132, IBG Monforts Gmbhi & Co, D-4050 Monchengladbach, Germany). The extruder was equipped with 254 mm barrel, a screw diameter of 200 mm and was fitted with a die nozzle of 4 mm diameter. The rehydrated samples were then extruded and the extrudates were cooled to room temperature and sealed in high density polyethylene films until measurements were taken.

Experimental design

A centre composite RSM design was used to show interactions of feed moisture, screw speed and temperature on the extrudates. This comprised of 20 runs, of which six were centre point and 14 for non-centre point (Stat-Ease, 2002).

Second order polynomial model was fitted to measure dependent variables (Y) such as bulk density (Y_1), expansion rate (Y_2), water absorption index (Y_3), water solubility index (Y_4), texture (Y_5). The following equation was used:

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2 +\beta_{12}X_1X_2+ \beta_{13}X_1X_3 + \beta_{23}X_2X_3$$

Where, β_0, β_1-β_3, β_{11}-β_{33} and β_{12}-β_{23} are regression coefficients for interception, linear, quadratic and interaction coefficients, respectively, X_1-X_3 are coded independent variables and Y is the response (Myers and Montgomery, 1995).

An ANOVA test was carried out using Design Expert 6.0.8 (Stat-Ease Inc., Minneapolis, USA) to determine the significance at different levels (0.1, 1 and 5%) (Stat-Ease, 2002).

Physical and functional properties of the extrudates

Bulk density

The bulk density was calculated by measuring the actual dimen-

Table 2. Chemical composition of cocoyam flour.

Composition	g/100g
Moisture	5.38
Protein	3.92
Fat	0.28
Fibre	1.56
Sugar	1.79
Starch	73.28
Carbohydrate	86.78

sions of the extrudates according to the method described by Ding et al. (2005). The diameter and length of the extrudates were measured using Vernier caliper. The bulk density was then calculated using the following formula,

$$\text{Density (g/cm}^3\text{)} = \frac{4 \times M}{\pi \times D^2 \times L}$$

Where: M = Mass (g), D = diameter (cm), L = length (cm). Six replicates of extrudate were randomly selected and an average taken.

Expansion ratio

The ratio of diameter of extrudate and the diameter of die was used to express the expansion of extrudate according to Ding et al. (2005). Six replicates of extrudate were randomly selected and an average taken.

Water absorption index (WAI) and water solubility index (WSI)

The WAI and WSI were measured using the method of Ding et al. (2005). The extrudate samples were milled and sieved through 600 µm sieve. 2.5 g samples was dispersed in 25 ml distilled water, using glass rod to break up any lumps and then stirred for 30 min, centrifuged at 4000 rpm for 15 min. The supernatant was decanted into an evaporating dish of known weight and dried at 105°C until constant weight. The weight of the gel remaining in the centrifuge tube was noted. The results were expressed as the average of the two measurements.

$$\text{WAI (g/g)} = \frac{\text{Weight gain of gel}}{\text{Dry weight of extrudate}}$$

$$\text{WSI (\%)} = \frac{\text{Weight of dry solids in supernatant}}{\text{Dry weight of extrudate}} \times 100$$

Determinations were made in triplicate.

Hardness

The hardness was determined by using Erweka (Gmbh D 63150, TBH200, Heusenstamm / Germany) hardness tester fitted with a 2 mm cylinder probe. The samples were punctured by the probe to a distance of 6 mm and the hardness recorded.

Nutrient composition of the extruded snack

Chemical composition

Moisture, protein, ash, fat and fibre contents were determined using AOAC (2000). Total sugar and starch contents were determined by the procedure described by Kayisu et al. (1981). Carbohydrate was estimated by difference and the energy were calculated using the Atwater factors of 4 X proteins, 4 X carbohydrates and 9 X fats. The samples were analyzed in triplicate.

Mineral analysis

Mineral elements were determined using AOAC (2000) methods. One gram of the sample was first digested with 20 ml of acid mixture (650 ml concentrated HNO_3; 80 ml per chloric acid (PCA); 20 ml concentrated H_2SO_4) aliquots of the diluted clear digest was used in atomic absorption spectrophotometer for determination of Ca, Mg, Zn, Cu, S and Fe. Flame analyzer was used for determination of K and Na using filters that match the different elements. Phosphorus (P) was determined by converting phosphate into phosphorus molybdate blue pigment and assayed at 7000 nm.

Vitamin analysis

Vitamin C, thiamine, riboflavin and niacin were determined using the method described by AOAC (2000).

Statistical analysis

The data obtained from the nutritional composition were subjected to One-Way-Anova [ANOVA] and the means of values were separated by Duncan Multiple Range Test using SPSS 16.0.

RESULTS AND DISCUSSION

Chemical composition of cocoyam flour used for extrusion is shown in Table 2. It contained moisture (5.38%), protein (3.92%), fat (0.28%), fiber (1.56%), sugar (1.79%), starch (73.28%) and carbohydrate (86.78%).

Effect of extrusion parameters on bulk density

The effect of extrusion parameters on extrudates density is shown in 3-D surface plot (Figure 1). It was observed that increase in feed moisture leads to increase in extrudates density at all temperature levels. However, increase in barrel temperature causes a decrease in the density of the extrudates. Asare et al. (2004) reported that bulk density has been linked with the expansion ratio in describing the degree of puffing in extrudates. Increase in feed moisture during extrusion of cocoyam flour could probably be due to a reduction in the elasticity of the

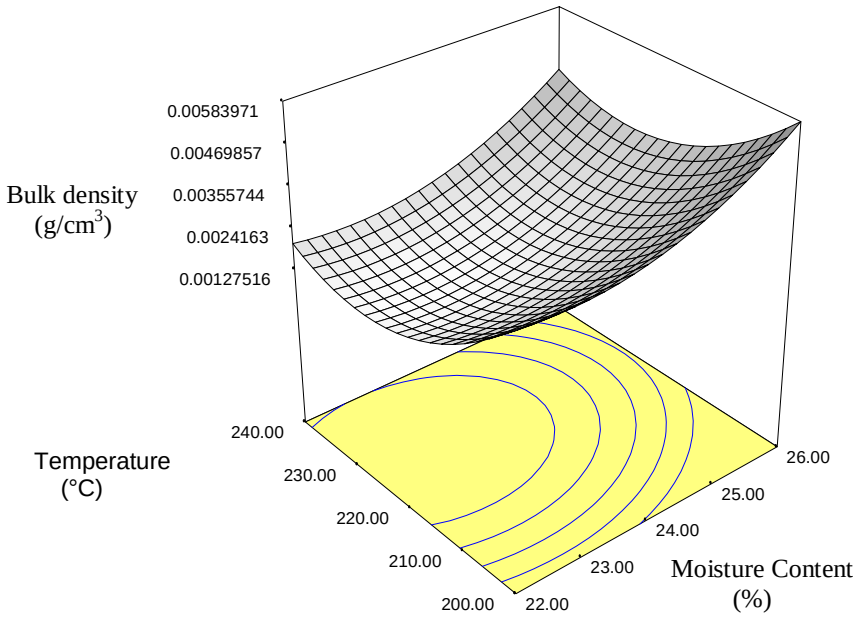

Figure 1. Effect of moisture content and barrel temperature at constant screw speed on the bulk density of cocoyam extrudates.

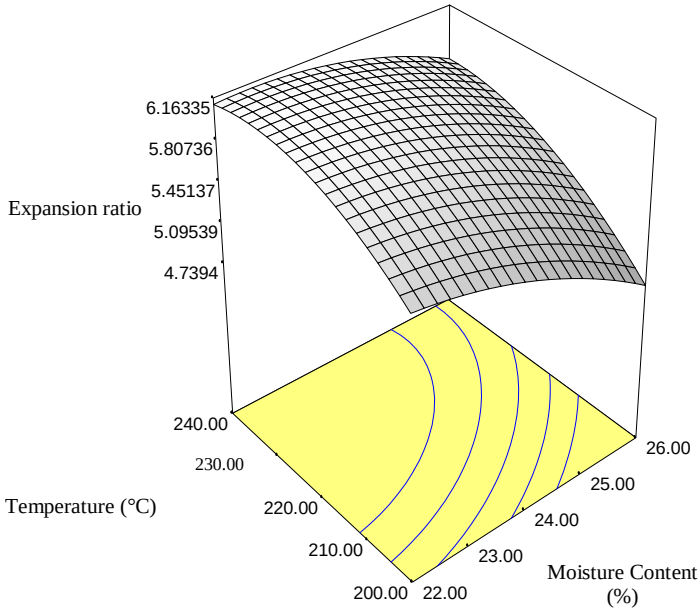

Figure 2. Effect of moisture content and barrel temperature at constant screw speed on the expansion ratio of cocoyam extrudates.

dough through plasticization of the melt, causing reduction in gelatinization and increase in the density of the extrudates. Increase in screw speed was observed in extrudates with lower density. Higher screw speed could have lowered the melting viscosity and increase the elasticity of the dough which results in reduction in the density of the extrudates (Ding et al., 2005).

Effect of extrusion parameters on expansion

The effect of extrusion parameters on the expansion of extrudate is shown in 3-D surface plot (Figure 2). Increase in feed moisture content caused decrease in the expansion ratio of the extrudates while increase in barrel temperature led to increase in the expansion ratio of the

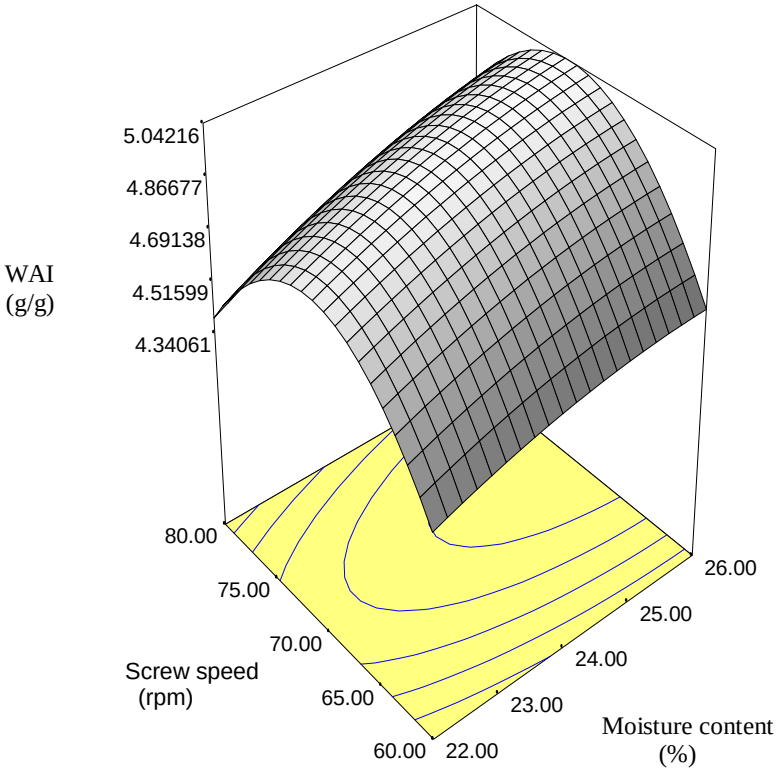

Figure 3. Effect of moisture content and screw speed at constant barrel temperature on the water absorption index of the cocoyam extrudates.

extrudates. Feed moisture content has been reported to have a highly significant effect on the radial expansion ratio. The radial expansion decreased with an increase in feed moisture content and it is most dependent on the melt elasticity (Launay and Lisch, 1983). Increase in feed moisture content during extrusion could have also lead to changes in the amylopectin networks and in the melting rheology characteristics leading to greater elastic effect and changes in product density and expansion.

Effect of extrusion parameters on WAI and WSI

The effect of extrusion parameters on the WAI and WSI of the extrudates are presented in 3-D surface plot (Figures 3 and 4). Increase in feed moisture content significantly increased the WAI of the extrudates. WAI also increased with increase in screw speed. Increase in barrel temperature was observed to cause a significant decrease in WAI. Increasing feed moisture content was observed to result in a significant decrease in WSI of the extrudates. However, increase in barrel temperature was observed to cause a significant increase in WSI of extrudates. The WAI measures the volume occupied by the starch after swelling in excess water, which maintains the integrity of starch in aqueous dispersion (Mason and Hoseney, 1986). WSI is often used as an indicator of degradation of molecular components (Kirby et al., 1988), and measures the degree of starch conversion during extrusion which is the amount of soluble polysaccharide released from starch component after extrusion. During the extrusion of the cocoyam flour, water is absorbed and bound to the starch molecule with a resulting change in the starch granule structure. Barrel temperature and feed moisture was observed to have the greatest effect on gelatinization. The maximum gelatinization occurs at low moisture and high temperature or vice versa (Ding et al., 2005). Decrease in WAI could probably be due to dextrinization, which also could have led to increase in WSI.

Effect of extrusion parameters on hardness

The effect of extrusion parameters on the hardness of the extrudates is shown in 3-D surface plot (Figure 5). An Increase in feed moisture content caused an increase in the hardness of the extrudates, while increase in screw speed and barrel temperature resulted in a decrease in hardness of the extrudates. The hardness is the average force required for a probe to penetrate the extrudate. The hardness of the extrudates increased as the feed moisture content increase which could be due to reduced expansion caused by the increase in moisture content

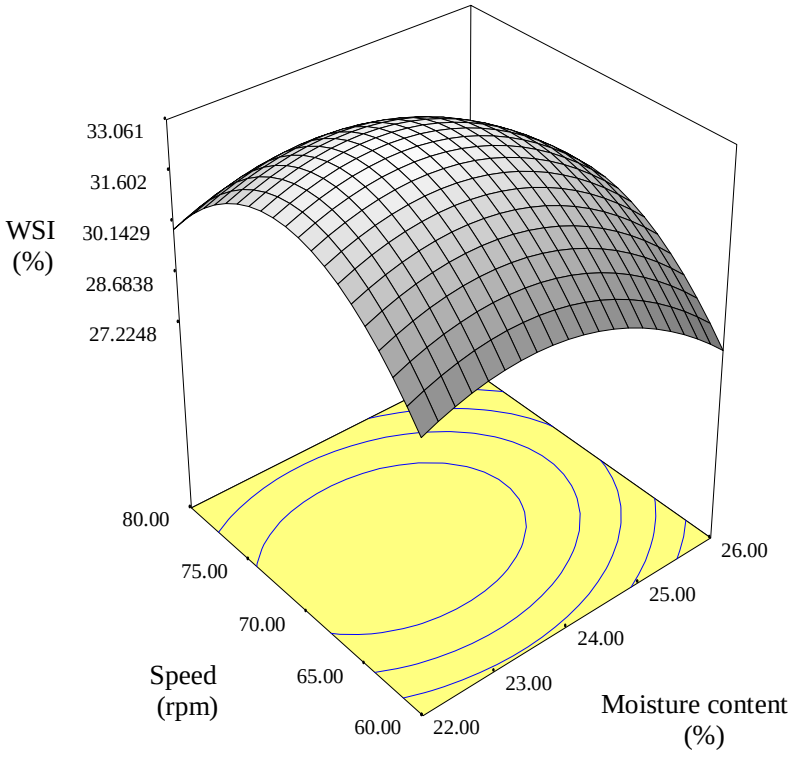

Figure 4. Effect of moisture content and screw speed at constant barrel temperature on the water solubility index of cocoyam extrudates.

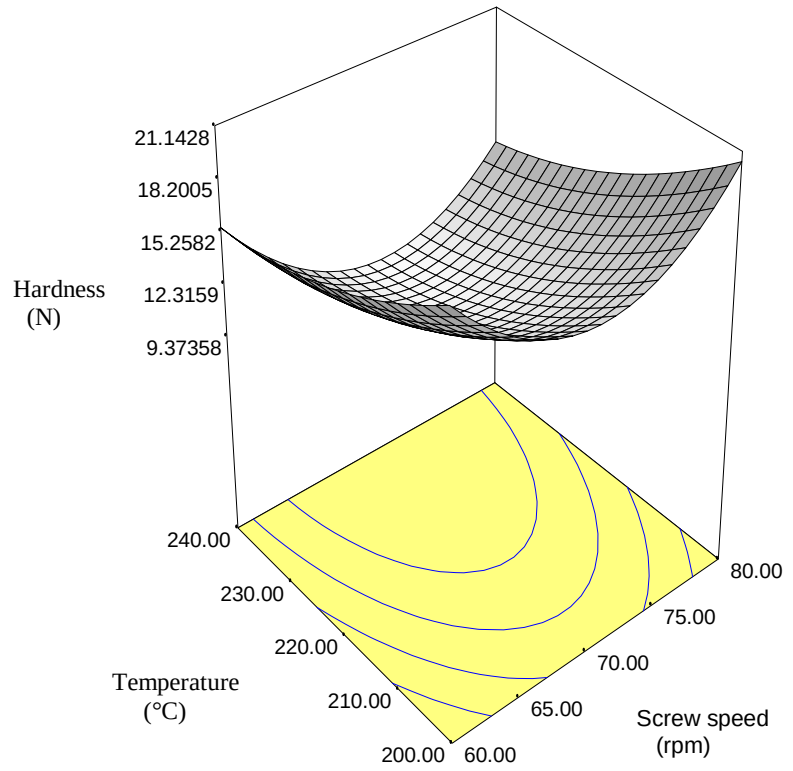

Figure 5. Effect of screw speed and barrel temperature at constant moisture on the hardness of cocoyam extrudates.

Table 3. Significant coefficients of regression equation for the responses

Coefficient	Bulk Density (g/cm^3)	Expansion Ratio	WAI (g/g)	WSI (%)	Hardness (N)
β_0	0.307**	-48.609*	-45.840**	-853.758***	851.298*
β_1	-0.009**	1.105*	0.236*	20.952**	-34.042**
β_2	-0.000	0.515	0.545	3.895	-6.812
β_3	-0.001**	0.207*	0.265**	4.441***	-1.771*
β_{11}	0.000*	-0.036	-0.010	-0.392***	0.648*
β_{22}	0.000	-0.003	-0.003**	-0.027***	0.049*
β_{33}	0.000*	-0.000	-0.000*	-0.009***	0.003
β_{12}	-0.000	-0.001	0.002	0.000	0.015
β_{13}	-0.000	0.002	0.000	-0.012	0.016
β_{23}	-0.000	0.000	-0.000	-0.000	-0.002
R^2	0.883	0.767	0.859	0.975	0.828

***Significant at the 0.1%; **Significant at the 1%; *Significant at the 5% .

Table 4. Chemical composition of extruded cocoyam snacks.

Parameters	Expanded cocoyam snack *
Moisture (%)	6.68±0.08
Protein (%)	3.76±0.01
Ash (%)	2.54±0.04
Fat (%)	1.11±0.03
Fibre (%)	6.41±0.01
Carbohydrate (%)	79.50±0.02
Energy (kj/100g)	343.03±0.33
Total sugar (%)	19.25±0.05
Starch (%)	17.33±0.05
pH	5.60±0.05
TTA (%)	0.39±0.01

*Each value represents the mean of triplicate determinations ± standard deviation

Table 5. Mineral and vitamin contents (mg/100g) of cocoyam snacks.

Parameter	Expanded cocoyam snack from Tannia*
Ca	238.50±1.87
Mg	113.71±1.48
K	283.77±2.69
Zn	7.51±0.09
Cu	7.85±0.23
Na	97.66±0.89
S	3.35±0.25
Fe	25.53±0.88
P	161.23±2.96
Vitamin C	1.03 ±0.01
Thiamine	ND
Riboflavin	ND
Niacin	0.78±0.04

*Each value represents the mean of triplicate determinations ± standard deviation.

(Lui et al., 2000). An increase in temperature resulted in a decrease in hardness which could be due to reduction in melting viscosity favouring bubble growth, increased expansion and lower density gave a softer extrudate. An increase in screw speed could also have lowered the melting viscosity of the mix resulting in a less dense and softer extrudates.

Effect of extrusion parameters on the nutrient composition of extruded cocoyam snack

The nutrient composition of the extruded cocoyam snack is shown in Tables 4 and 5. Extrusion cooking, like other food processing methods could have desirable and undesirable effects on the nutritional value of the extruded snacks. The food components that play an important role in the extrusion cooking processes are starch and proteins. During extrusion cooking, starch granules are disrupted and melted at low moisture contents, swelled and gelatinized at high moistures (Ilo et al., 2000). In both cases, starch conversion led to the loss of crystalline structure to form an amorphous phase, which in extrusion cooking of starch materials resulted in a fluid mass with starch biopolymers in the continuous phase. This help to retain the gases released during the expansion process at the extruder, enabling the formation of expanded foam structures. The amount of polymer which is found in the continuous phase determines the extensibility of bubble cell walls in the foam and the overall expansion of extrudates at the die (Ilo et al., 2000). Riaz (2000) reported that within the extruder barrel, unique chemical transformations occur. Different five chemical or physicochemical changes can occur during extrusion cooking such as binding, cleavage, loss of native conformation, recombination of fragments and thermal degradation. During extrusion cooking of the flour, there was a decrease in starch content and an increase in sugar content, this might be due to gelatini-

zation of starch that occurs at lower moisture levels (Qu and Wang, 1994). This agrees with the report of Hsieh et al. (1993) that during extrusion cooking, starch resulted into the production of maltodextrins and sugar. Proteins act as 'filler' in starch extrudates and are dispersed in the continuous phase of the extrusion melt, modifying the flow behaviour and characteristics of the cooled extrudates. Protein materials hydrate the mixing stage of the process and become soft viscoelastic doughs during formation of the extrusion melt. The shearing forces generated in the extruder cause breakage of the protein into small particles of roughly cylindrical and globular shapes and tend to reduce the extensibility of the starch polymer foam during its expansion at the die exit, reducing the degree of expansion (Brennan, 2006). Proteins are denatured by extrusion cooking process. Proteins are made of amino acids which are known as the building blocks of protein. Amino acids are held together by primary bonds whereas the molecules are held together by secondary bonds. The cooking action of the extruder breaks down the secondary bonds but does not create sufficient heat to destroy the amino acids or the primary bonds (INSTA-PRO, 2011). There was a decrease in protein content due to extrusion cooking which could have been due to denaturation of protein. Denaturation of protein has been reported to improve nutritional quality by making the molecules more accessible to proteases and more digestible (Brennan, 2006). It has been reported that most enzyme activities were lost within the extruder unless they were stable to heat and shear (Della Valle et al., 1994). Denaturation and loss of solubility was reported to be affected by increased barrel temperature (Della Valle et al., 1994). Area (1992) reported that during extrusion, disulfide bonds were broken and may re-form. Electrostatics and hydrophobic interactions favor formation of insoluble aggregates. The creation of new peptide bonds during extrusion was controversial and high molecular weight proteins can dissociate into smaller sub-units.

The exposure of enzyme susceptible sites improves digestibility. Maillard reactions may also occur particularly at higher barrel temperatures and lower feed moisture at which the flour was treated. Free sugars might be produced to react with lysine and other amino acids with free terminal amines. The starch and dietary fiber fragments as well as sucrose hydrolysis products were available for maillard reactions (Bates et al., 1994). The degree of fat complexing during extrusion depends on starch content in process material. This facilitates the formation of starch-lipid complexes. The presence of starch and protein in raw material favours the formation of starch-lipid and lipid-protein complexes (Sobota et al., 2010).

Most of the minerals and vitamins differ greatly in composition and stability, and also it varies during extrusion cooking. Minimizing temperature and shear within the extruder protects most vitamins. The water soluble vitamin most susceptible to thermal processing is thiamine. Thiamine stability during extrusion is highly variable as evidenced by Killeit (1994). He reported that losses ranged between 5-100%.

Conclusion

The functional and physical properties of cocoyam extrudates on single screw extrusion process were dependent on the process variables. The feed moisture and barrel temperature had significant effect on the extrudates properties, with feed moisture having the greatest influence. The high starch content of cocoyam flour makes it a great potential as a food ingredient in extruded products and can be successfully used in preparation of high quality snacks.

Conflict of Interests

The author(s) did not declare any conflict of interests.

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