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Review

Bael (*Aegle marmelos* Correa) products processing: A review

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Bael (*Aegle marmelos* Correa) being an indigenous fruit occupies an important place from medicinal point of view. The different varieties of Bael such as Mirzapuri, Kagzi Gonda, Kagzi Banarsi, Kagzi Etawah, Narendra Bael-1, Narendra Bael-2, Narendra Bael-5, Narendra Bael-9, Pant Shivani, Pant Sujata, etc are popular. Its nutritional and medicinal properties make this fruit one of the most valuable and a good source of nutrients, and qualities to cure diarrhoea, dysentery and other stomach ailments. This fruit have unlimited potential in its processed form. Bael can be processed to prepare jam, squash, nectar, toffee, slab, powder, ready-to-serve (RTS), wine, etc. The present paper therefore deals with the processing of bael into different products and the medicinal aspects together with physico-chemical changes in bael fruit.

Key words: *Aegle marmelos*, bael, medicinal properties, processing aspects, nutritional properties.

INTRODUCTION

The bael fruit (*Aegle marmelos* L. Correa) is known in India from prehistoric times. This fruit is native to Northern India, but widely found throughout the Indian Peninsula (Rahman and Pravin, 2014). It also grows in Sri Lanka, Pakistan, Bangladesh, Burma, Thailand and most of the southeastern Asian countries (Rakesh et al., 2005).

Roy et al. (1979) stated that the three lobed leaves of bael tree are traditionally used as sacred offerings to "Lord Shiva" according to Hindu customs. It has been said that this tree indicates the presence of underground

water. Bael is a sub-tropical tree but grows well both in tropical and sub-tropical climate up to an altitude of 1219 m. It is medium in size, about 12 to 15 m in height with short trunk, thick, soft, flaking bark and the lower ones drooping. It withstands temperatures as low as -8°C but under severe cold, it sheds its leaves. It is one of the choicest fruits of arid and semi arid zones due to its drought resistance and tolerance to temperatures upto 48°C. In Punjab, it grows up to an altitude of 4,000-ft (1,200-m) where the temperature rises to 120°F (48.89°C) in the shade in summer and descends to 20°F

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Table 1. Nutritional composition of bael fruit.

Nutrient	Amount
Moisture (g)	61.0
Protein (g)	1.6
Fat (g)	0.2
Mineral (g)	1.9
Fibre (g)	2.9
Calcium (mg)	80
Phosphorous (mg)	52
Iron (mg)	0.5
Carotene (µg)	55
Thiamine (mg)	0.12
Niacin (mg)	1.0
Vitamin C (mg)	8
Potassium(mg)	610
Copper (mg)	0.20

(-6.67°C) in the winter and prolonged droughts occur (Lambole et al., 2010).

Its mature leaf emits a disagreeable odour when bruised. Fragment flowers, in clusters of 4 to 7 along young branch lets, have 4 recurved, fleshy petals, green outside, yellowish inside and 50 or more greenish-yellow stamens (Patel et al., 2012). According to Chattopadhyay (1998), bael is a very hardy tree and grows in all type of soils. It thrives well even in swampy, alkaline and stony soils having pH range of 5 to 10. However, for good growth and yield, well drained, humus soils having pH range from 5.5 to 7.5 are best.

There are no standardized names for bael cultivars. They are named after the names of the locality where they are most easily available. Its main cultivated varieties in India are Mirzapuri, Kagzi Gonda, Kagzi Banarsi, Kagzi Etawah, Narendra Bael-1, Narendra Bael-2, Narendra Bael-5, and Narendra Bael-9. Some scientists have tried to identify and compare different varieties of bael fruits grown. Jauhari et al. (1969) surveyed the central and eastern Uttar Pradesh, western Bihar and selected seven varieties for physico-chemical studies. They found the "Kaghzi Etawah" as the best variety having 1893 g weight per fruit and 1583 g pulp per fruit with 36% TSS, 0.33% acidity and 21.7 mg ascorbic acid per 100 g edible pulp.

Bael fruit (*A. marmelos* L. *Correa*) belongs to the family *Rutaceae*, occupies an important place among the indigenous fruits of India. It is known by different names viz. Bael, Bel, Bengal Quince, Bil, Bilva, Bilpatre, Shul, Shaiphal, Vilvum, etc. In ancient Sanskrit poems, this fruit is considered auspicious, sacred and emblem of prosperity. This fruit is considered as of great mythological and religious significance (Sharma and Bhagwan, 1988). It is a large deciduous tropical tree, found all over India in Sub-Himalayan forests, Bengal,

Central and South India and also in Burma.

The utility of bael is mentioned in the Indian ancient system of medicine. Every part of the tree such as root, bark, leaf, flower, fruits, seed and even its latex are important in several traditional system of medicine, that is why it is one of the most important trees in India (Patel et al., 2012). The decoction of the root and root bark is useful in intermittent fever, hypo-chondriasis, melancholia and palpitation of the heart. The leaves and bark have been used in medicated enema. The nutritional composition of bael fruit is presented in Table 1.

The peel of the fruit which is a very hard shell and green to brown in color depends on ripening stage. The appearance of yellow or orange edible pulp is like a boiled pumpkin, possesses a slightly sweet taste and a characteristic floral, terpene-like aroma, very fragrant and pleasantly flavored. The ripe fruit of bael is sweet aromatic, nutritious and very palatable being highly esteemed and eaten by all classes of people (Charoensiddhi and Anprung, 2008). The fruit has excellent aroma which is not destroyed even during processing, thus there is untapped potential for processing bael into various products (Singh et al., 2014).

This fruit is used in Ayurvedic medicine to cure Vatha and Kaphay disturbances in the body. The fully ripened fruit is not much used in medicines. Half ripe fruits are mostly used in medicine (Kumar et al., 2012). The marmelosin ($C_{13}H_{12}O_3$) content which is found in this fruit is known as "panacea of stomach ailments". The following constituents are reported to be present in this fruit: aegelin, alloimperatorin, imperatorin, marmelosin, psoralen, scoparone, scopoletin, tannic acid, Umbelliferone, xanthotoxol and β -sitosterol (Kamalakkannan and Prince, 2005). Bael is reported to contain a number of coumarins, alkaloids, sterols and essential oils. Roots and fruits contain coumarins such as scoparone, scopoletin, umbelliferone, marmesin and skimming (Lambole et al., 2010). Its fresh, aqueous and alcoholic leaf extracts have been reported to have cardiogenic effects in mammals. This fruit also possess hypoglycemic activity, anti-spermatogenic activity, antioxidant activity and anticancer effect. Bael leaf enhances ability to utilize the external glucose load in the body by stimulation of glucose uptake similar to insulin (Dahanukar et al., 2000).

PHYSICO-CHEMICAL CHARACTERISTICS OF FRUIT

Physical characteristics such as average fruit weight, pulp, seed, fibre, rind thickness and shell percentage etc. have been studied by various researchers. Ram and Singh (2003) investigated four bael varieties viz. NB-5, NB-9, NS-1 and Kaghzi. Average fruit weight was varied from 1.011 to 2.09 kg. Cultivar NB-9 recorded highest value followed by cultivar, NS-1. Similar variability in bael

fruit weight was also reported by Jauhari and Singh (1971). It was observed that pulp (%) varies from 57-68.13% and cultivar NB-5 had the highest percentage of pulp (68.13%). Pulp of fruit exhibited various shades of yellow to orange colour. Seed content of bael fruit varied from 2.23 to 3.47%. Cultivar, Kaghzi has highest (3.47%) and cultivar NB-5 has lowest (2.33%) value for seed content. Fibre content varied from 9.91 (Kagazi) to 4.49% (NB-9). The highest percentage of shell was found in cultivar Kagazi (29.50%) while it was lowest in cultivar NB-5 (23.16%). Similar findings have been reported by Roy and Singh (1978).

Physical characteristics of bael cultivars PB7B, PB10, PB11, PB14, Pant Aparna, Pant Shivani, Pant Sujata and Pant Urvashi were studied by Singh et al. (2000). It was observed that the fruits vary in shape such as spheroid-oblong (PB7B and PB11), spheroid (PB10, Pant Shivani, Pant Urvashi), Ellipsoid (Pant Aparna), Oblate (Pant Sujata) and Pyriform (PB14). Maximum fruit length was observed in Pant Urvashi (53.27 cm) and minimum in PB14 (39.87 cm). Diameter was maximum in Pant Sujata (54.53 cm) and minimum diameter was in PB10 (37.37 cm). Fruit weight varied from maximum 2.06 kg in Pant Urvashi to 1.0 kg in PB 14. The mucilage content was maximum in PB7B (17.36%). Minimum pulp content was found in PB 7B (38.19%). Seed content was found maximum in PB11 (3.17%). Similar variation in fibre, mucilage, peel and pulp content was reported by Jauhari et al. (1969).

Prasad and Singh (2001) categorized bael fruit into extra large size (ELS) >1000 g; large Size (LS) >750 to 1000 g; medium Size (MS) >400 to 750 g and small size (SS) <400 g. The results showed that the polar diameter of ELS bael ranges from 13.57 to 17.36 cm. The transverse diameter varies from 13.50 to 17.60 cm. The peel thickness varies from 0.18 to 0.27 cm. The pulp content varies from 78.56 to 78.82%. Maximum sugar content, TSS and non reducing sugars were observed, 4.59%, 34.7^obrix and 14.93%, respectively.

Physicochemical characteristics of three genotypes of bael viz. NB-5, NB-9 and Kaghzi growing under rainfed conditions of Jammu was studied by Bhat and Kumari (2006). They showed variation in fruit weight, pulp, seeds, fiber, shell, TSS, acidity, sugars, ascorbic acid and phenols. NB-9 having larger sized fruits, more pulp, lesser seeds and fiber is ideal for processing. Pulp was extracted by adding water equal to the weight of the pulp and it was utilized for preparing beverages in different proportions. Organoleptic evaluation revealed that bael nectar consisting of 35% pulp, 25°Brix and 0.3% acidity was ideal combination whereas squash having 50% pulp, 1.5% acidity and 50°Brix was more acceptable.

The chemical composition of bael fruit was studied by Ram and Singh (2003). Moisture content of bael fruit varied from 59.0 to 66.67%. Cultivar Kagazi recorded highest moisture content (66.67%) followed by NB-5

(64.20%) and NB-9 (61.77%). The TSS varied from 30.50 (Kaghzi) to 38.50% in NS-1. Carotene content was highest in cultivar NB-9 followed by NS-I and varied from 85.0 to 97.0 IU/100 g. Highest acidity (0.40%) was found in NS-I while lowest was in Kagazi (0.32%). Ascorbic acid content varied from 73.30 to 17.25% and was highest in NB-9. Phenol content varied from 2.38 to 2.87% and was lowest in NS-I. Highest reducing sugar (4.87%) and non-reducing sugar (15.57%) content was found in NB-5 and NB-9, respectively. Total sugar content varied from 16.84 to 19.44% and was highest in cultivar NB-9. Similar observations were also reported by Roy and Singh (1978).

Studies were carried out on bael seed protein concentrate (BSPC) to evaluate the proximate, mineral and amino acid composition, nitrogen extractability and functional properties. The protein content was found to be 70.8 g/100 g BSPC. Calcium and phosphorus were observed in major quantities. The bael seed meal (BSM) lipid is found to be rich in unsaturated fatty acids (75%). Essential amino acids occurred in good quantities in BSPC. Nitrogen extractability of BSPC in water was found to be higher at 1:40 (w/v) ratio and an extraction time of 40 min. Minimum and maximum nitrogen extractability as 14 g/100 g protein and 97 g/100 g protein were observed at pH 4 and 12, respectively. In the presence of sodium chloride (0.1 and 0.5 M), the nitrogen extractability was found to be increased between pH 4-10. Protein precipitability was maximum (90 g/100 g protein) at pH 5.5. SDS-PAGE of BSM and BSPC showed different polypeptides with molecular weights from 205 to 12 kDa (Rao et al., 2011). Volatile compounds in bael fruit pulp were analyzed using the solid-phase micro extraction (SPME)/ gas chromatography (GC)/ mass spectrometry (MS) method (Charoensiddhi and Anprung, 2008). A total of 28 volatile compounds were identified, and the dominant components were monoterpenes and sesquiterpenes. Among these components, limonene was the major constituent producing the characteristic bael fruit flavor.

BIOCHEMICAL CHANGES IN BAEI FRUIT DURING DEVELOPMENT AND RIPENING

The changes in physico-chemical characteristics of bael fruits in their green and ripening stages were studied by Kaushik et al. (2000). The results showed that there is a significant difference ($P < 0.05$) in fruit weight (650 g at green to 764.7g at ripe stage) whereas the length and the breadth did not differ significantly as the fruit turned to ripe from green stage. Specific gravity of the fruit decreased from 1.0 for green stage to 0.94 for ripe stage. Biochemical constituents such as TSS, TSS : Acid ratio, ascorbic acid and carotenoids were significantly higher in ripening stage as compared to green stage. Acidity

Table 2. Chronological progression of physicochemical and biochemical characteristics of bael fruit.

Researchers	Bael varieties	Characteristics
Jauhari and Singh (1971)	NB-5, NB-9, NS-1 and Kaghzi	NB-5 had the highest % of pulp while other varieties like Kaghzi had highest seed content (3.47%). The least seed content was observed in case of NB-5
Pande et al. (1986)	Mirzपुरi, Desi Kanpur local variety	Phenolic constituents and ascorbic acid was present in major amount in both the varieties
Singh et al. (2000)	PB7B, PB10, PB11, PB14, Pant Aparna, Pant Shivani, Pant Sujata and Pant Urvashi	Maximum mucilage content and least pulp content was observed in the case of PB7B
Ram and Singh (2003)	NB-5, NB-9, NS-1 and Kaghzi	NB-9 was reported to have highest value of average fruit weight followed by NS-1
Ram and Singh (2005)	NB-5, NB-9, NS-1 and Kaghzi	Highest carotene and phenolic content was observed in case of NB-9
Bhat and Kumari (2006)	NB-5, NB-9 and Kaghzi	NB-9 contains maximum pulp, lesser seeds. Its fiber is considered ideal for processing

decreased from green stage (0.606%) to ripening stage (0.476%). Significant decrease from 3.8 to 3.50, 6.03 to 4.54 and 3.84 to 2.87% was observed in the case of crude protein, crude fibre and pectin content. Marmelosin content decreased from 0.432 to 0.244%.

The changes in the biochemical composition of the bael fruits at the subsequent stages of fruit development in Mirzपुरi and Desi (Kanpur local) varieties were studied by Pande et al. (1986). The results showed that the dry matter and TSS content of pulp increased gradually with the advancement of fruit. The sugar content increased with the increase in fruit age. The increase in non-reducing sugar content was however poor. The acidity of the both varieties reduced gradually with the maturity of the fruits. Sugar : acid ratio was increased. Crude protein content was decreased rapidly in both cultivars. The ascorbic acid content, tannins of the fruits increased with the maturity. Pectin content increased as the fruit grew and thereafter gradual fall was recorded. Marmelosin content was observed highest in matured fruits. The phenolic constituents were found maximum in immature fruit and a sharp decline was observed with advancement of fruits.

The physical, physiological and biochemical changes in bael fruits during development and ripening were also studied by Roy and Singh (1980). They stated that the diameter (transverse and polar) and weight increased rapidly upto December. The change in weight was observed during ripening after harvest. Physical characters of bael fruit showed a single sigmoid growth curve. The specific gravity was found to be high initially and then it fell gradually. The respiration pattern of the fruit on the tree does not show climatic pattern but on 8th day after harvest climatic is shown. The moisture content of the peel decreases gradually and the peel becomes

dry and woody. The mucilage was found to have increased rapidly up to October and then remained constant. Crude protein fell progressively. Total sugars and non-reducing sugars showed increasing trend during development and there was a steep rise during ripening. Total phenolics fell during development and ripening. The falling trend in acidity was also observed. The chronological progression of physicochemical and biochemical characteristics of bael fruit is presented in Table 2.

PROCESSING OF BAEI FRUIT

Tropical fruits, which are at present under-utilized, have an important role to play in satisfying the demand for nutritious, delicately flavored and attractive natural foods of high therapeutic value. They are in general accepted as being rich in vitamins, minerals and dietary fibre and therefore are an essential ingredient of a healthy diet. Apart from nutritive, therapeutic and medicinal values, quite a few of these tropical fruits have excellent flavour and very attractive colour. Bael fruit is not an easy to eat out of hand item. The bael fruit can be processed for preparation of various products. For all type of products, bael pulp is the first requisite.

As the storage quality of the whole fruit cannot be maintained for long period of time, improvement in the post harvest processing will enhance the effective utilization of the fruit. Because of its hard shell, mucilaginous texture and numerous seeds, it is not popular as a fresh fruit. The fruit has excellent aroma which is not destroyed even during processing. Therefore, there is tremendous potential for processing this fruit into various products. It is usually processed into products like preserves, refreshing beverages, powder,

leather, squash, nectars, toffee, jam, syrup. These products being highly nutritive and therapeutically important can be very easily popularized in internal as well as international markets (Kaushik et al., 2000).

Extraction of bael pulp

Ripened bael fruits are used for extraction of pulp. The important factors in consideration for ideal extraction of pulp are incorporation of water into pulp, inactivation of enzymes by application of heat and pH adjustment. Extraction of bael pulp was obtained successfully by the addition of water to pulp successfully in the proportion of 1:1 and 2:1. A clear juice was obtained by centrifugation of pulp (fruit: water, 1: 2) at 4000 rpm for 10 min. The power law model was used to determine the viscometric constants. Residual enzyme activity was determined for pulp and juice. Pectin methyl esterase activity was minimum (1.163 U) for juice and was maximum (1.375 U) for pulp (fruit: water, 1:1) (Ghosh and Gangopadhyay, 2002). Bael fruit pulp was successfully extracted by addition of water to pulp in proportion of 1:1 and 2:1 and a clear juice was obtained by centrifugation of the pulp (Fruit-water, 1:2) at 4000 rpm for 10 min.

The extraction of pulp from bael fruit is the main hindrance to the processing. Shrestha (2000) reported that the bael fruit pulp extracted by passing through the sieve without addition of water results in very sticky pulp. The pulp so obtained is unfit for handling and nearly 10% loss of pulp results during extraction, partly left with the pomace and partly sticking to the sieve. This may be due to mucilage content of the pulp. Incorporation of water and application of heat results in dilution of mucilage considerably and make the pulp possible to extract commercially.

Moisture desorption isotherm of bael pulp and adsorption isotherm of pulp powder were determined at 20, 30, 40 and 50°C. Static gravimetric method was used by exposing the samples to controlled atmospheres maintained by saturated salt solutions. The isotherms were found to be of type II sigmoid. The isosteric heat of sorption varied between 47.5 - 44.55 kJ g⁻¹ mol⁻¹ at moisture levels 0.5 - 3.5 g/g dry matter for bael pulp and 46.12 - 44.40 kJ g⁻¹ mol⁻¹ at moisture level between 0.25 - 1.125 g/g dry matter for powder (Bag et al., 2009).

The characterization of bael fruit hydrolysate treated with commercial pectinase enzyme was investigated by Charoensiddhi and Anprung (2010). The characteristics of bael fruit hydrolysate showed that bael fruit hydrolyzed at time longer than 2 and 4 h gave greater total carotenoids and antioxidant activities, respectively. Hydrolysis of bael fruit at 6 h resulted in the smallest particle size at 79.92 µm. The hydrolysis of bael fruit resulted in higher soluble dietary fiber and volatile compounds, but it did not affect the prebiotic activity score as compared to non treated sample.

The antioxidant potential of bael fruit pulp extracts was studied by Rajan et al. (2011). Results of phytochemical screening of the aqueous extract revealed the presence of steroid, terpenoid, saponin, tannins, lignin and flavonoids. Alcoholic extract showed the availability of alkaloids and devoid of saponin. *In vitro* antioxidant activity of the plant extract revealed that both extracts showed good antioxidant power.

Sujatha et al. (2011) conducted pharmacognostical and preliminary phytochemical studies on bael fruit base pulp. Fruit pulp revealed the presence of steroids, terpenoids, flavonoids, saponins, phenolic compounds, lignins, fat and oil, proteins, carbohydrates, amino acids and reducing sugars. These results would be of immense value in the botanical identification and standardization of drugs in crude form.

Bag et al. (2011) studied the foam expansion and foam stability of the bael fruit pulp foam. Foams were prepared from various pulp concentrations (PC) by adding different concentration of glycerol monostearate (GMS) and methyl cellulose (MC) at different whipping time (WT). Response surface methodology was used to predict the foam stability and expansion. The optimum conditions achieved after the numerical and graphical optimization for maximum foam expansion and stability was: GMS (3.10 g/100 g pulp), MC (0.32 g/100 g pulp), PC (13.2°Bx), and WT (2 min).

Singh et al. (2012) studied the effect of incubation temperature (28.18-61.82°C), incubation time (97.5-652.5 min) and pectinase concentration (0.64-7.36 mg/25 g bael pulp) on juice yield, viscosity and clarity of juice. The recommended enzymatic treatment conditions were incubation time (425 min), incubation temperature (47°C), pectinase concentration (5.0 mg/25 g bael pulp) and the juice yield, viscosity and clarity under these conditions were 84.5%, 1.35 cps and 22.43%, respectively.

Singh et al. (2014) studied the effect of pre-treatment on various physical and thermal properties of bael pulp. The fruit pulp of bael fruit was extracted and TSS of the extracted pulp was raised to 25°Brix by adding 65°Brix sugar syrup. The pH of pulp was set at 3.0 and 3.5, which was heated at 80- 85°C for 15, 20 and 25 min and kept at refrigerated conditions for 80 days. The TSS, pH, titratable acidity, colour-L*, a*, b*, thermal conductivity and specific heat ranged between 18-25°Brix, 2.6-3.5, 0.15-0.35%, 20.32-56.87, 2.95-20.28, 23.58-64.01, 0.37-0.76 w/m°C, 1.73-2.50 J/g°C respectively. Minimum colour change and maximum sensory score was observed at pH 3 and 15 min heating under refrigerated storage. The zero or first order models were well fitted for the responses of the bael pulp (3 pH, 15 min) stored under refrigerated conditions.

Bael juice

Due to refreshing effect and therapeutic value of bael

fruit, its juice can be commercialized. Singh et al. (2013) has optimized the extraction of juice from bael fruit by using commercial and crude pectinase enzymes. As juice extraction is difficult due to the presence of pectins that hold the water, use of enzymes effectively increase the yield and clarity of juice. Singh et al. (2013) studied the effect of incubation time, incubation temperature, and crude enzyme concentration on the yield, viscosity and clarity of the juice obtained from bael fruit pulp. The recommended enzymatic treatment conditions from the study were incubation time 475 min, incubation temperature 45°C, and crude enzyme concentration 0.20 mL/25 g bael fruit pulp. The recovery, viscosity and clarity of the juice under these conditions were 82.9%, 1.41 cps and 21.32%T, respectively. The variables, clarity, and yield were found as principal components for comparing different samples of the juice treated with enzyme.

Bael fruit beverages

Due to increasing demand of health drinks based on indigenous fruits, this fruit can be processed for making beverages. Various researchers have successfully prepared different beverages from bael fruit. A whey protein enriched bael fruit beverage was prepared by Singh and Nath (2004) by adding whey protein concentrate, pectin and carboxy methyl cellulose. The beverage having 16°Brix, 25% bael fruit pulp, pH 3.9 and 1.75% protein was found the best concerning the overall acceptability. Blended beverages from bael and guava were prepared by Nidhi et al. (2008) by extracting the pulps of both fruits. Bael pulp was extracted by adding equal amount of water to the crude mass and heating it to 80°C for 1 min and passing through pulper. Guava pulp was obtained by adding fruit slices with 25% water, heating at 80°C for 5 min and passing through pulper to obtain homogenous mass without seed and fibres. These pulps were blended in 100:0, 75:25, 50:50, 25:75 and 0:100 proportions. The RTS beverage with pulp 15 and 20%, TSS 15% and acidity 0.26 were prepared by using these blends.

RTS drink, nectar and squash from bael fruit were prepared by Verma and Gehlot (2006). For preparing these products, bael pulp was extracted by adding 1 L of water per kg of crude mass and heating it at 70°C for 1 min followed by cooling and passing through muslin cloth. The TSS and acidity were analyzed in extracted pulp and requisite amount of sugar and citric acid, dissolved in water, were added to pulp as per requirement to meet the specifications. The prepared RTS and nectar were thoroughly homogenized and sterilized. The bael fruit squash was also homogenized and sodium benzoate @ 1 g/ L of squash was added as a chemical preservative after dissolving in small amount of water. The squash was also sterilized after filling in bottles.

Therapeutic beverages from bael fruit were prepared by Verma and Gehlot (2007). Unripe bael fruit is useful in curing dysentery and diarrhoea whereas ripe fruit acts as laxative and good tonic for heart, brain and to cure dyspepsia. Keeping all this in mind, researchers prepared RTS drink, nectar, squash and syrup from the bael fruit. For RTS, they used 1 L of pulp, 1.2 kg sugar, 28 g citric acid and 7.7 L of water. Recommended colour and essence were added after dissolving them in small quantity of prepared RTS. The RTS was pasteurized at 85-88°C for 30 min. For nectar, 1 L bael pulp, 650 g sugar, 15 g citric acid and 3.3 L water were used. For squash, 1 L pulp, 1.5 kg sugar, 20 g citric acid, 1 L water and sodium benzoate @ 1 g/L of finished product were used. The pulp content was maintained at minimum of 25% and TSS at 40%. The fruit syrup should contain 25% minimum fruit portion and 65% TSS. For syrup preparation, 1 L bael pulp, 2 kg sugar, 20 g citric acid, 500 ml water was added. For syrup, generally no preservative is required but for long storage; sodium benzoate can be used.

The wild bael fruit was utilized by Kenghe et al. (2009) for value addition. They prepared squash from this fruit by adjusting the TSS of the pulp and by adding the preservatives. The pulp was obtained by adding water to the crude mass in the equal amount, heating at 80°C for 1 min and passing through muslin cloth. The squash was filled in bottles, crowned and pasteurized at 80°C for 30 min followed by cooling and wax sealing to ensure tightness.

The blended bael RTS was successfully prepared by Kenghe and Zambare (2009). For this fresh, matured, raw bael fruits were taken, cleaned and cut into halves by using knife. The seeds and pulp were removed from the rind, which is boiled with a little quantity of water for a period of time. Then such pulp was filtered. The sugar syrup along with measured quantity of citric acid was added with the pulp to prepare RTS beverage of specific TSS and pulp percentage as per standard and sensory acceptability. The bael RTS is then blended with tamarind RTS with 1:1, 1:5 and 1:9 proportions to ensure the feasibility of acceptance of blended RTS. Jalgera @ 15 ml/ L of RTS, KMS @ 0.9 mg/L of RTS were added in different lots. One lot was kept as control sample. The results suggested that 1:9 ratio is the best for blending and jalgera can be used as a source of natural preservative.

RTS beverage from bael and citrus fruit blends was prepared by Nagpal and Rajyalakshmi (2009). Bael pulp was extracted for this purpose by adding equal amount of water to the crude mass, blended and passed through 20 mesh stainless steel sieves to obtain thick, uniform pulp free from seeds and fibres. Lime and sweet oranges were cut into halves and the juice was extracted using a wooden lime squeezer and juice extractor, respectively. Mandarin oranges were peeled and the segments fed

into a screw type juice extractor to obtain juice. Pineapple was peeled, cut into small pieces, blended and filtered. Bael pulp was blended with the juice of lime, mandarin oranges, sweet orange and pineapple in different proportions. The RTS beverages were prepared by adding sugar, citric acid and water so as to obtain the desired level of TSS, acidity and dilution. The prepared beverages were filled in sterilized glass bottles and pasteurized, cooled and stored at ambient temperature.

Bael wine

Wine is one of the functional fermented foods and has many health benefits. Bael fruit having sufficient sugar content can be used for wine making. Singh et al. (2006) prepared wine from this fruit by adjusting the TSS of pulp to 24°Brix with the help of sucrose and added potassium metabisulphite (KMS) to maintain SO₂ @ 100 ppm along with cyclodextrins @ 0.4% for preparation of must. This was added with the 10% yeast starter culture. The starter culture was prepared by adding 0.05% yeast extract and 0.1% ammonium phosphate at pH 5 to the boiled and cooled bael pulp. The fermentation was carried out at 25°C. The maximum alcohol percentage in the wine was upto 10.08% after fermentation for 88 h at pH 5.

Bael preserve

Green bael fruits are used for preparing preserves (murabba) which is an important Ayurvedic medicinal product and generally prescribed for all types of digestive troubles. This fruit preserve was prepared by Kaushik et al. (2002). The peeling was done by knife by making a slit at the stellar end of the fruit and then inserting the pointed end of the knife under the rind to crack it and remove the rind in pieces. The peeled fruit was sliced crosswise into the pieces of about 2 cm thickness and then washed with water. The slices were pricked at both side with stainless steel forks and blanched in boiling water for 5 min or until they become soft. Sugar syrup of 40% concentration was prepared and citric acid @ 0.6% was added to the syrup. The treated fruits were placed in stainless steel container and sugar syrup was added. The next day, syrup was decanted and its strength was increased by adding 300 g of additional sugar to that by boiling. This was repeated on 4th and 6th day and the syrup was heated to bring the concentration of sugar to 70% and the preserve was stored in glass jars at ambient temperature.

A recipe for preparation of preserve (murabba) from bael fruit was also given by Rakesh et al. (2005). They used 1 kg mature bael fruit slices of 2 cm thickness, 1.25 kg sugar, citric acid 2-3 g and water 1 L. The slices were pricked with stainless steel fork from both sides and

dipped in 2% lime solution for 2-3 h. After removing the slices from the lime water, they were washed thoroughly in running water and then put in boiling water to make them soft. Softened slices dipped in 40% sugar syrup were prepared by using 700 g sugar. The next day, the sugar syrup was drained and half of the remaining sugar was added to the syrup, concentrated and again added to the slices. After 2 days, same procedure was repeated and the TSS of syrup was maintained at 70%.

Bael candy

The candy from bael fruit was prepared by Rakesh et al. (2005). For candy preparation, the recipe was kept same as for bael preserve. The slices in sugar syrup of 70% were drained out and dried at 55-60°C for 8-10 h in oven. The fruits of bael cultivar (NB-9) was used for the preparation of candy and stored in glass jars and polythene pouches at ambient temperature. The changes during storage in quality were judged at monthly interval. It was observed that %TSS, acidity and browning of candy were increased while ascorbic acid was decreased during storage in both types of containers. The study indicated that a good quality of candy can be prepared by bael cultivar NB-9 and in polythene pouches it can be stored for 4 months without any spoilage of organoleptic quality (Mishra et al., 2013).

Toffee is a confection made by caramelizing sugar or molasses along with butter and generally flour. The mixture is heated until its temperature reaches the hard crack stage of 300 to 310°F (100 to 154°C). A recipe for preparation of bael toffee was formulated by Rakesh et al. (2005). It was prepared by using fruit pulp, sugar, glucose, skim milk powder (SMP) and butter. Water, 750 ml was then added for each 1 kg of fruit pulp followed by mixing and heating to up to 80°C. It was screened and the fine pulp was obtained. 1 kg of pulp was cooked until one third of its original volume remained. 500 g sugar, 100 g glucose and 100 g butter were added to the cooking mass. Now SMP was added to the cooking mass after adding small amount of water. Cooking was continued until cooking mass starts leaving the sides of pan. Cooking was stopped and the cooked mass was spread uniformly in 0.5-0.75 mm thick layer. It was allowed it to cool, and cut into pieces and wrapped in moisture proof or butter paper.

Bael slab

Slab, leather or paper can also be prepared from bael fruit. This product was formulated by Rakesh et al. (2005). For this, they added 200-300 ml water to each kg of fruit crude mass. Agitation was carried out, followed by heating it upto 30°C. Sieve analysis was then carried out

using stainless steel sieve. Sugar, citric acid and KMS was added so that the treated pulp contains 35% TSS, 0.5% titratable acidity and 0.07% KMS. Then treated pulp was boiled and spread on aluminium trays smeared with butter. It was then dried at 55-60°C for 15-16 h to achieve a moisture content of 14.5%. Cut slabs were then wrapped in butter paper and packed in polyethylene bags.

Bael fruit jam

The bael fruit can be processed to prepare jam. A mix fruit jam from bael and mango was prepared by Mishra and Chopra (2006). The pulp of this fruit was extracted by adding the water to equal amount of crude mass, followed by heating at 80°C for 1 min and passing through 20 mesh stainless steel sieves. Mango pulp was added to bael fruit pulp. Various combinations of bael and mango pulp were prepared. The final optimized jam had 45% mixed pulp (Bael: mango: 1:1), 70% TSS and 0.5% acidity based on its organoleptic characteristics

A recipe for bael fruit jam preparation has been reported by Rakesh et al. (2005). This consisted of addition of 750 g sugar and 3-4 g citric acid to the 1 kg strained pulp without any seed and fibres. It was then cooked with continuous stirring using a spoon until end point is reached. End point can be judged by doing sheet test of boiling mass. The cooking was then stopped after end point. Hot jam was then filled into clean and well sterilized jam bottles. Jam filled bottles was inverted for 10 min for sterilization of their caps.

Dehydrated bael

According to Rakesh et al. (2005), mature, green bael fruit can be dehydrated by slicing in 1-1.5 cm thick slices and fumigating these slices with SO₂ for ½ h in sulphur box and then dehydrating in an oven at 55-60°C upto constant weight.

Bael powder

Bael powder can be prepared by grinding the dried fruit slices in a grinder. The ground bael powder is packed in polyethylene bags and stored in dry places after proper sealing (Rakesh et al., 2005). A method to prepare bael fruit powder was developed by Rastogi et al. (2005) that consists of spray drying a fruit pulp of bael to obtain a free-flowing, yellow-red powder with good shelf life.

The comparative study of minerals, fat and protein content in green bael and ripe bael powder was carried out by Islam et al. (2011). The analysis showed that the concentration of Na, K, Ca, Mg, Zn and Cu (119, 4821,

92.9, 259, 1.69 and 1.34 ppm) in ripe bael powder is more than that of green bael powder (55.6, 1356, 78.9, 142, 0.66 and 0.67 ppm). Fe and carbohydrate is more in green bael powder (19.3 ppm, 93.73%) than in ripe bael powder (16.22 ppm, 90.33%). Vitamin C is almost same in both types of powders.

The moisture desorption isotherm of bael fruit pulp and adsorption isotherm of pulp powder at 20, 30, 40 and 50°C were determined by Bag et al. (2009). Static gravimetric method was used by exposing the samples to controlled atmosphere maintained by saturated salt solution. The isotherms were found to be of type-II sigmoid. Experimental data were fitted to four isotherm models viz. Anderson, Guggenheim-Anderson-DeBoer (GAB), Oswin and Peleg Models. It was found that Peleg model fits best describing the equilibrium moisture content and equilibrium relative humidity (EMC-ERH) for both the bael pulp and pulp powder. The isosteric heat of sorption varied between 47.5-44.55 kJ/g/mol at moisture levels 0.5-3.5 g/g dry matter for bael fruit pulp and 46.12-44.40 kJ/mol/g at moisture level between 0.25-1.125 g/g dry matter for powder. The chronological progression of different processing aspects of Bael fruit is presented in Table 3.

Bael panjiri

This product is highly nutritive, restorative and is prescribed for stomach ailments. Rakesh et al. (2005) used bael powder 1 kg, desi ghee (butter oil) 1 kg, sugar powder 1.5 kg, wheat flour and dry fruits as per taste for preparation of bael panjiri. Bael powder is roasted in desi ghee. Roast wheat flour was added in small amount as per taste in desi ghee. All the ingredients were then mixed.

Utilization of processing waste of bael

The bael peel and pomace that constitute the 35-40% of the total fruit weight were utilized by Saini et al. (2002) for cattle feed purpose. One hectare bael plantation leaves behind nearly 22-27 quintals of dried waste. They dried the peel and pomace to a constant weight in a tray drier at 60°C. It was ground to powder in a grinder. Ground samples were analyzed for various biochemical constituents. Peel contains 27.34% crude fibres, 31.85% crude protein, 1.0% Ca and 0.14% phosphorus whereas pomace contains 9.98, 10.5, 1.2 and 0.1% of crude fibre, crude protein, calcium and phosphorus, respectively. Palatability of these wastes was adjusted by feeding to the cows after mixing with cattle feed in a ratio 1:3. After mixing with cattle feed, wastes were readily acceptable to the cows, which confirmed that it can be used as animal feed. *In vitro* digestibility of various constituents was also

Table 3. Chronological progression of different processing aspects of bael fruit.

Researcher	Salient features	Processed products
Shrestha (2000)	Incorporation of water and application of heat results in dilution of mucilage	Bael pulp extraction
Ghosh and Gangopadhyay (2002)	Pectin methyl esterase activity was minimum for juice and maximum for pulp	Bael pulp extraction
Kaushik et al. (2002)	Syrup was brought up to the desired concentration of 70%	Bael preserve
Singh and Nath (2004)	Beer having 16°Brix, 25% Fruit Pulp, pH 3.9, 1.75% protein is acceptable	Bael fruit beer
Rakesh et al. (2005)	All products were acceptable	Bael preserve, candy, toffee, slab, jam and panjiri
Verma and Gehlot (2006)	Product was acceptable which was further homogenized and sterilized	Bael RTS drink, nectar and squash
Singh et al. (2006)	Maximum alcohol % was found upto 10.08% after fermentation for 88 h at pH 5	Bael wine
Mishra and Chopra (2006)	Optimized jam having 45% mixed pulp containing bael and mango was acceptable	Bael fruit jam
Verma and Gehlot (2007)	Bael nectar prepared with 20% bael pulp, 15% total soluble solids and 0.25% acidity was found most acceptable among all the treatments.	Therapeutic bael nectar
Kenghe and Zambave (2009)	Jalgerra can be used as a source of natural preservatives	Blended bael RTS
Nagpal and Rajyalakshmi (2009)	The RTS beverages were prepared by adding sugar, citric acid and water so as to obtain the desired level of TSS, acidity and dilution	Bael RTS Beverage
Charoensiddhi and Anprung (2010)	The bael fruit hydrolyzed at time longer than 2 and 4 h gave greater total carotenoids and antioxidant activities, respectively. Hydrolysis of bael fruit at 6 h resulted in the smallest particle size, 79.92 µm.	Bael fruit hydrolysate
Rajan et al. (2011)	Phytochemical screening of the alcoholic extract revealed the presence of steroids, saponins and tannins etc. In vitro antioxidant activity of the plant extracts revealed good antioxidant power.	Bael fruit pulp
Bag et al. (2011)	The optimum conditions for maximum foam expansion and stability was: GMS (3.10 g/100 g pulp), MC (0.32 g/100 g pulp), PC (13.2°Bx), and WT (2 min).	Bael fruit pulp foam
Singh et al. (2012)	The recommended enzymatic treatment conditions were incubation time (425 min), incubation temperature (47°C), pectinase concentration (5.0 mg/25 g bael pulp) and the juice yield, viscosity and clarity under these conditions were 84.5%, 1.35 cps and 22.43% respectively.	Bael juice
Singh et al. (2013)	Optimized juice extraction was carried out using crude enzymes. The recommended enzymatic treatment conditions from the study were incubation time 475 min, incubation temperature 45°C, and crude enzyme concentration 0.20 mL/25 g bael fruit pulp.	Bael juice
Singh et al. (2014)	The pulp can be stored for 60 days under the refrigerated conditions. Minimum colour change and maximum sensory score was observed of the sample at 3 pH and 15 min heating.	Bael pulp

analyzed and it was observed that 40.1% of total peel and 69.6% of the pomace was digestible. Use of these wastes as animal feed not only reduce the feeding and processing costs but also prevent health hazards and environmental pollution.

MEDICINAL USES OF BAEI

The bael fruit is very well known for its medicinal importance. Morton (1987) explained the medicinal composition and uses of bael fruit. The fresh ripe pulp of the higher quality cultivars, and the "sherbet" made from it are taken for their mild laxative, tonic and digestive effects. A decoction of the unripe fruit, with fennel and ginger, is prescribed in cases of hemorrhoids. It is employed in the treatment of leucoderma.

Marmelosin derived from the pulp is given a laxative and diuretic effect. Stem bark and root of aqueous extracts have similar phytochemical compounds (Nirupama et al., 2012). In large doses, it lowers the rate of respiration, depresses heart action and causes sleepiness. A number of chemical constituents from different parts of plant have been extracted like aegelin marmelosin, coumarin, β -sitosterol and alkaloids. The plant is a rich source of amino acid, galactose and fatty acids. The compound isolated from plant show a variety of pharmacological activity.

For medicinal use, the young fruits, while still tender, are commonly sliced horizontally and sundried and sold in local markets and are exported. Because of the astringency, especially of the wild fruits, the unripe bael is most prized as a means of halting diarrhea and dysentery, which are prevalent in India during the summer season. Bitter, light-yellow oil extracted from the seeds given in 1.5 g doses render a purgative effect. Bael herb contains 15.6% palmitic acid, 8.3% stearic acid, 28.7% linoleic and 7.6% linolenic acid. The seed residue contains 70% protein. Leaves, fruits, stems and roots of bael fruit have been used in ethno medicine to exploit its medicinal properties including astringent, anti-diarrheal, anti-inflammatory activities. Compounds purified from bael have been proven to be biologically active against several major diseases including cancer, diabetes, etc (Maity et al., 2009). In Ayurveda, this fruit is used to cure problems related to heart, stomach, intestine, chronic constipation and dysentery; some forms of indigestion, typhoid, debility, fever, hemorrhoids, hypochondria, melancholia and for heart palpitation (Kumar et al., 2013).

The essential oil isolated from the leaves of bael tree has proved to antifungal activity against animal and human fungi like *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum gypseum*, *Microsporum audouinii*, *Microsporum cookie*, *Epidermophyton floccosum*, *Aspergillus niger*, *Aspergillus flavus* and *Histoplasma capsulatum* (Dhankhar et al.,

2011). Satyal et al. (2012) collected the bael leaves to obtain, analyze, and examine the bioactivity from its essential oil. The essential oil from leaves was obtained by hydro-distillation. The chemical composition, determined by GC-MS, revealed 82 compounds, with 81 components being identified. The major component was limonene (64.1%), with the other two abundant components being (*E*)- β -ocimene (9.7%) and germacrene B (4.7%). The essential oil of *A. marmelos* showed remarkable brine shrimp lethality. Antimicrobial and antifungal activity of the leaf oil was negligible. Most of the observed biological activity was apparently due to the relatively high level of limonene (64.1%) in the essential oil of the leaf of *A. marmelos*.

The bitter, pungent leaf juice, mixed with honey, is given to allay catarrh and fever. With black pepper added, it is taken to relieve jaundice and constipation accompanied by edema. The leaf decoction is said to alleviate asthma. A hot poultice of the leaves is considered an effective treatment for ophthalmia and various inflammations, also febrile delirium and acute bronchitis.

A decoction of the flowers is used as eye lotion and given as an antiemetic. The bark contains tannin and the coumarin, aegelinol; also furocoumarin, marmesin; umbelliferone, a hydroxy coumarin; and the alkaloids, fagarine and skimmianine. The bark decoction is administered in cases of malaria. Decoctions of the root are taken to relieve palpitations of the heart, indigestion, and bowel inflammations; also to stop vomiting. The fruit, roots and leaves have antibiotic activity, the root, leaves and bark are used in treating snakebite. Chemical studies have revealed the following properties in the roots: psoralen, xanthotoxin, O-methylscopoletin, scopoletin, tembamide, and skimmianin; also decursinol, haplopin and aegelinol. Scientific studies have validated many of the ethnomedicinal uses of bael fruit and reports indicated that the fruit possesses broad range of therapeutic effects that includes free radical scavenging, antioxidant, inhibition of lipid peroxidation, antibacterial, antiviral, anti-diarrheal, gastro protective, anti-ulcerative colitis, hepatoprotective, anti-diabetic, cardio protective and radio protective effects (Baliga et al., 2011).

Diabetes mellitus is a heterogeneous metabolic disease characterized by altered carbohydrate, lipid and protein metabolism. So many traditional herbs are being used by diabetic patients to control the disease. But very few studies are performed to investigate the efficacy of these herbs clinically. An attempt has been made to investigate clinically the antidiabetic activity of bael leaves individually and collectively with the standard oral hypoglycemic therapy in non insulin dependent diabetes mellitus (NIDDM) patients (Ismail, 2009). Antidiabetic effect was more markedly observed when it was combined with the oral hypoglycemic therapy. Bael leaves can be combined in high dose with oral hypoglycemic agents to bring the blood glucose to normal

levels in patients whose diabetes is not controlled with these agents or in those patients in whom these drugs produce adverse effects on dose increments. The unripe or half-ripe fruit is good for digestion. It is useful in preventing or curing scurvy. It also strengthens the stomach and promotes its action. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems (Kumar et al., 2012). The antifungal potential of bael fruit was studied by Parihar and Kumar (2013). The results were referenced against glucanazole antifungal agent. Methanol fruit extract showed strong antifungal activity against most of the strains whereas moderate antifungal potential was shown by leaf extract in aqueous solution.

CONCLUSION

Bael (*A. marmelos*) fruit has a lot of potential to be processed for value addition. Its flavor is acceptable by almost all classes of the people as a refreshing drink in the summer. The bael is still being used only by unorganized sector and is not being given much emphasis for its commercial utilization in terms of value added products. The selected pharmacological studies have been conducted on different parts of this fruit and the literature supports the potential of bael fruit to be processed and formulated to prepare number of products. The focused research is required in the field to investigate the unexplored and unexploited potential of this fruit on the commercial scale. The health and functional foods from the bael is an area which still requires the attention of scientific fraternity.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Retention of nutrients of pearl millet using conventional and solar cooking

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Comparative estimation of nutrients of pearl millet was studied by adopting two methods of cooking, that is, conventional cooking on liquefied petroleum gas (LPG) stoves and solar cooking. Starch, total soluble carbohydrate, protein, calcium and iron were determined for raw, conventional and solar cooked pearl millet. It was found that retention of starch was 98.58% for solar cooked and 74.51% for conventional cooked pearl millet. Retention of total soluble carbohydrate was 31.22% for solar cooked and 28.96% for conventional cooked pearl millet. Retention of protein was 98.00% for solar cooked and 97.24% for conventional cooked pearl millet. Retention of calcium was 83.3% for solar cooked and 77.8% for conventional cooked pearl millet. Retention of iron was 98.27% for solar cooked and 95.66% for conventional cooked pearl millet. The results in the study revealed that solar cooking is better than conventional cooking because more nutrients are retained in solar cooking than conventional cooking.

Key words: Solar cooking, retention of nutrient, pearl millet.

INTRODUCTION

The genus *Pennisetum* is distributed throughout the tropics and subtropics of the world. It includes about 140 species. One African species, *Pennisetum purpureum* Schumacher became widely distributed as a tropical forage grass (Bruken, 1977). *Pennisetum glaucum* is widely distributed in south of Sahara, in the semi-arid Sahel and bush from Senegal to Eritrea in Ethiopia. It was domesticated along the southern margins of the Saharan central highlands at the onset of the present dry phase some 4000-5000 years ago (Clark, 1962; Davies, 1968; Munson, 1975). Pearl millet is the most drought tolerant of all domesticated cereals, and soon after its domestication it became widely distributed across the

semi-arid tropics of Africa and Asia. It is the principal food crop across sub-Saharan Africa and North-Western India. It is a premier crop of Rajasthan. Of the Kharif crop grown in the state, this crop alone accounts for 47% area and contributes 37% to the total Kharif production in the state. Of the Kharif cereals nearly 71 and 56% production is shared by pearl millet. It is important for Rajasthan agriculture chiefly because it is highly adapted to harsh environmental conditions in the vast dry land areas; it succeeds as a fodder crop for sustaining livestock health and productivity which in turn, sustains the livelihood of resource-poor farmers in most parts of the state. It is, therefore, of permanent importance for food and fodder

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security in the state. For this reason alone, the crop has stayed in Rajasthan agriculture for hundreds of years. And it will continue to remain important for the state for hundreds of years to come as well. Pearl millet as a grain crop occupies an important position in the national context. It is the fourth important cereal crop in the country, next to rice, wheat and sorghum. Major states growing pearl millet are Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana. The two states, Rajasthan and Maharashtra, together occupy 65% of the total area under pearl millet cultivation in the country. Rajasthan alone occupies 49% of the area and contributes nearly 30% of the total pearl millet production in the country. Total area under pearl millet cultivation is 9.1 million hectare and production is 7.3 million tons and the productivity is 780 kg/ha. Pearl millet flour is eaten in the form of chapati and broken pearl millet is eaten in the form of *daliya*.

George and Ogale (1987) found that protein retention in selected cereals, pulses and vegetables that were solar cooked were higher than those cooked by absorption method in saucepan. Solar cooked green gram dhal, red gram dhal, brinjal, kawai and cluster beans contained thiamine in a higher percentage than the same foods cooked in saucepan. Protein retention in selected pulses, vegetables, cereals that were solar cooked was higher than those cooked by pressure cooker (Devdas and Venmathi, 1992). Solar cooker was superior due to better retention of carotene and vitamin C as compared to microwave oven (Eswaran and Kalpana, 1998). Study was conducted by Chandrasekhar and Kowsalya (1998) on nutrient retention cooked taking two methods (conventional cooking and solar cooking) as compared to the raw amaranths, the percent loss of protein and riboflavin in both methods of cooking was found to be similar, while there was no loss of phosphorus in the solar cooking, percentage loss of calcium and ascorbic acid in the solar cooked sample was more as compared to that of the cooked sample by absorption method. Srivastava and Aakanksha (2009) measured retention of nutrient in moth bean in conventional and solar cooking and found that retention of starch was 81.26% for solar cooked and 76.30% for conventional cooked moth bean. Retention of total soluble carbohydrate was 45.41% for solar cooked and 40.34% for conventional cooked moth bean. Retention of protein was 99.48% for solar cooked and 97.23% for conventional cooked moth bean. Retention of calcium was 84.7% for solar cooked and 80.0% for conventional cooked moth bean. Retention of iron was 96.9% for solar cooked and 94.08% for conventional cooked moth bean. The results of the study revealed that solar cooking is better than conventional cooking because more nutrients are retained in solar cooking than conventional cooking. In this paper, retention of nutrient viz., starch, total soluble carbohydrates, protein, calcium and iron in pearl millet has been studied using conventional and solar cooking.

MATERIALS AND METHODS

Conventional cooking

100 g fresh pearl millet sample (three replications) were cooked with distilled water in stainless steel saucepan on LPG stove. Fresh samples as well as cooked samples were dried at 48°C in an infrared oven. Dried samples were grounded to powdered form and sealed in a polythene bag.

Solar cooking

100 g fresh pearl millet samples (three replications) were cooked with distilled water in stainless steel cooking utensil. Hot box solar cooker with double reflector was used for cooking so that tracking towards the sun was avoided for three hours. The device consisted of a double walled hot box. The outer and inner boxes were made of aluminium. The space between them was filled with glass wool insulation and separated by a wooden frame. The inner box was painted with black board paint. Two clear window glass panes of 4 mm thickness were fixed over it with a wooden frame, which can be opened. Two 4 mm thick plane mirror reflectors were fixed over it. Fresh samples as well as cooked samples were dried at 48°C in an infrared oven. Dried samples were grounded to powdered form and sealed in a polythene bag.

Biochemical analysis

Determination of total soluble carbohydrates and starch by anthrone method

The concentration of pentoses, hexoses, disaccharides including sucrose, lactose, maltose and hexuronic acids present either freely or along with polysaccharides can be estimated using this method. Anthrone, 10-keto-9, 10-di hydro anthracene, a reduction product of anthroquinone, reacts by condensing with carbohydrate furfural derivative to produce a green colour in a dilute solution and a blue colour in a concentrated solution.

50 mg of oven dried samples (three replications) were extracted in 80% ethanol. The homogenate was centrifuged at 5000 rpm for 10 min and the residue was re-extracted and the supernatants were pooled. Final volume was made up to 25 ml. The supernatant was used for estimation of total soluble carbohydrates, while residue was used for the estimation of starch (Yemn and Wills, 1954).

1.0 ml of aliquot of supernatant was evaporated to dryness in the test tube at 60°C in a water bath. After allowing it to cool the residue was dissolved in 1.0 ml distilled water. To this, 4 ml of Anthrone reagent (0.2 g/100 ml concentrated sulphuric acid) was added and heated in boiling water bath for 10 min. The tubes were removed and allowed to cool before recording the optical density (OD) at 620 nm by spectrophotometer (Figure 3) against a reagent blank. The amount of soluble carbohydrate present in the extract was calculated by using a standard curve prepared with graded levels of glucose (10-100 mg/l).

Procedure for starch

Residue left after 80% ethanol extraction was used for starch analysis by anthrone method after its acid hydrolysis following the method (Clegg, 1955). Four milliliters of 26% perchloric acid was added to the tubes containing pellets left after ethanol extraction and left overnight at 4°C. These were then centrifuged at 5000 rpm for 15 min. Supernatant was collected in the test tube while the residue was washed with 26% perchloric acid and centrifuged again. Supernatants were pooled and the volume was made to 10 ml with 26% perchloric acid. 0.2 ml of the supernatant was diluted

to 1.0 ml with distilled water and then 4.0 ml of anthrone reagent (0.2 g/100 ml concentrated sulphuric acid) was added. This reaction mixture was heated in a boiling water bath for 10 min, cooled to room temperature and the optical density (OD) was recorded at 620 nm. Concentration of starch was calculated using standard curve prepared by using graded quantity of glucose and then multiplying the values by 0.9. Results were expressed as mg g⁻¹ dry weight of tissue.

Determination of protein by Kjeldahl method

The nitrogenous compounds were converted into ammonium sulphate by boiling with concentrated sulphuric acid. It is subsequently decomposed by addition of excess of alkali and liberated ammonia absorbed into a boric acid solution containing bromocresol green and alcoholic methyl red mixed indicator by steam distillation. Ammonia forms a loose compound, ammonium borate with boric acid which is titrated directly against standard sulphuric acid. Protein (N x 6.25) content of food samples can be determined by Kjeldahl method using Tecator's Kjeltec System-II. For the analysis, 0.5 g oven dried food samples were taken in digestion tubes and digested with 10 ml concentrated sulphuric acid and a digestion tablet (containing 3.5 g potassium sulphate and 3.5 mg selenium) in Tecator's 1015- Digestion system (block digester) for two hours [First at moderate (250°C), followed by a high temperature (350°C)]. The digested samples are cooled at room temperature and 75 ml of distilled water is then added in each tube. The content of each digested samples were allowed to cool and sample tubes were individually transferred to the Tecator's Kjeltec system-II, 1003- distillation unit, where 50 ml of 40% sodium hydroxide (NaOH) was automatically added into the tube. The contents of the tube were steam distilled and about 50 ml distillate containing evolved ammonia, was collected in 25 ml of 2% boric acid, which was later titrated against 0.1 N sulphuric acid.

The protein content in the sample was calculated as follows:

1ml of 0.1 N H₂SO₄ = 1.4 mg of nitrogen or 0.0014 g nitrogen

Protein of such type of vegetable material, on an average contain 16% of nitrogen (100/16 = 6.25)
Protein content = 6.25 x nitrogen

$$\text{Protein, g/100 g sample} = \frac{A \times 0.0014 \times 6.25 \times 100}{B}$$

Where, A = volume (ml) of 0.1 N sulphuric acid consumed; B = weight of sample taken for analysis (0.5 g)

Determination of minerals

Preparation of ash for calcium and iron

Wet ash method was used for the preparation of ash solution because this method is most suitable for estimation of minerals, as it is less time consuming and gives more accurate results.

1.0 g of oven dried powdered materials were (three replications) taken into a dry 100 ml micro Kjeldahl flask. 5.0 ml of concentrated nitric acid (HNO₃) was added and kept on a digestion rack. The food samples were heated and then these were dissolved, 5.0 ml of perchloric acid was added and heated till the particles were completely digested and cleared. The flask was removed after digestion was completed from the heating source and the volume was made to 30 ml with double distilled water. The solution

prepared was kept in dry glass bottles and kept in a dust free chamber. This solution was used for estimation of minerals like calcium and iron.

Determination of calcium by titrimetric method

Calcium is precipitated as oxalate and is titrated with ethylene diamine tetra acetate (EDTA) (Cheng and Bray, 1951). The calcium content was calculated by using the following formula:

Meq./litre of Ca⁺⁺ = ml versanate solution (EDTA) required x normality of versanate solution (define Meq) (Meq is a unit of concentration of an ion in the solution that is defined as a measure of concentration of a solute in a solution obtained by dividing the concentration in mg per litre by equivalent weight of the ion.)

Determination of iron by atomic absorption spectrophotometer

Computer attached atomic absorption spectrophotometer model GBC 932AA was used. Standard blank solutions were aspirated into the flame directly. Optimum operating conditions recommended by the instrument manufacturer was used. Standard solutions were read before and after the sample readings. Burner was flushed with deionised water between samples and checked for 0 setting. Calibration curve was prepared from readings of standards. The concentration of samples were determined from the standard graph.

Calculation

$$\text{ppm minerals} = \frac{(\mu \text{ g mineral /ml}) \times \text{dilution factor}}{\text{ml aliquots} \times \text{g sample}}$$

RESULTS AND DISCUSSION

Time taken for cooking

Average time (three replication) taken for cooking 100 gm broken pearl millet by conventional cooking was 30 min while it was 90 min by solar cooking.

Temperature variation while cooking in solar cooker

The average temperature (three replications) at the time of loading in solar cooker was 115°C, which was reduced to 90°C after loading and again increased to 110°C when cooking was complete.

Biochemical analysis of nutrient values of pearl millet

Carbohydrates

Carbohydrates are a class of energy yielding substances by the process of respiration and include starch, glucose, sucrose, lactose, etc. Calcium foods and roots and tubers

Table 1. Starch present in pearl millet, with regards to raw, conventional and solar cooking.

Type of food	Sample no.	Starch (g/100 g)					
		Raw		Conventional cooked		Solar cooked	
		Measured value	Mean value	Measured value	Mean value	Measured value	Mean value
Broken pearl millet	I	39.90	40.96	31.60	30.52 (74.51)*	41.94	40.38 (98.58)
	II	41.46		30.04		39.86	
	III	41.52		29.92		39.34	

*Values in parenthesis indicate percent retention.

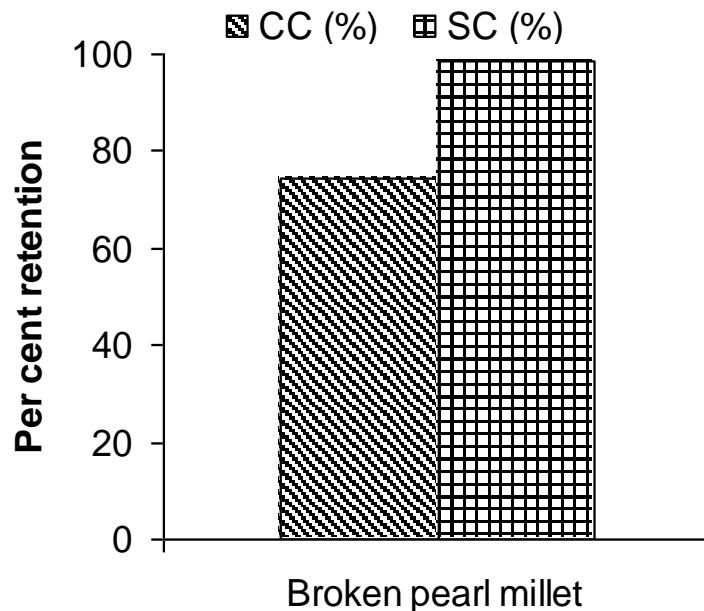


Figure 1. Starch retention in conventional cooked (CC) and solar cooked (SC) pearl millet.

are largely composed of starch, a complex carbohydrate. Food ingredients like simple sugars namely cane sugar and glucose are pure carbohydrates. Starch is a complex carbohydrate made up of glucose units. Glucose derived from starch and other sugars present in the diet is the main sources of energy in the body. Carbohydrates derived from cereals are chief source of energy in the Indian diets. Starches when eaten in a cooked form are completely digested in the gastro intestinal tract and the released glucose is absorbed and metabolised in the body to yield energy. Starches are almost completely utilised and there is no difference between starches derived from different sources.

Starch

The results of the proximate analysis of pearl millet for starch estimate are shown in Table 1. The samples were

analysed thrice and arithmetic mean of these values are shown in Table 1.

The percent retention of starch in conventional cooking and solar cooking in pearl millet is shown in Figure 1. From Table 1 and Figure 1, it is clear that retention of starch is more in solar cooking as compared to conventional cooking. 98.58% starch was retained in solar cooking as compared to 74.51% by conventional cooking on liquefied petroleum gas (LPG) stove.

When raw and conventional cooking is compared for retention of starch as shown in Table 2, calculated values of t was 13.781 which is significantly greater than table value for 4 degrees of freedom at 1%, that is, 4.604 so difference between two means is highly significant, showing significantly lost of starch in cooking.

For retention of starch when conventional cooking and solar cooking were compared (Table 1), calculated values of t was 10.441 which is greater than table value for 4 degrees of freedom at 1%, that is, 4.604, so

Table 2. Total soluble carbohydrates in pearl millet along with percent loss in both method of cooking

Type of food	Sample No.	Total soluble carbohydrates (g/100 g)					
		Raw		Conventional cooked		Solar cooked	
		Measured value	Mean value	Measured value	Mean value	Measured value	Mean value
Broken millet	I	2.27	2.21	0.660	0.64 (28.96)*	0.684	0.690 (31.22)
	II	2.19		0.635		0.710	
	III	2.17		0.625		0.676	

*Values in parenthesis indicate percent retention.

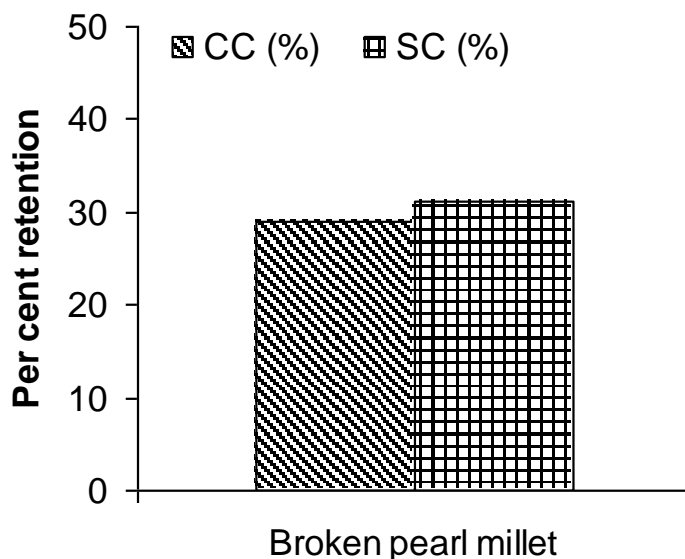


Figure 2. Total soluble carbohydrate retention in conventional cooked (CC) and solar cooked (SC) pearl millet.

difference between two means is highly significant, showing solar cooking is significantly better than conventional cooking for retention of starch in pearl millet.

Total soluble carbohydrates

The results of proximate analysis of total soluble carbohydrates (TSC) in raw, conventional cooked and solar cooked pearl millet are shown in Table 2. The samples were analysed thrice and arithmetic mean is shown in Table 2.

The retention of total soluble carbohydrates by both method of cooking as compared to total soluble carbohydrates present in raw food is shown in Figure 2. The retention of total soluble carbohydrate in broken pearl millet was 31.22% in solar cooking as compared to 28.96% by conventional cooking.

For retention of TSC when raw and conventional cooking is compared as shown in Table 2, calculated values of t were 52.669 which is significantly greater than

table value for 4 degrees of freedom at 1%, that is, 4.604 so difference between two means is highly significant.

When conventional cooking and solar cooking are compared for retention of starch as shown in Table 2, calculated values of t is 3.421 for pearl millet which is significantly greater than table value for 4 degrees of freedom at 5%, that is, 2.776 so difference between two means is significant.

Protein

Proteins are vital to any living organism. Proteins are important constituent of tissues and cells of the body. They form the important component of muscle and other tissues and vital body fluids like blood. The proteins in the form of enzymes and hormones are concerned with a wide range of vital metabolic process in the body. Proteins supply the body building material and replace the loss due wear and tear. Proteins are antibodies that help the body to defend against infection. Thus, the

Table 3. Proteins present in pearl millet , with regards to raw, conventional and solar cooking.

Type of food	Sample No.	Protein (g/100g)					
		Raw		Conventional cooked		Solar cooked	
		Measured value	Mean Value	Measured value	Mean value	Measured value	Mean value
Broken pearl millet	I	10.55	10.49	10.19	10.20 (97.24)*	10.27	10.28 (98.00)
	II	10.47		10.23		10.31	
	III	10.45		10.18		10.26	

*Values in parenthesis indicate percent retention.

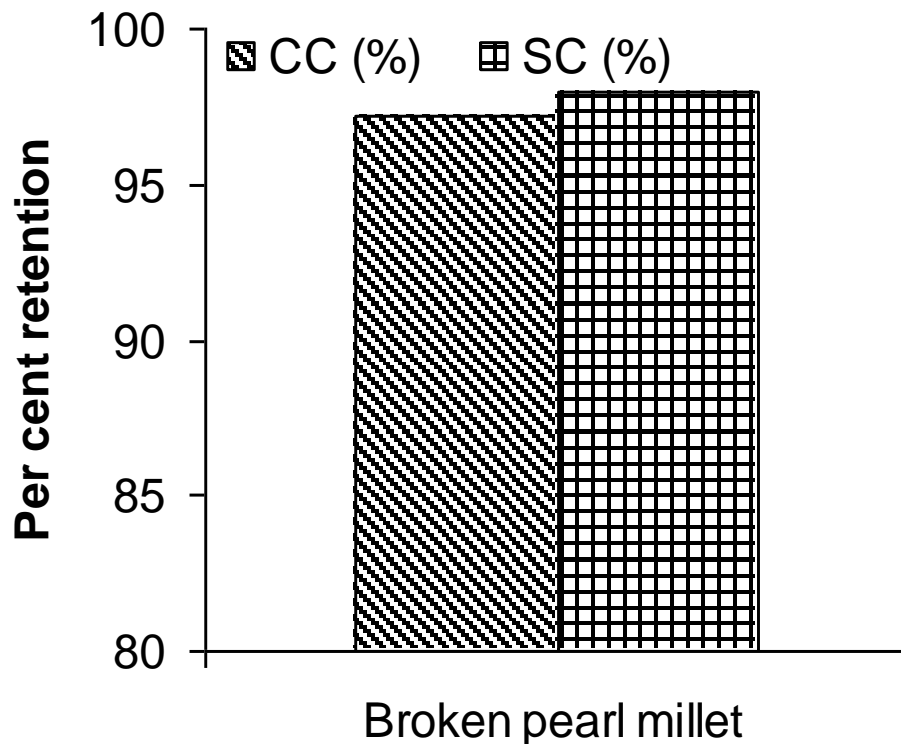


Figure 3. Protein retention in conventional cooked (CC) and solar cooked (SC) pearl millet.

proteins are one of the most important nutrient required by the body and should be supplied in the adequate amounts in the diet. The protein needed by the body has to be supplied through the diet we consume. The adequacy of protein in the diet is an important measure of adequacy and quality of a diet.

The results of proximate analysis of protein in raw, conventional cooked and solar cooked pearl millet are shown in Table 3. The samples were analysed thrice and arithmetic mean is shown in Table 3.

The percent retention of protein in conventional cooked and solar cooked pearl millet is shown in Figure 3. From the Table 3 and Figure 3, it is clear that retention of protein is more in solar cooked food as compared to

conventional cooked pearl millet. 98.0% protein was retained in solar cooking while it was 97.2% in conventional cooking. Similar results were obtained by George and Ogale (1987) in green gram, kidney bean; Devdas and Venmathi (1992) in red gram dhal and rice and by Chandrasekhar and Kowsalya (1998) in beans.

When raw and conventional cooking are compared for retention of protein from the Table 3, calculated values of t were 8.950 for pearl millet, which is significantly greater than table value for 4 degrees of freedom at 1%, that is, 4.604 so difference between two means is highly significant, indicating protein is lost in cooking.

For retention of protein when conventional cooking and solar cooking are compared as shown in Table 3,

Table 4. Calcium present in pearl millet in raw form, conventional and solar cooked.

Type of food	Sample No.	Calcium (g/100g)					
		Raw		Conventional cooked		Solar cooked	
		Measured value	Mean value	Measured value	Mean value	Measured value	Mean value
Broken pearl millet	I	44.0	43.2	33.1	33.6 (77.8)*	35.6	36.0 (83.3)
	II	42.2		34.7		35.4	
	III	40.4		33.0		37.0	

*Values in parenthesis indicate percent retention.

Table 5. Iron present in pearl millet in raw form, conventional and solar cooked.

Type of food	Sample No.	Iron (g/100 g)					
		Raw		Conventional cooked		Solar cooked	
		Measured value	Mean Value	Measured value	Mean value	Measured value	Mean v value
Broken pearl millet	I	10.34	10.38	10.02	9.93 (95.66)*	10.16	10.20 (98.27)
	II	10.48		9.88		10.14	
	III	10.32		9.89		10.30	

*Values in parenthesis indicate percent retention.

calculated values of t was 3.135 which is greater than table value for 4 degrees of freedom at 5%, that is, 2.776, so difference between two means is significant, showing solar cooking is significantly better than conventional cooking for retention of protein in pearl millet.

Minerals

A large number of minerals and trace metals are present in the body. Some of these form part of body structural component and some other acts as catalytic agents in many body reactions. Bones and skeleton are made up of mainly calcium, magnesium and phosphorous, and iron is a component of blood.

Calcium

Calcium is an essential element required for several life processes. As the structural component, calcium is required for the formation and maintenance of skeleton and teeth. It is also required for a number of other essential processes. It is required for normal contraction of muscles to make limbs move, contraction of heart for its normal function, nervous activity and blood clotting. These later function are carried out by ionised calcium present in the cells. The calcium levels in cells and plasma are well maintained. Calcium present in bones helps to maintain the calcium level in plasma in the face of dietary calcium deficiency.

The results of analysis of calcium in pearl millet raw

form, conventional cooked and solar cooked are shown in Table 4. Samples have been analysed thrice and their arithmetic means of these values are shown in Table 4.

The percent retention of calcium in conventional and solar cooked pearl millet is shown in Figure 4. From Table 5 and Figure 4, it is clear that retention of calcium is more in solar cooked pearl millet as compared to conventional cooked. 83.3% calcium was retained while it was 77.8% in conventional cooking. Similar results were obtained by Chandrasekhar and Kowsalya (1998) in carrot, beans and cabbage.

When raw and conventional cooking is compared for retention of calcium from the Table 4, calculated values of t was 7.649 which is significantly greater than table value for 4 degrees of freedom at 1%, that is, 4.604 so difference between two means is highly significant, showing lost of calcium in cooking.

For retention of calcium when conventional cooking and solar cooking are compared from Table 4, calculated values of t was 3.220 for pearl millet which is greater than table value for 4 degrees of freedom at 5%, that is, 2.776 so difference between two means is significant, indicating solar cooking is significantly better than conventional cooking for retention of calcium.

Iron

Iron is an essential element for formation of haemoglobin of red cells of blood and plays an important role in the transport of oxygen. Tissues also require iron for various oxidation reduction reactions. Most of the iron in the body

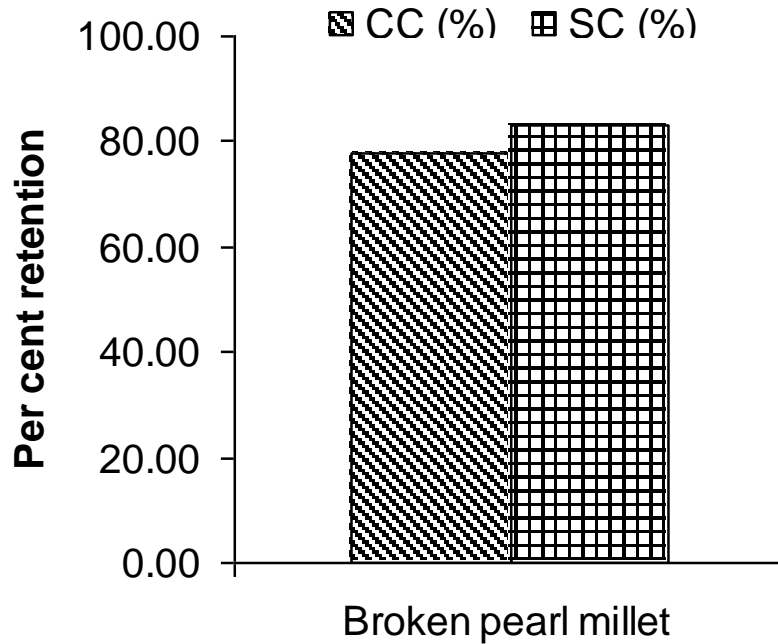


Figure 4. Calcium retention in conventional cooked (CC) and solar cooked (SC) pearl millet.

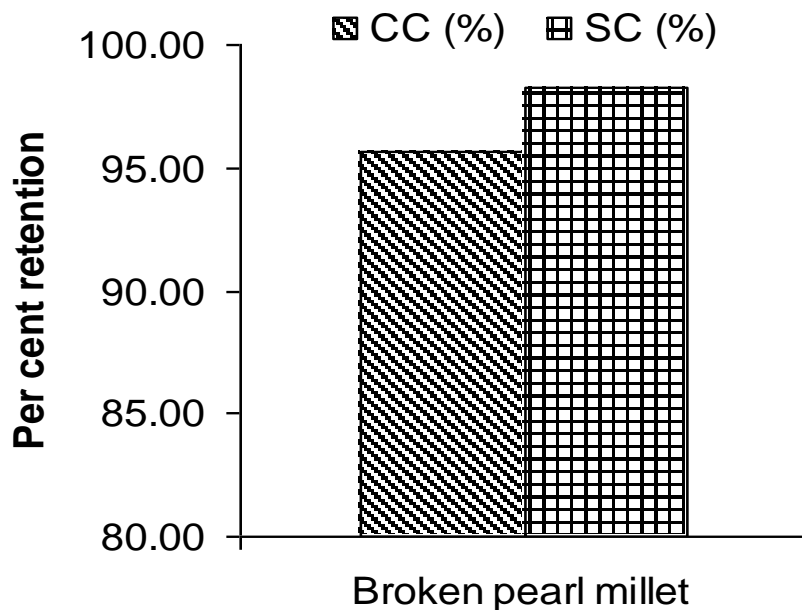


Figure 5. Iron retention in conventional cooked (CC) and solar cooked (SC) pearl millet.

is utilised and some of the body iron is also stored in liver and spleen. The amount of iron to be absorbed from the daily diet is quite small. Since there is limited capacity to absorb dietary iron, diet should contain 10-25 fold iron required daily.

Results of analysis of iron in pearl millet in raw form, conventional cooked and solar cooked is shown in Table 5. Samples were analysed thrice and their arithmetic means of these values are shown in Table 5.

The percent retention of iron in conventional and solar

cooked pearl millet is shown in Figure 5. From Table 6 and Figure 5, it is clear that retention of iron is more in solar cooked food as compared to conventional cooked food. 98.27% iron was retained in solar cooking while it was 95.66% in conventional cooking. Similar results were obtained by Chandrasekhar and Kowsalya (1998) in different vegetables viz. amaranthus, beans and cabbage.

When raw and conventional cooking is compared for retention of iron as shown in Table 5 calculated values of *t* was 6.669 which is significantly greater than table value for 4 degrees of freedom at 1%, that is, 4.604 so difference between two means is highly significant indicating lost of iron in cooking.

For retention of iron when conventional cooking and solar cooking are compared as shown in Table 5, calculated values of *t* was 4.001 which is greater than table value for 4 degrees of freedom at 5%, that is, 2.776, therefore, difference between two means is significant, showing solar cooking is significantly better than conventional cooking for retention of iron.

Conclusions

Comparative estimation of nutrients of pearl millet by two methods of cooking, that is, conventional cooking on LPG stove and solar cooking revealed that:

1. Retention of starch was 98.58% for solar cooked and 74.51% for conventional cooked pearl millet.
2. Retention of total soluble carbohydrate was 31.22% for solar cooked and 28.96% for conventional cooked pearl millet.
3. Retention of protein was 98.00% for solar cooked and 97.24% for conventional cooked pearl millet.
4. Retention of calcium was 83.3% for solar cooked and 77.8% for conventional cooked pearl millet.
5. Retention of iron was 98.27% for solar cooked and 95.66% for conventional cooked pearl millet.
6. It can be concluded that solar cooking is better than conventional cooking because more nutrients are retained in solar cooking than conventional cooking.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of antioxidant and hypolipidaemic effects of fermented *Parkia biglobosa* (Jacq) seeds in tyloxapol-induced hyperlipidaemic rats

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Globally, fermented foods form an intricate part of the staple diet of people. This study investigated the hypolipidaemic potential of fermented seeds of *Parkia biglobosa* (African locust bean/*iru*), a popular condiment by supplementing (20% w/w) in animal feed. Animals (n=5) in six treatment groups received; standard rat diet (control); *iru*-supplemented feed; standard rat feed with tyloxapol administered at the end of the experimental period (tyloxapol control); *iru* supplemented feed and triton at the end of the experimental period; standard feed with administration of fluvastatin sodium (40 and 80 mg/kg body weight) accordingly. Hyperlipidaemia was induced and ascertained by single intraperitoneal injection of 250 mg/kg triton WR 1339 (tyloxapol) constituted in normal saline. It was administered after six weeks experimental period to respective groups. The results revealed that addition of the fermented condiment into animals' feed mitigated increased lipid levels [total cholesterol (TC) and Low-density lipoprotein-cholesterol (LDL-C); triglyceride (TG)] triggered by injection of tyloxapol. On the other hand, *iru* caused a significant decrease in plasma and liver total cholesterol (TC), triglyceride (TG), LDL-C (p< 0.05) and increased high-density lipoprotein-cholesterol (HDL)-C levels (p< 0.05). The condiment showed a competitive hypotriglyceridaemic and greater hypocholesterolemic activity in the plasma when compared with fluvastatin at both concentrations. The condiment showed reasonable activities for the entire *in vitro* antioxidant assays done. Histopathologic examination revealed its hepatoprotective capability. Regular consumption of this condiment may represent a good dietary alternative for control of hyperlipidaemia and associated conditions.

Key words: *Parkia biglobosa*, hyperlipidaemia, total cholesterol, high density lipoproteins-cholesterol, low density lipoproteins-cholesterol, triglyceride, fluvastatin, tyloxapol.

INTRODUCTION

Fermentation is described as the process of anaerobic or partial anaerobic oxidation of carbohydrate material, during which process; enzymes elaborated by micro-organisms break down carbohydrates or carbohy-drate-

like materials (Odufa, 1985). The deliberate fermentation of foods by man predates written history and is possibly the oldest method of preserving perishable foods. Evidence suggests that fermented foods were

consumed as far back as 7,000 years ago in Babylon (Battcock and Aza-Ali, 1998).

Fermented foods are generally produced using plant or animal ingredients in combination with fungi or bacteria which are either sourced from the environment, or carefully kept in cultures maintained by humans. Fermented foods are noted for enhancement of diet through development of flavour, aroma and texture in food substrates. They also preserve foods and increase their shelf-life through production of various chemical substances such as lactic acid, alcohol, acetic acid and alkaline fermentation. Fermented foods enhance food quality with protein, essential amino acids, essential fatty acids and vitamins, improving digestibility and nutrient availability. Very often, detoxification of anti-nutrients in food occurs through food fermentation processes, and there can also be a decrease in cooking time and fuel requirement. Some inedible seeds and fruits are made edible through fermentation processes (Evans et al., 2013).

Globally, fermented foods whether from plant or animal origin form an intricate part of the staple diet of people and the raw ingredients are indigenous in that particular place. In Africa, the art of fermentation is widespread including the processing of fruits and other carbohydrate sources to yield alcoholic and non alcoholic beverages (Adewusi et al., 1991, 1992), they therefore play a very important role in the socioeconomics of developing countries as Evans et al. (2013) reported.

Fermented African locust beans (Dadawa or *Iru*) are made by a natural un-inoculated solid - substrate fermentation of the boiled and dehulled cotyledon of the seeds of *Parkia biglobosa* (African locust-bean). They are one of the most important food condiments in Nigeria and many countries of West and Central Africa (Evans et al., 2013). Dadawa or *Iru* are used in much the same way as bouillon cubes are used in the Western world as nutritious flavouring additives. They are rich in fat (39 to 40%) and protein (31 to 40%) and contributes significantly to the energy intake, protein and vitamins, especially riboflavin, in many countries of West and Central Africa (Achi, 2005; Daramola et al., 2009). The major fermenting organisms are the *Bacillus* and *Staphylococcus* species (Omafuvbe et al., 2004; Achi, 2005). Fermented seeds of *P. biglobosa* are used in all parts of Nigeria and, indeed, the west coast of Africa to season traditional soups (Ajaiyeoba, 2000; Agunu et al., 2005).

Introduction of foreign high technology products especially processed ones because of globalization and liberalization of the economy is radically changing the Nigerian food culture into a mixed grill of both foreign and local dishes (Ojo, 1991). Achi (1992), reported an

ambivalent attitude in terms of consumers' tastes and preferences for fermented foods in Nigeria. Arogba et al. (1995), reported that the traditional condiments in Nigeria have not attained commercial status, yet anecdotal evidences abound in areas where these indigenous condiments are heavily consumed that there are low incidences of dyslipidaemias. Antihypertensive (Assane et al., 1993; Ajaiyeoba, 2000), analgesic, anti-inflammatory (kouadia et al., 2000), antidiabetic, antidiarrhoeal (Agunu et al., 2005; Odetola et al., 2006) activities of different extracts from *P. biglobosa* seeds and trees have been reported. Ognatan et al. (2011), reported a low incidence of hypertension cases in a community where this condiment is heavily consumed in Togo, within the African continent.

The present study was therefore designed to rationally investigate the hypolipidaemic effects of regular intake of this condiment in tyloxapol-induced hyperlipidaemia in rats. The effects were compared with that from fluvastatin, a commonly prescribed antilipidaemic drug. This is with a view to establishing scientifically, the benefits (if any) of its intake as a means of controlling hyperlipidaemia and related conditions given the importance and widespread popularity of this indigenous condiment. Figure 1 shows clumps of *iru* seeds.

MATERIALS AND METHODS

Chemicals

Triton WR 1339 (tyloxapol) was obtained from Sigma Chemical Company, St. Louis Missouri, USA. Diagnostic kits for cholesterol, triglycerides and high-density lipoprotein (HDL) precipitants were purchased from Randox Laboratories (Antrim, United Kingdom). All other reagents were of analytical grade and of purest quality.

Fermented condiment

Traditional method of production of locust bean condiment (*iru*) as reported by Omafuvbe et al. (2004) was adopted.

Preparation of feed

Feeds supplemented with *iru* was prepared by thoroughly mixing weighed condiment and standard rodent feed in a ratio 1:4 respectively to give a twenty percent (20% w/w) *iru*-supplemented feed. The feed was pelletized by adding 4-5 drops of water, and passed through a roller to cake; and thereafter placed in the oven set at about 40°C for drying and caking.

Experimental animals

Thirty albino rats (both sexes), weighing between 75 and 110 g, were used. After 2 weeks acclimatization, rats were kept in

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Figure 1. Clumps of *iru* seeds.

standard animal cages in an animal house maintained at 26-29°C with a 12 h light–dark cycle. Animals were fed on standard laboratory chow purchased from Bendel Feeds and Flour Mills limited, Benin Road, Ewu, (Edo State, Nigeria). The feed contained 14.5% crude protein, 4.8% crude fat, 7.2% crude fiber, 8.0% crude ash, 0.6% phosphate, 0.3% available phosphorus, 0.6% lysine, 0.3% methionine, 0.5% methionine + Cystine, 8,000 I.U vitamin A, 2,400 I.U vitamin D₃, 15.0 mg vitamin E, 4.0 mg vitamin B₂, 50.0 mg Vitamin C, 30.0 mg Manganese, 30.0 mg Zinc, 0.2% sodium. Rats had access to feed and water *ad libitum*. Animals were distributed randomly into six different groups with five animals in each group. The experimental/treatment period was six weeks and animals were weighed daily.

Group A served as the control group and received standard feed (no treatment). Group B received *iru* supplemented feed; C was given standard feed with tyloxapol administered at the end of the experimental period (tyloxapol control), D received *iru* supplemented feed with tyloxapol administered; Groups E and F received standard feed with tyloxapol administered and fluvastatin sodium (40 and 80 mg/kg body weight respectively).

Administration of tyloxapol

At the end of the six weeks experimental period, animals were deprived of food for 24-h, after which hyperlipidaemia was induced by a single intraperitoneal injection of 250 mg/kg body weight of rats (5) to groups C, D, E and F.

Administration of fluvastatin sodium

Two doses of the drug, that is, 40 mg and 80mg/kg body weights of rats were prepared and administered 12 h after injection of tyloxapol to two different groups (E and F) orally with an intubator. The caplet was dissolved in distilled water; the needed concentrations were prepared based on weights of the rats.

Sample collection

Blood samples were withdrawn from 48-h fasted rats by cardiac puncture using needles and syringes into heparinised bottles.

These were centrifuged with a bench centrifuge at 3000 revolutions per minute for 15 min to separate the plasma from the blood cells. Plasma samples were used for the assays. Animals were sacrificed by cervical dislocation and their livers were removed.

Preparation of liver homogenate

Liver samples were washed with normal saline and blotted with soft tissue paper. 1 g each was homogenized in 10 ml of 0.25 M sucrose solution to give 10% (w/v) homogenates.

Lipid profile estimation

Plasma triglyceride and cholesterol levels were assayed using commercial kits (Randox Laboratories). The HDL was measured using the enzymatic colorimetric method. After centrifugation at 3000 *g* for 10 min at 25°C, the clear supernatant contained the HDL fraction, which was assayed for cholesterol using the kit. Low-density lipoprotein-cholesterol (LDL-C) was calculated according to the formula of McNamara et al. (1990).

In vitro antioxidant assays

The antioxidant activity of the condiment was determined by different *in vitro* methods such as, the DPPH free radical scavenging assay (Blois, 1958; Brace, 2001), nitric oxide radical inhibition activity (Marcocci et al., 1994), lipid peroxidation assay (Nabasree and Bratati, 2002), ascorbic acid equivalent (Benzie and Strain 1999) methods. All the assays were carried out in triplicates and average values were considered.

Histopathological investigation

Histopathological investigation was performed by fixing the representative liver tissues from each group in 10% formal saline by total immersion for 48 h after which they were processed via paraffin wax embedding method of Drury and Wallington (1980). The staining procedure of H and E as described by Drury and Wallington (1980) was also adopted. This was done at the Department of Anatomy and Cell Biology of the Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Statistical analysis

Statistical evaluation of the data was done with Graph Pad Prism version 4.0 for Windows, Graph Pad Software, San Diego California USA (Ansarullah et al., 2009). The difference between groups was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison Test with 5% level of significance ($P < 0.05$) considered significant (Gaudiot et al., 2000). Values are expressed as mean \pm SEM (Standard Error of Mean).

RESULTS

Effect of condiment supplementation on animal weekly weight changes

Average weights of rats' increased with the progression in experimental week based on the diet received.

Table 1 Effect of *iru* supplementation on animal weekly weight changes.

Time (weeks)	Control	<i>iru</i> -supplemented feed	Tyl. control	<i>iru</i> -supplemented feed +Tyl.	<i>iru</i> -supplemented feed + Tyl. + FLU (40 mg)	<i>iru</i> -supplemented feed+Tyl.+FLU (80mg)
1	93.25 ± 5.51	85.73 ± 3.39	117.90 ± 1.99	77.14 ± 3.02	110.80 ± 5.09	115.20 ± 3.31
2	104.40 ± 5.30	98.73 ± 2.33	125.90 ± 2.09	89.27 ± 3.81	110.90 ± 3.78	120.40 ± 4.09
3	108.20 ± 3.42	107.40 ± 2.91	127.70 ± 3.44	100.80 ± 2.06	111.30 ± 4.47	120.50 ± 4.81
4	110.20 ± 4.01	105.70 ± 5.01	129.50 ± 2.41	111.90 ± 2.27	111.00 ± 2.48	122.00 ± 3.41
5	105.30 ± 3.30	103.90 ± 3.64	123.30 ± 3.51	114.80 ± 5.41	110.90 ± 5.13	117.50 ± 5.50
6	107.20 ± 5.19	110.10 ± 2.11	124.00 ± 3.98	128.90 ± 3.99	111.10 ± 2.39	121.20 ± 2.05

Table 2. Antioxidant assay result of *iru*.

DPPH		Nitric oxide		Lipid peroxidation		Ascorbic acid equivalent	
Concentration (mg/ml)	Inhibition (%)	Concentration (mg/ml)	Inhibition (%)	Concentration (mg/ml)	Inhibition (%)	Concentration (mg/ml)	(µgVitCequiv/g)
25.00	64.65 ± 4.27	100.00	46.73 ± 2.67	100.00	24.93 ± 2.06	100.00	63.86
12.50	41.92 ± 1.11	50.00	40.43 ± 3.12	50.00	18.79 ± 3.79	50.00	62.84
6.25	25.15 ± 0.12	25.00	36.41 ± 0.10	25.00	10.10 ± 2.04	25.00	60.03
IC₅₀	17.41		128.03		229.96	Mean ± SEM	62.24 ± 1.15

Values are Mean±SEM of triplicate tests.

Supplementation of rats feed with *iru* resulted in improved growth better than the control group in this study (Table 1). While weight change declined from the 4th week and gradually normalizes in control rats, sharp weight gain was seen in *iru*-fed rats. The increase became evident in test rats from the 3rd week.

Antioxidant assay results

Antioxidant assay results are presented in Table 2. The condiment showed nitric oxide and DPPH radical scavenging activities. It also inhibited lipid peroxidation *in vitro*. For each of the assays, the results were concentration dependent, in other words, the higher the concentration, the more the radical scavenging potential. Ascorbic acid equivalent test also showed that antioxidant activity from 1 µg ascorbic acid (standard antioxidant) is equivalent to that from 62.24±1.15 g of *iru*.

Effects of condiments supplementation on plasma and liver lipids of rats

Plasma and liver lipid profile

Total cholesterol estimation in plasma (Figure 2), shows that triton induced a statistically significant increase ($P < 0.05$) in the plasma total cholesterol, causing a 23.86% increase. Fluvastatin administered at 40 and 80 mg/kg

body weight induced reductions of 2.92 and 6.88%, respectively. In group D animals that were fed the *iru*-supplemented diet, the percentage reduction in total cholesterol was found to be 32.29. This implies that pretreatment of the rats with *iru* resulted in reduction of total cholesterol that was much better than what was obtained with Fluvastatin.

Triton again induced an increase of 88.19% in liver total cholesterol concentration (Figure 3) which was again ameliorated in the group pre-treated with the condiment. Fluvastatin at both concentrations of 40 and 80 mg/kg body weight caused significant reductions of 4.37 and 34.04% respectively. There was no significant difference ($P > 0.05$) between groups D and E (3.8 and 4.37% decrease, respectively), this suggests the percentage reduction elicited by 40 mg/kg body weight, fluvastatin can be likened to that of the condiment supplemented group in the liver.

In the case of plasma triglycerides (Figure 4), injection of triton elicited a percentage increase of 38.96% in triglyceride level (group C). This increase was mitigated in group D pre-treated with condiment supplemented feed (5.46% decrease). This implies pre-treatment of rats with condiment-containing diet prevents the rise in plasma triglycerides level elicited by administration of triton. Interestingly, in the group fed the condiment alone without the induction of hyperlipidaemia, there was a 23.77% reduction in triglycerides concentration. A similar result was obtained with liver triglycerides concentrations (Figure 5). Triton induced a 38.39% increase in liver

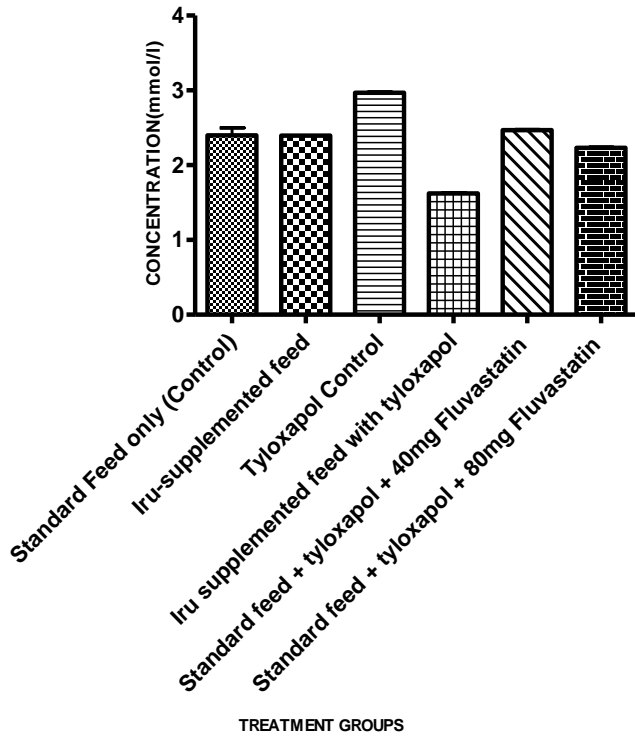


Figure 2. Effect of *iru* supplementation on plasma total cholesterol levels following triton-induced hyperlipidaemia. All values are expressed as mean \pm SEM. n=5.

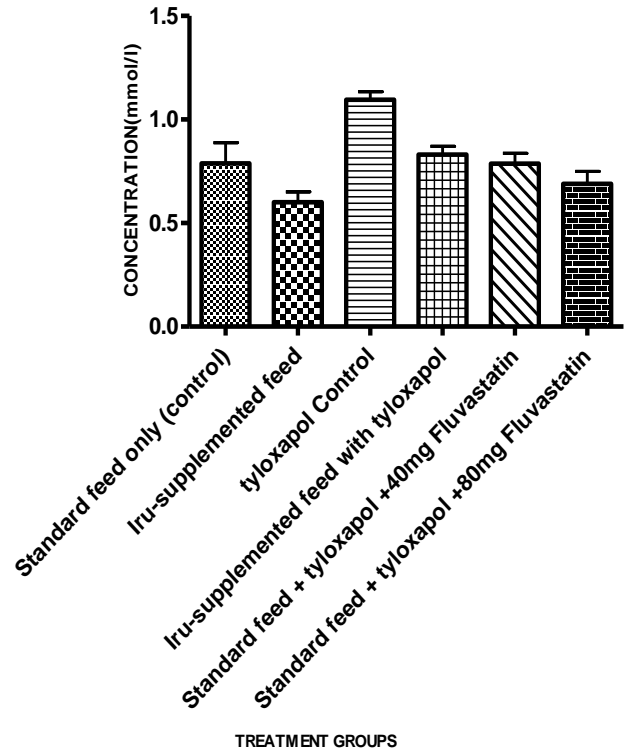


Figure 4. Effect of *iru* supplementation on plasma triglycerides (mmol) levels following triton-induced hyperlipidaemia in experimental and control rats. All values are expressed as mean \pm SEM.

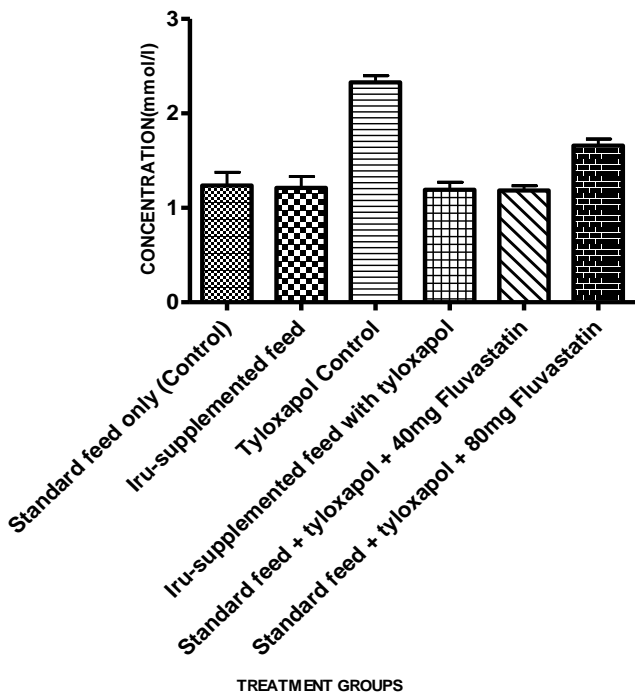


Figure 3. Effect of *iru* supplementation on liver total cholesterol concentrations in triton-induced hyperlipidaemia in experimental and control rats. All values are expressed as mean \pm SEM.

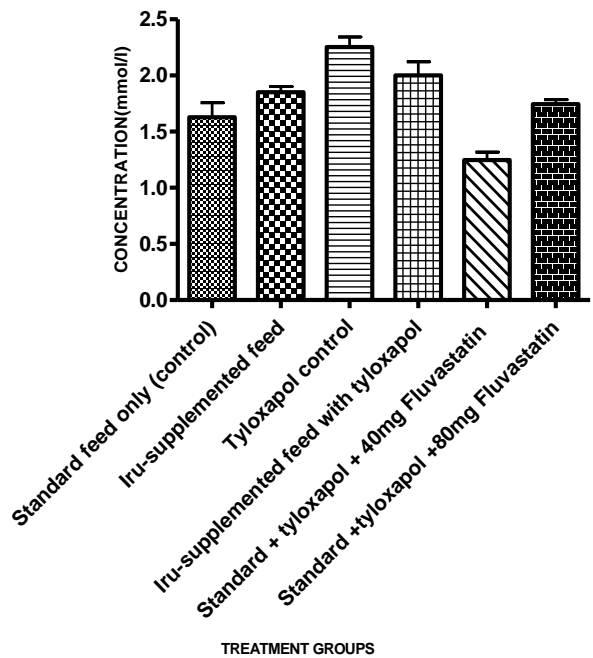


Figure 5. Effect of *iru* supplementation on liver triglyceride levels in triton-induced hyperlipidaemia in experimental and control rats. All values are expressed as mean \pm SEM. n=5.

triglycerides concentration. The condiment treated group and the fluvastatin 40 mg/kg treated groups were not significantly different from each other; there was a 22.91 and 23.40% decrease, respectively.

Plasma and liver lipoproteins

Figure 6 presents the results for the plasma LDL-Cholesterol; triton again induced a significant increase of 67.27%. Fluvastatin at 40 mg reduced plasma LDL-C by 25.28% while 80 mg/kg body weight fluvastatin reduced it by 16.43%. *Iru*-supplemented feed caused a percentage decrease of 66.32. This reduction is statistical significant ($P < 0.05$), and also higher than that from both concentrations of fluvastatin. Again in the absence of triton, the condiment elicited a reduction of 9.51%.

The plasma HDL-cholesterol results are shown in Figure 7. In the tritonised group of rats (Group C), there was a significant reduction (28.61%) in the HDL-C concentration. 40 and 80 mg/kg body weight fluvastatin induced increases of 3.75 and 8.61% respectively, while *iru* condiment supplemented feed induced an increase of 18.33%. This was much higher than the value at both concentrations of fluvastatin. Again in the absence of triton, pre-treatment with the condiment gave rise to an increase of 29.44% in HDL-cholesterol concentration. As shown in Figure 8, triton induced 20.18% decrease in liver HDL-C concentration. Fluvastatin at 40 and 80 mg/kg body weight gave rise to percentage increases of 7.45 and 75.30 respectively, while the condiment brought about an increment of 0.90%.

Histopathology results

Figure 9 shows the photomicrographs of representative liver sections from the experimental groups. The staining and magnification used is hematoxylin and eosin at 400x.

In group A rats, architecture of the liver appears normal. The hepatocytes (H) are arranged in plates around the central vein (CV) with sinusoids (Sn) in between the plates. In group B rats (treated with the condiments alone), the hepatocytes (H) are arranged in plates with poorly defined sinusoids (blue arrow) in between the plates. Architecture of the liver of group C rats (triton-treated) appears abnormal showing necrosed hepatocytes with poorly defined sinusoids in between the plates. There are also signs of fibrosis (F) and degeneration (D) in the liver section. The architecture of the liver of *iru*-fed rats appears normal. In the fluvastatin (40 mg) treated rat (group E) liver section, the hepatocytes in the periportal area of the liver section with sinusoids (Sn) in between the plates were seen, as well as the portal area (PA). The hepatocytes appear normal. In the fluvastatin (80 mg) treated rats (group F), the architecture of the liver appears normal.

DISCUSSION

High nutritive values of fermented foods have been well documented (Steinkraus, 1997; Gadaga et al., 2004).

The results of this study (Table 2) suggest that the condiment is a potential source of antioxidants of natural origin. The condiment possessed significant DPPH radical and nitric oxide scavenging potentials. This suggests that regular intake of this condiment would be of immense health benefit since oxidative processes have been proposed to have a causative and/or contributory role in an increasingly growing number of diseases, including certain types of cancers, neurodegenerative disorders, stroke, diabetes, etc.

Triton WR 1339, in agreement with literature (Otway and Robinson, 1967; Abe et al., 2007; Ngoc et al., 2008) induced significant increase in total cholesterol and triglycerides in the present study. The results of this study further showed that, the condiment caused a significant ($P < 0.05$) increase in the plasma level of HDL-C, which is usually termed the 'good cholesterol' (Agbedana, 1999). The resultant effects of increased HDL-C and decreased LDL-C (bad cholesterol), resulted in an increased HDL-C/LDL-C ratio in the test animals, this has been shown to be beneficial and is indicative of a lower risk of coronary heart diseases (Castelli, 1984).

In increasing the good cholesterol, the condiment performed much better than the standard medicine used (Figure 7). The effect on lowering plasma LDL-C was also significantly ($p < 0.05$) better than fluvastatin sodium (Figure 6), while a comparison of the hypotriglyceridemic effects of the standard drug and condiment also shows that the condiment compares favorably with the standard drug.

Hepatic examination reveals that the liver sections of *iru*-supplement fed animals were comparable with standard control rats. The effect of tyloxapol was highly pronounced on the hepatocytes of group C rats, as deadness and degeneration were observed. This is in agreement with literature (Otway and Robinson, 1967; Abe et al., 2007; Ngoc et al., 2008). This effect was however mitigated in group pretreated with the condiment and also those administered fluvastatin at both concentrations (Figure 9). This suggests that the condiment has hepatoprotective potential as well as ability to ameliorate liver cells fibrosis and necrosis due to chronic hyperlipidaemic agent in rats.

Conclusion

In conclusion, whole *iru*-supplemented feed showed hypolipidemic activity in tyloxapol-induced hyperlipidemia in rats. Regular intake of a good quantity would be useful in protecting the human body system against changes that would elicit high cholesterol, LDL-C, and triglycerides levels while significantly boosting HDL-C. Even in the absence of an hyperlipidaemic state, its consumption is

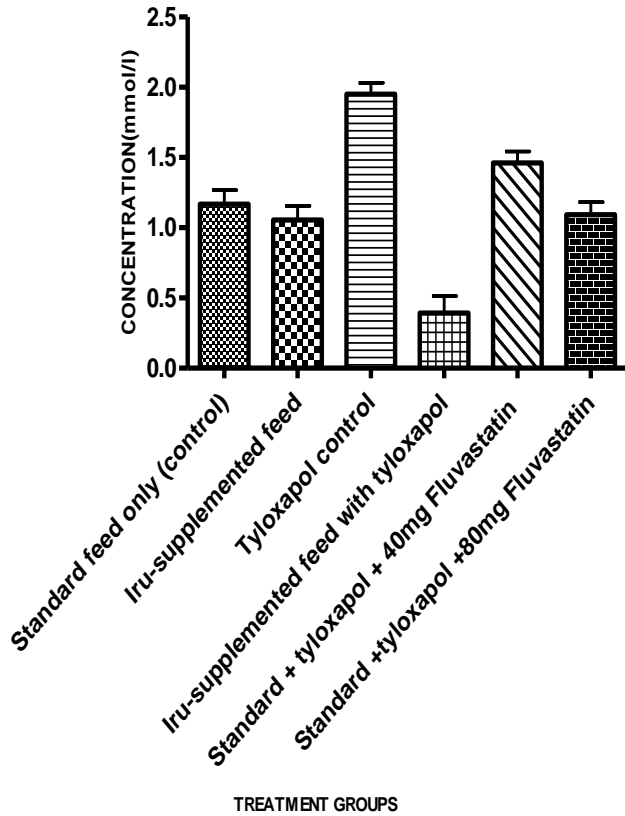


Figure 6. Effect of *iru* supplementation on plasma LDL-cholesterol concentrations in triton-induced hyperlipidaemia. All values are expressed as mean±SEM. n=5.

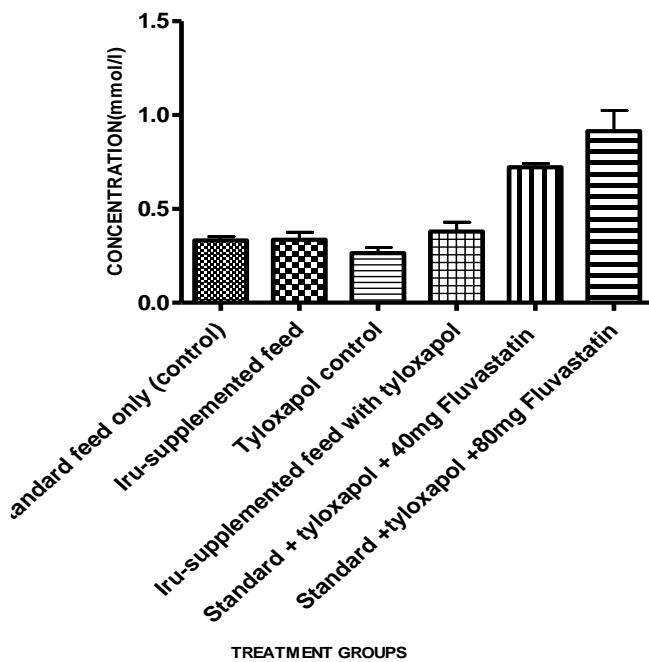


Figure 7. Effect of *iru* supplementation on plasma HDL-Cholesterol levels in triton-induced hyperlipidaemia with the various treatments administered. All values are expressed as mean±SEM. n=5.

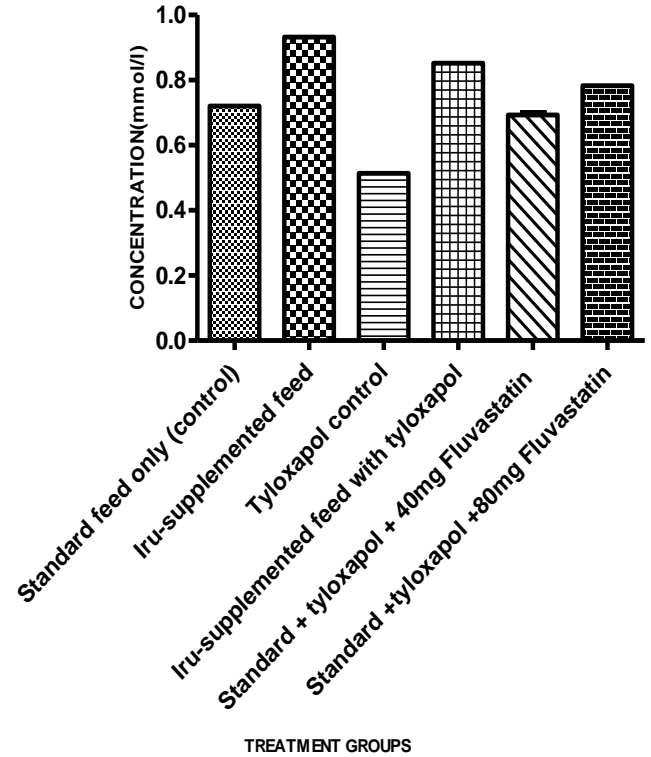


Figure 8. Effect of *iru* supplementation on liver HDL-cholesterol in triton-induced hyperlipidaemia in experimental and control rats. All values are expressed as mean±SEM. n=5.

also proven to be beneficial in the animal model used. Therefore, regular consumption of the condiment may represent a new alternative way to control or protect against common disorders of lipid metabolism which are associated with hyperlipidaemia and oxidative stress. It may also be considered a cheap and accessible source of antihyperlipidaemics. The use of the condiment could also be advocated as a food ingredient for processed and canned foods in order to further increase their acceptability, versatility and utility.

Work is ongoing in our laboratory on identification and isolation of specific antioxidant and hypolipidaemic component(s) present in the condiment as this may lead to chemical entities with huge potential for clinical use.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

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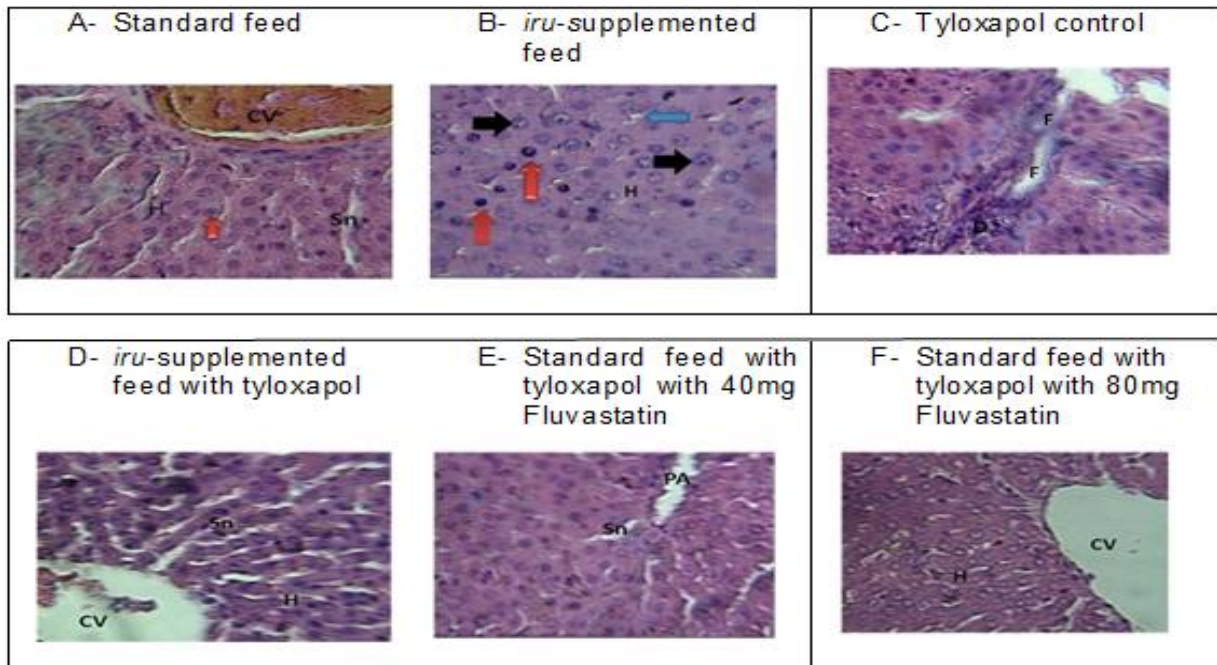


Figure 9. Changes in hepatocytes of representative rats from groups A-F in triton-induced hyperlipidaemia after treatment with condiment, triton and fluvastatin sodium. F- fibrosis, D- degeneration, Sn-sinusoids, H- Hepatocytes, CV- Central vein, PT- portal area. (hematoxylin and eosin400 x).

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Full Length Research Paper

Optimization of taste and texture of biscuit produced from blend of plantain, sweet potato and malted sorghum flour

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Biscuits were produced from blends of plantain, sweet potato and malted sorghum flour. This study was carried out to evaluate the effect of varying the proportions of these flour mixtures on the taste and texture of the produced biscuits. Mixture response surface methodology was used to model the taste and texture of the biscuits with single, binary and ternary combinations of the three flours. The optimum taste and texture of the biscuit were targeted and the responses optimizer of Minitab software (version 14.0) was used to obtain the mixture that gave these targets. Result showed that the taste and texture of biscuits samples differed significantly ($p < 0.05$). Sweet potato flour had the highest positive influence on the taste, while malted sorghum mostly improved the texture. The regression coefficients showed that the mixture of plantain and malted sorghum flour decreased both the taste and the texture of the biscuit, while the blend of sweet potato and malted sorghum flour had negative influence on the texture of the biscuit. The mixture of the three flours improved both the taste and the texture of the biscuit more than their single or binary combinations. Biscuit of acceptable taste and texture could be produced from the blend of these three flours.

Key words: Plantain flour, sweet potato flour, malted sorghum flour, biscuit, taste, texture.

INTRODUCTION

Among ready to-eat snacks, cookies and biscuits are widely consumed throughout the world. They are sold at markets, street shops and hawked at motor parks and schools where they could be bought and consumed by

people of all ages (Lorenze, 1983). The fact remains that wheat is the choice flour for baked products because of its gluten content which other cereals lack. It has been reported that wheat is not sufficiently produced in most

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countries of the tropics making them to rely on imported and expensive wheat flour (Holt et al., 1992; Eneche, 1999).

Products made from non wheat flour or from composite flour are latest trend in producing baked goods. Users of composite flour aim at mitigating the poor properties of flour produced from other non wheat grains. The substitute for wheat flour should be the flour that is readily available, cheap and able to replace wheat flour in terms of functionality. Some times, the interest in non-wheat products is based on their nutritional, health benefits and sensory properties. Celiac disease is a food induced immunological disease of the upper intestine triggered by the ingestion of gluten containing cereals in genetically susceptible individuals and these individual often resort to gluten-free baked goods. Researchers have developed gluten-free biscuits and cookies comparable in quality to ones produced from wheat (Okorie and Onyeneke, 2012).

Sorghum (*Sorghum guinea*) is readily available and acceptable in Nigeria. Even though it compares favorably with other cereals in terms of nutrient composition, many developed countries of the world still regard the grain as inferior (FAO, 1995). Malted sorghum has higher metabolisable energy, protein, soluble sugar and lysine than the unmalted sorghum and it has also been reported that malting reduces the tannin content of sorghum (Magness et al., 1971; Barrett and Larkin, 1974; Wu and Well, 1980). Sorghum has been reported as the fifth most important cereal among the world cereals (Dogget, 1989). But for tropical Africa, it is ranked the first with about six million tones produced yearly in Nigeria, this being the largest in Africa (Etuk et al., 2012).

Sweet potato (*Ipomea batata*) is abundant in many tropical and subtropical regions (Horton and Fano, 1985). Sweet potato is an important crop for food security in Nigeria where it is usually eaten boiled, or fried as chips. It is rich in nutrients especially dietary fibre, vitamins and minerals (Okorie and Onyeneke, 2012). It has been reported that incorporation of 40% sweet potato flour to wheat flour produced cookies with improved nutritional and sensory qualities (Singh et al., 2008).

Plantain (*Musa paradisca*) is one of the major sources of carbohydrate for millions of people in Africa, Caribbaean, Latin America, Asia and the Pacific (FAO, 1989). Unripe plantain is processed into flour in Nigeria and some other African countries and stirred in boiling water to form an elastic paste that is eaten with soup in Nigeria and has been used in making biscuits and cookies (Ishiwu, 2005; Ngalani and Crouzet, 1995).

Mixture response surface methodology (MRSM) is a statistical technique that can be used to determine the effect that components in a mixture have on the attributes of a finished product (Cornell, 1979; Myers et al., 2009; Okpala and Okoli., 2012).

The present study was undertaken with the aim to produce biscuits from blends of plantain flour, sweet potato flour and malted sorghum flour which are gluten-

free flours and use MRS regression to study the effects of blends on taste and texture of the biscuits, and thereby optimize these attributes in the biscuits.

MATERIALS AND METHODS

Source of raw material

Sweet potato tubers (*I. batata*), sorghum grain (*S. guinea*), plantain (*M. paradisca*) and wheat flour were bought from Ogbete main market in Enugu, Nigeria.

Production of malted sorghum flour (MSF)

Sorghum grains were malted using the modified method for germinated cowpea described by Hallen et al. (2004) and Okpala and Okoli (2012). The cleaned grains were steeped in 1% sodium hypochlorite solution for 30 min to prevent mould growth. The steeped grain were again thoroughly washed and re-soaked in water (10 h). After soaking, the hydrated grains were spread on a moist jute bag which had been previously sterilized by boiling for 30 min and the grains were allowed to germinate for 4 day after which the ones that failed to germinated were discarded. The seeds germinated seeds were dried at 60°C in an oven (Gally kamp). Thereafter, the formed roots were manually rubbed off before milling and sieving through a sieve (Mesh no 80) and packaged in an air tight container until it is used.

Production of sweet potato flour (SPF)

The procedure reported by Adeyemi and Ogazi (1985) for the production of sweet potato flour was used with little modification. Four kilograms of sound tubers was weighed out after the potato had been cleaned, hand peeled, washed sliced into chips of varied length and weight (4 mm thickness) and blanched in hot water at 90°C for 30 s. Excess water on the chips were moped with hand towel before drying them at 60°C for 12 h in a hot air oven (Gallen kamp). The dried chips were milled and sieved using a laboratory test sieve (mesh no. 80). The flour was packaged with air tight plastics bucket.

Production of plantain flour (PF)

Unripe plantain was peeled, sliced (4 mm) and wrapped in muslin cloth and blanched for 30 s in hot water (90°C). After blanching, excess water on the slices was moped using a hand towel. The slices were further dried at 60°C in a hot air oven (Gally kamp) for 12 h. The dried slices were milled and sieved through sieve (no 80 mesh) to obtain fine flour. The flour was packaged in an air tight container until it was used.

Experimental design

The design was a three-component augmented simplex centroid design which was carried out using statistical software (Minitab version 14.0) as shown in Table 1. The three mixture components in this study were plantain flour (x_1), sweet potato flour (x_2) and malted sorghum flour (x_3). The proportion of each flour was expressed as a fraction of the mixture and for each treatment combination giving the sum of the component proportion as 100 where:

Table 1. Experimental design used to produce the flour blends.

Sample (S/N)	Plantain (g) (X ₁)	Sweet Potato (g) (X ₂)	Malted Sorghum (g) (X ₃)
1	50.000	50.000	0.000
2	16.667	16.667	66.667
3	66.667	16.667	16.667
4	33.333	33.333	33.333
5	0.000	100.000	0.000
6	16.667	66.667	16.667
7	50.000	0.000	50.000
8	100.000	0.000	0.000
9	0.000	0.000	100.000
10	0.000		
11	(100% wheat)	50.000	50.000

$$\sum X_i = X_1 + X_2 + X_3 = 100 \quad (1)$$

In this design, the number of runs was 10 (Table 1). A sample making the total number of samples 11.

Biscuit preparation

The ingredients used were: Flour 100 g, hydrogenated vegetable fat 40 g; sugar 10 g; salt 0.5 g; baking powder 2 g; water varied between 20 and 30 ml. Fat, salt and sugar were mixed in a Hobart mixer. The baking powder and sifted flour were added to the mixture and manually mixed. Water was carefully added in bits to form dough. The dough was rolled and cut in to circular shapes of 5 cm diameter. Baking was carried out at 180°C for about 30 min. Biscuit samples produced were cooled and stored in polyethylene bags and again in a wide screw mouth glass bottle until analyzed. Biscuit was made from 100 % wheat flour with the same amounts of ingredients above to serve as a control.

Determination of taste and texture

In order to evaluate these attributes, sensory evaluation was conducted on the biscuit samples: A total of twenty semi-trained panelists were recruited from staff and students of Nnamdi Azikiwe University, Awka. Each panelist evaluated all the samples on the attributes of taste and texture in one session. A 9-point hedonic scale was used with: 1 = dislike extremely; 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely (Ihekoronye and Ngoddy, 1985; Iwe, 2002). Samples were coded with 3 alphabets and randomly presented to the panelists. The panelists were instructed to rinse their mouths with water after evaluating each sample.

Statistical analysis

Data generated from the sensory evaluation of taste and texture of the samples were subjected to analysis of variance (ANOVA) using a statistical software (SPSS version 17.0). Significant differences between the samples were determined at $P < 0.05$. Minitab version 14.0 was also used to analyze the data for the regression coefficients used to form the mathematical models that explained the relationship between the independent variable: plantain flour (x_1), sweet potato flour (x_2) and malted sough flour (x_3) and the

response variables: taste (y_1) and texture (y_2) of the biscuit samples. The model search was started with linear, through full quadratic and the equation that gave high R^2 adj was selected as shown in Equation 1 below:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (2)$$

Where y is the predicted responses (taste and texture); β is the parameter estimate (coefficient) for each linear and cross product term for the prediction model; x_1 , x_2 , x_3 , x_1x_2 , x_1x_3 and $x_1x_2x_3$ are the linear terms for plantain, sweet potato and malted sorghum and their cross product terms.

Graphical optimization of taste and texture

In order to optimize the taste and texture of the biscuit, a degree of likeness was targeted as 7.0 which represents like moderately in the hedonic scale. The response optimizer in Minitab 14.0 was clicked and high value (8.0) which represents like very much, the target (7.0) and low value (6.0) which represents like slightly were typed into the provided boxes to generate the flour ratio that would produce the biscuit with the targeted sensory score (7.0) for taste and texture.

RESULTS AND DISCUSSION

The mean values of the sensory scores for taste and texture of the biscuits are shown in Table 2. Significant differences ($P < 0.05$) existed among the blends, this is in agreement with the earlier reports (Capitani et al., 2009; Okpala and Okoli., 2012) which stated that the minimum requirement for modeling a surface is that variation among the samples should be observed.

Table 3 shows the coefficients estimates, adjusted regression coefficients (R^2 adjusted) for taste and texture for the biscuits, the multiple regression analysis showed that the full quadratic model was significant in predicting the taste and the texture of the biscuits. The model that explained the relationship between the taste of the biscuits and the independent variables (x_1 , x_2 and x_3) is shown in Equation 3:

Table 2. Mean scores of taste and texture of biscuits produced from the flour blends and the control.

Sample (S/N)	Plantain (g) (X ₁)	Sweet potato (g) (X ₂)	Malted sorghum (g) (X ₃)	Taste (mean)	Texture (mean)
1	50.000	50.000	0.000	5.40 ^c	5.55 ^c
2	16.667	16.667	66.667	6.00 ^b	6.25 ^b
3	66.667	16.667	16.667	6.00 ^b	4.75 ^d
4	33.333	33.333	33.333	6.75 ^a	6.25 ^b
5	0.000	100.000	0.000	6.75 ^a	5.50 ^c
6	16.667	66.667	16.667	6.50 ^b	5.25 ^c
7	50.000	0.000	50.000	4.75 ^c	3.75 ^d
8	100.000	0.000	0.000	5.50 ^c	5.00 ^c
9	0.000	0.000	100.000	5.98 ^b	5.68 ^b
10.	0.000	50.000	50.000	6.50 ^b	5.20 ^c
11. (Control)				8.00 ^w	7.00 ^a

Sample 11 = Control sample (100% wheat flour biscuit). Scores are based on 9-point hedonic scale. Data are mean scores by the panelists. Data in the same column bearing different superscript differ significantly ($p < 0.05$).

Table 3. Coefficient estimates and adjusted regression coefficient ($R^2_{adj.}$) for taste and texture of biscuit produced from blends of plantain, sweet potato and malted sorghum flours.

Variable	Taste	Texture
X ₁	5.494	4.98
X ₂	6.744	5.48
X ₃	5.974	5.66
X ₁ X ₂	-2.925	0.98
X ₁ X ₃	-3.985	-6.44
X ₂ X ₃	0.515	-1.64
X ₁ X ₂ X ₃	108.032	121.53
R-Sq (adj.)	98.10%	86.62%

X₁ = Plantain flour (PF); X₂ = sweet potato flour (SPF); X₃ = malted sorghum flour (MSF).

$$y_1 = 5.494x_1 + 6.744x_2 + 5.974x_3 - 2.925x_1x_2 - 3.985x_1x_3 + 0.515x_2x_3 + 108.032x_1x_2x_3 \quad (3)$$

The positive (+) sign in the equation means that the response variable (y_1) increased with increase of the variable bearing that positive coefficient whether it is linear, binary or ternary combinations while the negative (-) sign means that the response variable decreased with increase of the variable bearing the negative coefficient thereby producing antagonistic affect. From the equation, it was observed that increasing the linear, binary and ternary combinations where sweet potato flour was incorporated increased the taste of the biscuit, but the binary combination of plantain and malted sorghum decreased the taste of the biscuit. Sweet potato flour

exhibited the highest effect in increasing the taste of the biscuit than plantain or malted sorghum flour having the highest positive coefficient than the other two flours. The combination of plantain and malted sorghum flour resulted in decrease in taste since the coefficient is negative. The blend of plantain, sweet potato and malted sorghum produced biscuit whose taste and the texture were liked slightly. The $R^2_{adj.}$ for the taste was 98.10%. This high value suggests that the effect of the independent variables contributed 98.10% of the observed changes in the taste while the remaining 1.90% changes in the taste was caused by other variables not factored in the experiment. Similarly, it was an indication that the model is adequate for predicting the taste of the biscuit under these experimental conditions. Biscuit produced with 100% wheat flour rated highest in terms of taste preference. Its taste was like very much. The biscuit produced with flour ratio: PF = 33.333, SPF = 33.333 and MSF = 33.333 was liked slightly and ranked next to 100% wheat flour biscuit in terms of taste. Also, there was no significant difference ($p < 0.05$) between its taste and the taste of biscuit produced with 100% sweet potato flour which was also like slightly. The regression model for predicting the texture of the biscuit samples was shown in Equation (4) below:

$$y_2 = 4.98 x_1 + 5.48 x_2 + 5.66 x_3 + 0.98 x_1x_2 - 6.44 x_1x_3 - 1.64 x_2x_3 + 121.53 x_1x_2x_3 \quad (4)$$

From the regression equation, linear, binary and ternary combinations of the three flour components had influence on the texture of the biscuits. Combination of plantain and sweet potato flours increased the texture of the biscuit whereas combination of plantain and malted sorghum or sweet potato and malted sorghum had antagonistic or

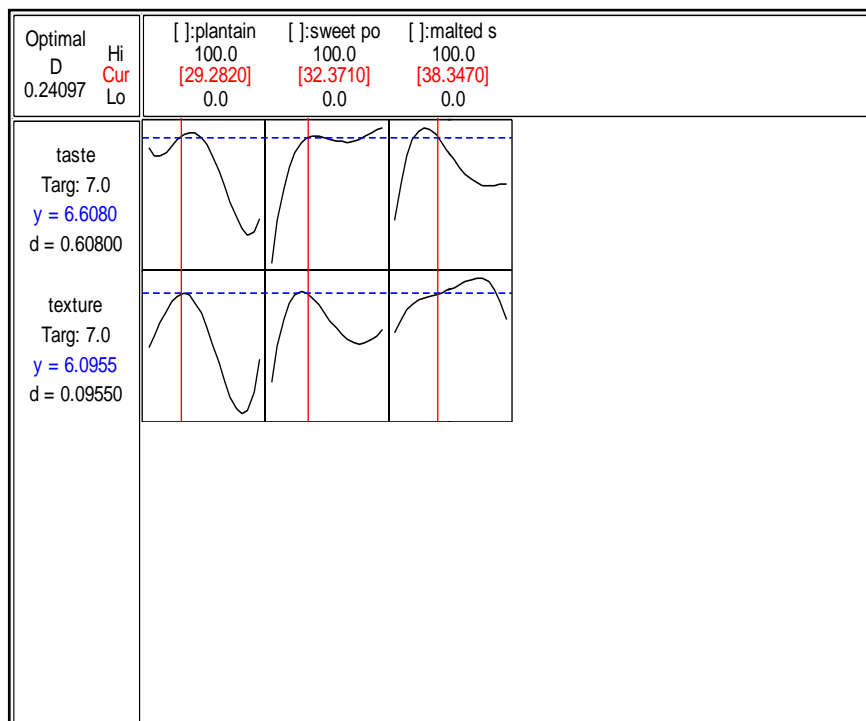


Figure 1. Optimization plot for targeted taste and texture of the biscuit.

negative effect on the texture of the biscuit. However, the combination of the three flour components positively influenced the texture of the biscuits. The R^2_{adj} was 86.62%, suggesting that the model was adequate in predicting the texture of the biscuits up to 86.62%, while 3.38% of the changes in the texture of the biscuits were caused by extraneous variables not considered in the experiment. Biscuit produced from 100% wheat flour exhibited the highest textural quality when compared with other samples. Its texture was like moderately, while the texture of the biscuit sample produced with equal proportion of the three flour blend was like slightly, and ranked next to 100% wheat flour biscuit in texture. Significant differences in texture existed among samples ($p < 0.05$).

Figure 1 presents the graphical optimization of the taste and texture of the biscuit. The optimum taste and texture for the biscuit produced from the three flour blend were targeted to be like moderately. The current blend, the one written in red ink, PF = 29.2820, SPF = 32.3710, MSF = 38.3470 was arrived at as the closest ratio that could be mixed and used to produce biscuit that would hit the targeted sensory score of like moderately (7) for both the taste and texture.

Conclusion

MRSM was successfully used to identify the optimum combination of plantain, sweet potato and malted

sorghum flour for biscuit production. The final goal was to obtain a novel biscuit produced from blend of gluten-free flours with an acceptable taste and texture. The biscuit produced from the blend of PF (29.2820), SPF (32.3710) and MSF (38.3470) will exhibit the taste and texture that would be liked slightly if the blend is used in producing biscuit as evidenced from the graphical optimization. The modeling of experimental data generated useful equations for use in predicting the taste and texture of the biscuit under these three different flour combinations.

The terms selected to fit the regression model was based on significant model ($p < 0.05$) and high R^2_{adj} . (Cornell, 1986; Opkala and Okoli, 2012).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Evolution of biochemical and physical parameters of two fresh-cut fruits over storage at 4°C

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Biochemical and physical changes of two types of fresh-cut fruits packaged in plastic containers and stocked at 4°C were studied over a period of six days. The first type of container was film-lidded and contained orange juice, pomelo, kiwifruit and orange slices. The second type was plastic-lidded and contained orange juice, apple, kiwifruit and orange slices. Color, vitamin C and carbohydrates changes were investigated. In film-lidded containers, the results showed significant variations of brightness in kiwifruit and pomelo. Carbohydrates decreased in orange juice in plastic-lidded containers. Vitamin C content did not significantly change. These results suggest that carbohydrates oxidation could be prevented by vitamin C and low oxygen observed in containers atmosphere which avoids enzymatic browning in most fruits. Overall, these results indicate a relative good stability of nutrients and well preserved organoleptic qualities of these fresh-cut fruits during the storage.

Key words: Fresh-cut fruits, orange juice, carbohydrates, color, vitamin C.

INTRODUCTION

Fresh-cut fruits and vegetables uses fresh, uncooked, peeled, carved, without preservative and ready to eat fruits and vegetables (Watada and Qi, 1999). A minimal processing of the raw fruits and vegetables is needed to keep the freshness of these products as well as supply them in a convenient form that allows preserving their nutritional quality (Soliva-Fortuny et al., 2002). Processing of fresh-cut fruits and vegetables includes peeling, carving and other mechanical actions and can

cause loss of physical and biochemical parameters such as color, texture, aroma and nutrients (Watada et al., 1990). Nevertheless, because of their freshness and high content of sugars, organic acids, vitamins and minerals, fresh-cut fruits and vegetables are considered as being more nutritious than canned and frozen foods (Klein, 1987). Fresh-cut fruits undergo a wounding stress in the cut tissues as a consequence of mechanical injury, leading to an increase in their respiration rate (Watada et

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al., 1996). Respiration oxidizes molecules such as carbohydrates and organic acids. Moreover, the destruction of fruit cellular compartments causes the oxidation of phenol compounds by polyphenol oxidase that leads to enzymatic browning. To extend their shelf life, defined as the time required for nutritional and physical parameters loss to an acceptable level, fresh-cut fruits and vegetables are generally packaged in film bags or containers which create a modified atmosphere within the package. Low atmosphere in oxygen and high carbon dioxide are used to extend the shelf life of these products, by reducing respiration and ethylene production (Yang and Hoffman, 1984). Because oxygen is needed for enzymatic browning reactions, atmosphere with low oxygen and high carbon dioxide levels can contribute to reduction of browning in fresh-cut fruits and vegetables. Further studies showed that atmosphere containing high carbon dioxide levels within the package could inhibit the biosynthesis of phenolic compounds (Ke and Saltveit, 1989).

Physiological and biochemical mechanisms, such as enzymatic browning that leads to appearance change and nutrients loss, generally limits fresh-cut fruits and vegetables nutritional quality and shelf life (Toivonen and Brummel, 2008). With the aim to study changes of appearance and nutrients contents of two kinds of fresh-cut fruits salads, we measured the color, pH, ascorbic acid (vitamin C) and carbohydrates content over a storage period of six days at 4°C.

MATERIALS AND METHODS

Fresh-cut fruits salads samples

Two types of fruit salads were packaged in plastic containers by the Civial firm (France). The first type contained slices of kiwifruit (France), orange (Navel, France), pomelo, orange juice and coated with a film lid. The second one was made of slices of kiwifruit, orange (Navel, France), apple (Granny Smith), orange juice and coated with a plastic lid. The containers weight was adjusted at 125 g by adding fresh orange juice during the preparation. They were then shipped by cool boxes at our laboratory. The marketing conditions of these products were recreated in the laboratory by stocking them at 4°C. Two experiments were carried out on each type of container. For each experiment, the slices of each type of fruits and juice from 10 film-lidded or from 8 plastic-lidded containers were mixed and analyzed to obtain daily measurements of parameters (color, pH, carbohydrates and vitamin C contents) over 6 days. In total, 120 film-lidded and 96 plastic-lidded containers were analyzed.

Determination of carbohydrates by high pressure liquid chromatography (HPLC) and pH measurements

Orange and pomelo slices were grinded. Kiwifruit and apple slices were first boiled in ethanol bath. After alcohol evaporation under vacuum at 37°C, kiwi and apple extracts were dissolved in water (20 and 50 ml for kiwifruit and apple, respectively) to obtain water-soluble extracts. The grinding of orange and pomelo, orange juice and water-soluble extracts of apple and kiwifruit were centrifuged at 10.000 rpm for 30 min. The supernatants were then filtrated

through cellulose (Spartan 30/B, Schleicher and Schuell, 0.45 µm filter, 30 mm diameter). The filtrates (20 µL) were analyzed by HPLC. The HPLC system consisted of a LC-6A pump and a C-R5A Chromatopac data processor (Shimadzu, Japan) equipped with ions exchange column (temperature, 35°C) and a differential refractometer (Beckman 155, USA) that allowed detection of carbohydrates (sucrose, glucose and fructose). The column was eluted with sulfuric acid (8.10^{-2} N) with a flow rate of 0.4 ml.min⁻¹. The results were expressed in mg.100 g⁻¹ of fruit fresh weight. The pH was measured on orange juice and fresh-cut fruits supernatants obtained as described above, using a pH meter (Schott, Germany).

Color analysis

Fruit slices and juice color was measured with a Minolta colorimeter CR200 (Minolta Corp., USA) using Hunter L^* , a^* , b^* scale (Hunter, 1958), where L^* is the brightness index; and the chromatic components a^* and b^* are the red-greenness and the blue-yellowness, respectively. The fruit slices were grinded and the color values (L^* and a^*) were immediately measured to limit modifications due to enzymatic browning.

Vitamin C (ascorbic acid) assay

Ascorbic acid concentration was determined on orange juice and fresh-cut fruits supernatants, obtained as described in carbohydrates assay section, by spectrophotometry at 525 nm as previously described (Delaporte and Macheix, 1968) using an UV-Vis spectrophotometer (Uvikon, Germany). The reaction is based on the reduction of dichlorophenol indophenol (DCPIP) by vitamin C in acid medium, in the presence of phenolphthalein. The results of vitamin C content were expressed in mg.100g⁻¹ of fruit fresh weight.

Statistical analysis

The data show the daily values of each parameter (pH, vitamin C, sucrose, glucose, fructose and color) studied per experiment in fresh-cut fruits and orange juice, as described above. Differences in daily values were compared between the both film-lidded or plastic-lidded by using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA), and subjected to ANOVA variance procedure. The Fischer least significant difference method was used to determine difference among means. A p value < 0.05 was considered statistically significant.

RESULTS

pH analysis

Overall, the pH fluctuations were measured between 3 and 3.5. Significant variations were found in slices of kiwifruit ($p < 0.01$), orange ($p < 0.05$) packaged in film-coated containers and in slices of apple ($p < 0.05$) packaged in plastic-coated containers. In contrast, the pH did not significantly change in pomelo and orange juice whichever the type of container (Figure 1).

Vitamin C (ascorbic acid)

Vitamin C was determined by spectrophotometry at 525

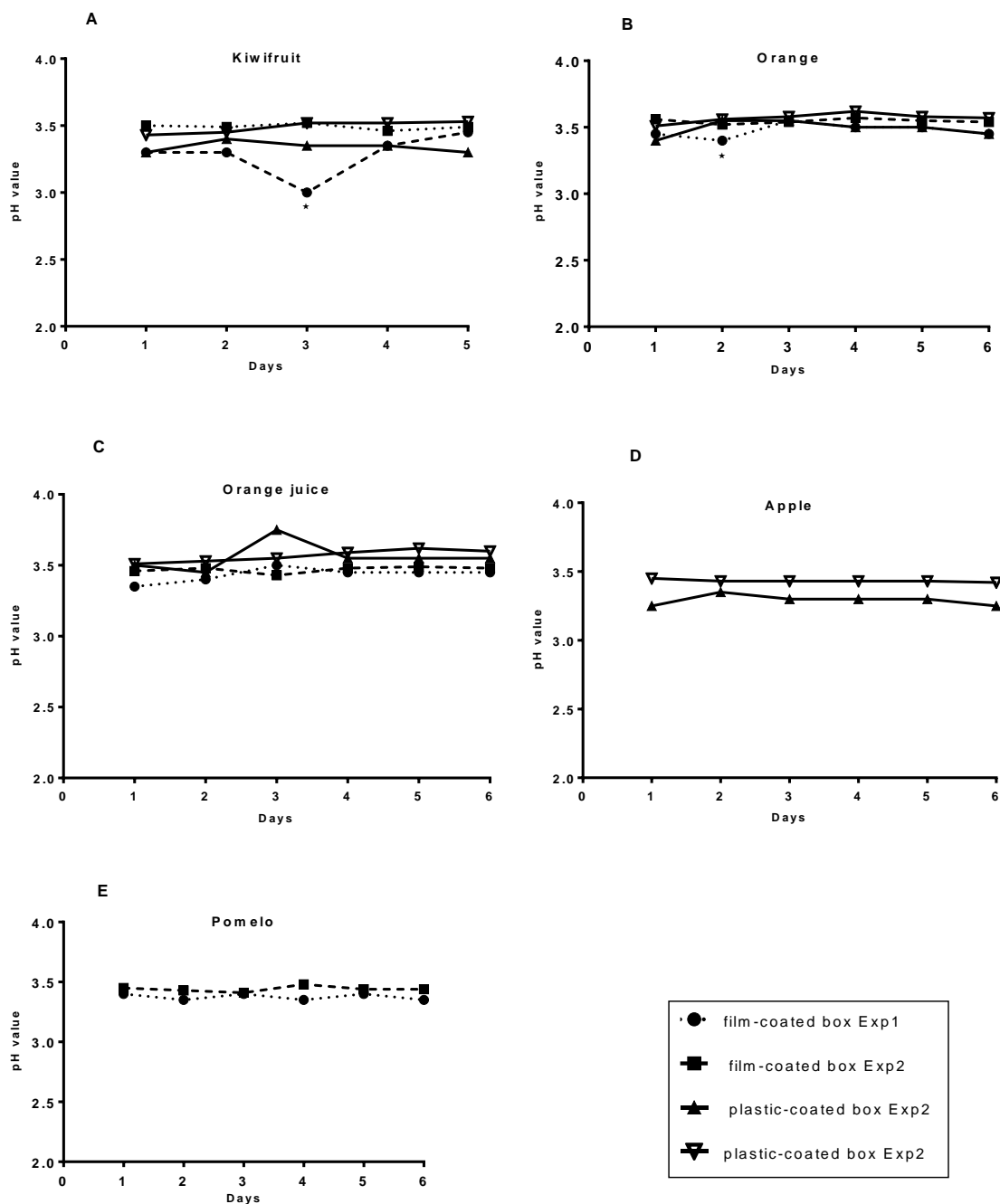


Figure 1. pH time variations measured in fresh-cut fruits and orange juice during storage.

nm and the content was expressed in $\text{mg}\cdot 100\text{ g}^{-1}$ of fruit fresh weight. The results showed a slight but not significant loss of vitamin C in all the fresh-cut fruits and orange juice in both film-coated and plastic-coated containers (Figure 2).

Carbohydrates

The carbohydrates (sucrose, glucose and fructose) were

obtained by HPLC and expressed in $\text{mg}\cdot 100\text{ g}^{-1}$ fw. (Figures 3, 4 and 5, respectively). The results showed a significant decrease ($p < 0.01$) of sucrose, glucose and fructose levels in orange juice packaged in plastic-coated containers from days 3 to 6 (Figures 3C, 4C and 5C, respectively); whereas the changes were not significant in orange juice packaged in film-coated containers. In contrast, carbohydrates content did not significantly vary in fresh-cut fruits in both film-coated and plastic-coated containers.

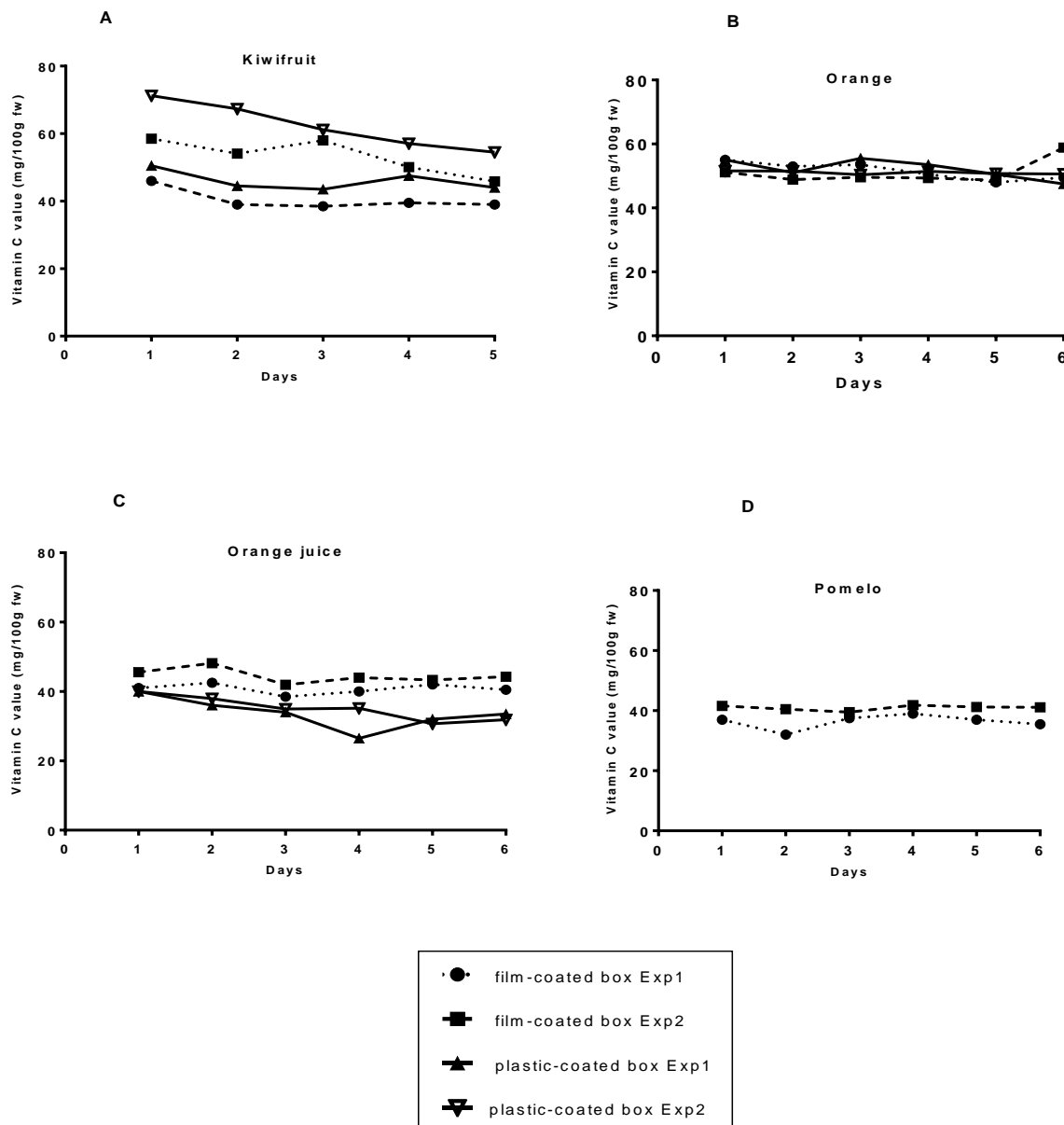


Figure 2. Vitamin C time variations in fresh-cut fruits and orange juice during storage.

Color analysis

Color measurements were performed using a colorimeter, based on L^* , a^* , and b^* color parameters. Except in film-coated containers (Figure 6A and D), where the brightness value (L^*) of kiwifruit and pomelo significantly changed ($p < 0.05$), the chromatic component value (a^*) and brightness value (L^*) did not significantly change in the other fresh-cut fruits slices and orange juice whichever the type of container (Figures 6 and 7).

DISCUSSION

In film-lidded containers, color variation was observed in

the brightness (L^*) in kiwifruit and pomelo slices ($p < 0.05$) whereas no change of color was observed in plastic-lidded containers. Color variation is due to enzymatic browning caused by the oxidation of phenolic compounds due to the effect of polyphenol oxidase. To avoid oxidation in these products and to extend their shelf life, low atmosphere in oxygen and high carbon dioxide are used to reduce respiration, which leads consequently to ethylene production (Yang and Hoffman, 1984). The color change in kiwifruit and pomelo observed in film-lidded containers could be suggested as a consequence of exposure of these fruits to atmospheric oxygen further to the breaking of the film lid over the transportation or storage. Such a hypothesis can be supported by our

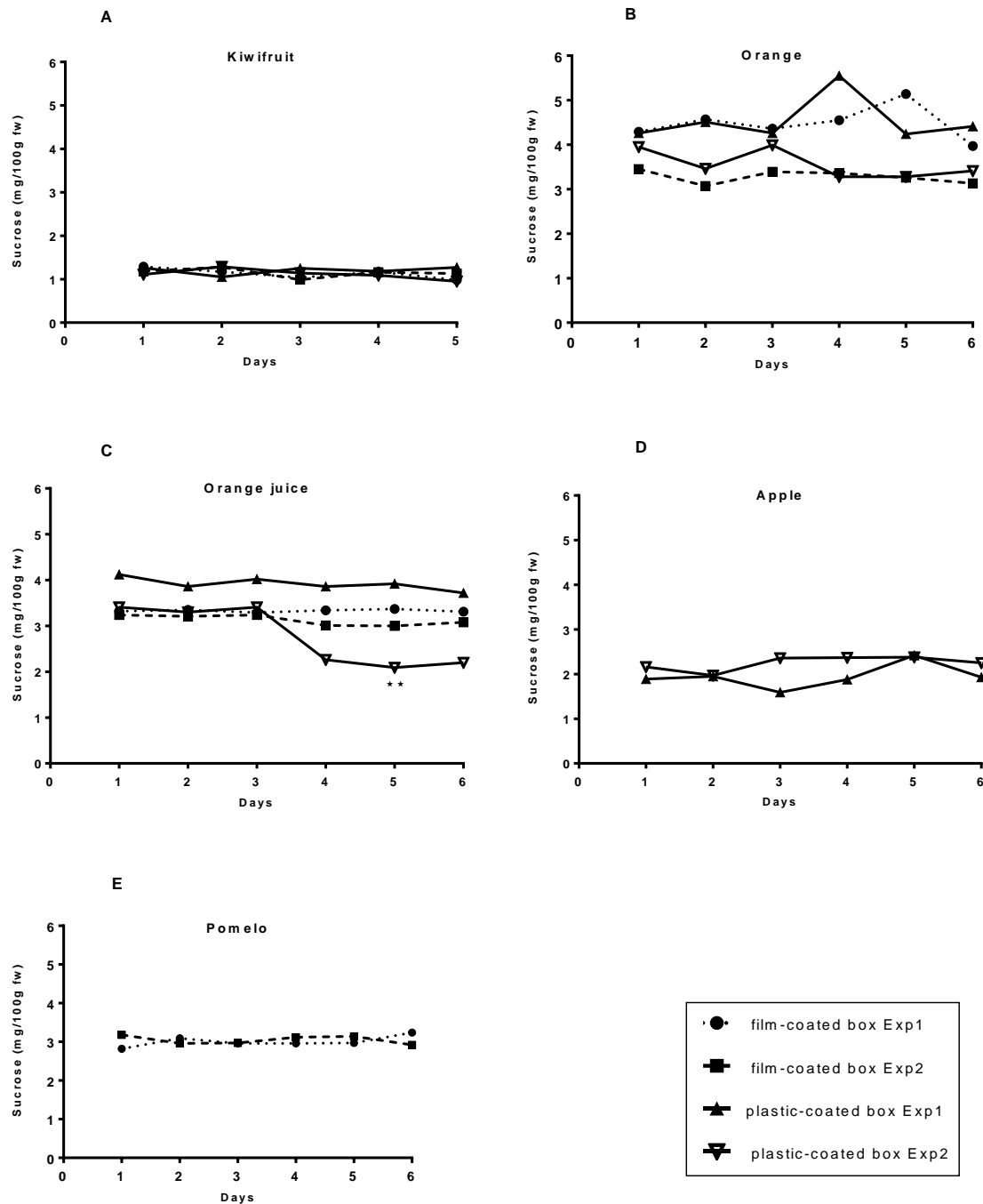


Figure 3. Sucrose time variation in fresh-cut fruits and orange juice during storage.

results obtained in plastic-lidded containers in which fruits color did not change. A low oxygen concentration in combination with moderate carbon dioxide rate is used to maintain the visual appearance of fresh-cut fruits (Agar et al., 1999). In fresh-cut apple such as Fuji apple variety, such an atmosphere combination cannot prevent enzymatic browning because of their high phenols content (Rojas-Graü et al., 2007, 2008). In contrast, in

Granny Smith apple variety, the phenolic compounds have been reported to decrease during the development period and during a cold storage (Pérez-Illzarbe et al., 1997). Our results agree with these previous studies since a slight but not significant color variation was observed in Granny Smith apple slices used in our study.

Ascorbic acid content did not significantly change in all fruit slices and orange juice in all the types of packaging.

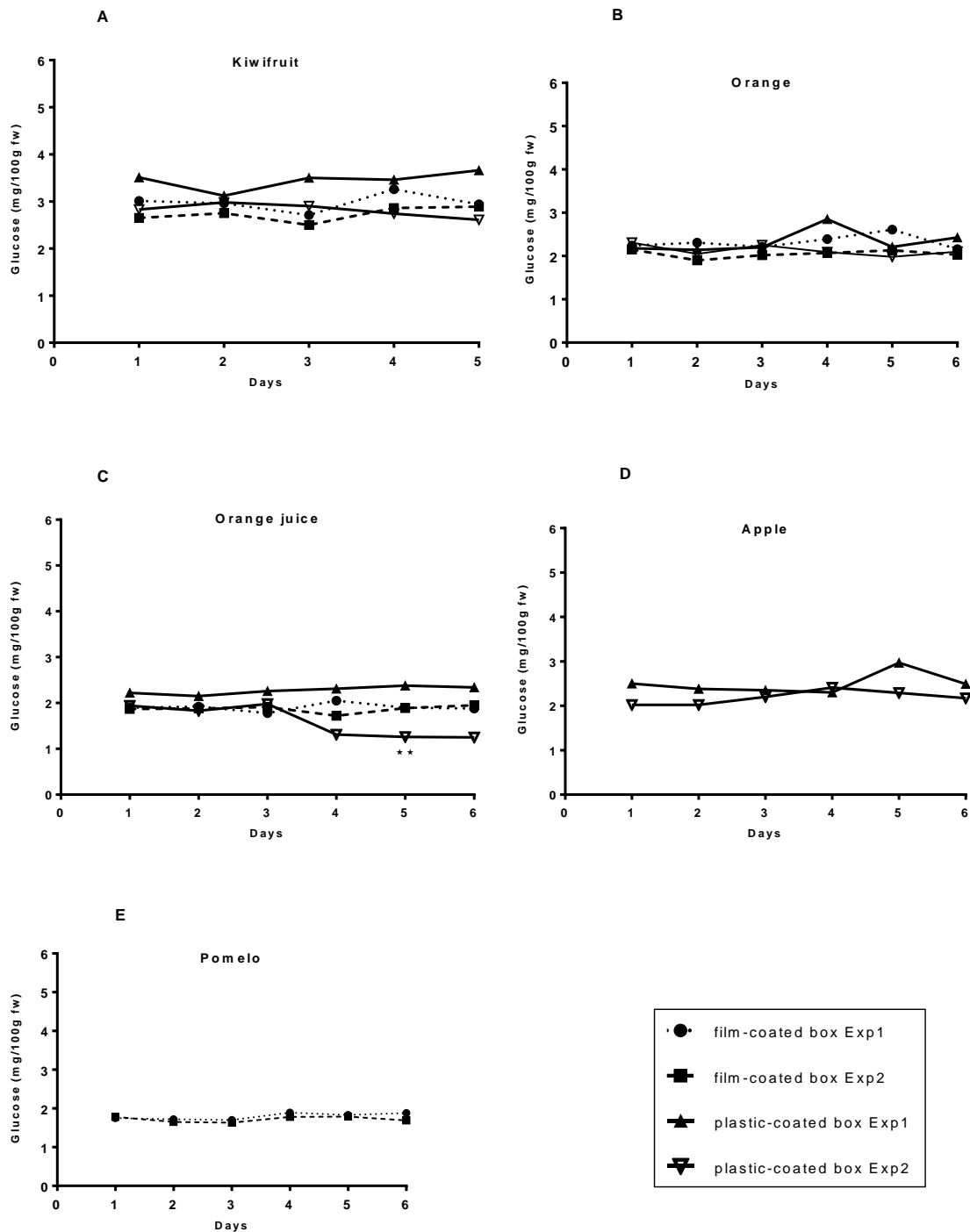


Figure 4. Glucose time variation in fresh-cut fruits and orange juice during storage.

These results agree with a previous study reported by Rivera-Lopez et al. (2005) showing that ascorbic acid content was significantly affected by temperature and storage period. Indeed, these authors observed that vitamin C content did not change in fresh-cut papaya slices stored at 5°C over 6 days but decreased at 10 and 20°C or after 18 days of storage at 5°C. We suggest that

such a slight variation of ascorbic acid could be due to its renewal by a reducing system, as the glutathione, produced in the tissues is still alive over a short storage period. Other studies reported strong decrease of ascorbic acid levels in whole kiwifruit at the end of a long time of a cool storage (Tavarini et al., 2008) and a rapid decrease in fresh-cut fruits when compared with whole

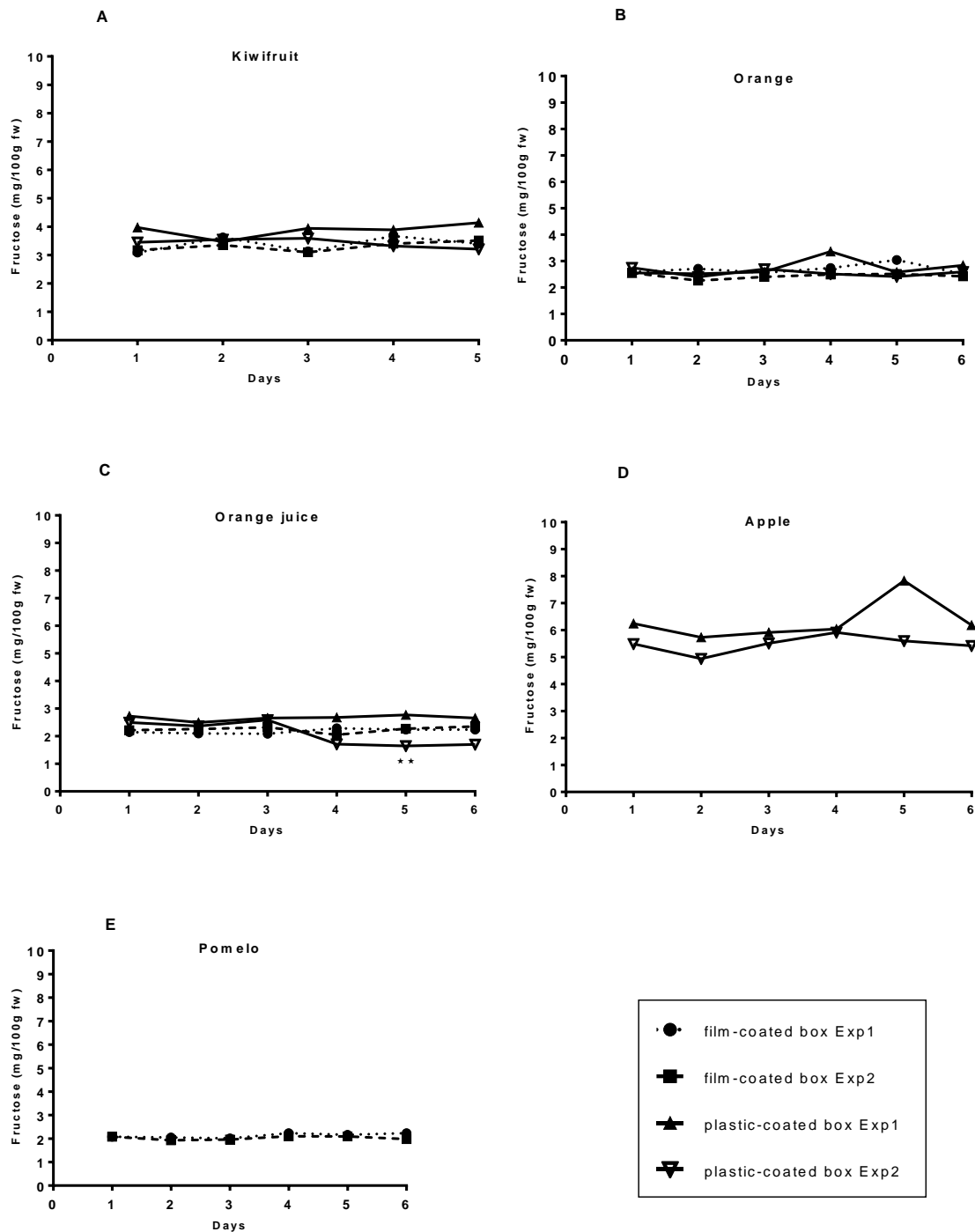


Figure 5. Fructose time variation in fresh-cut fruits and orange juice during storage.

fruits (Allong et al., 2000). In fresh-cut kiwifruit slices, a loss of 12% in vitamin C content was reported (Gil et al., 2006).

Loss of vitamin C content of about 22% has been also described in fresh-cut navel orange slices (Rocha et al., 1995) and in orange juice (Klimczak et al., 2007; Lee and Coates, 1999) over a long storage period. Such

decreases of ascorbic acid could be caused by its use to avoid enzymatic browning by reducing quinones to phenolic compounds before they undergo reactions to produce pigments as previously described (Iyengar and McEil, 1992).

The pH significantly changed in kiwifruit and orange slices in the film-lidded containers ($p < 0.01$ and $p < 0.05$,

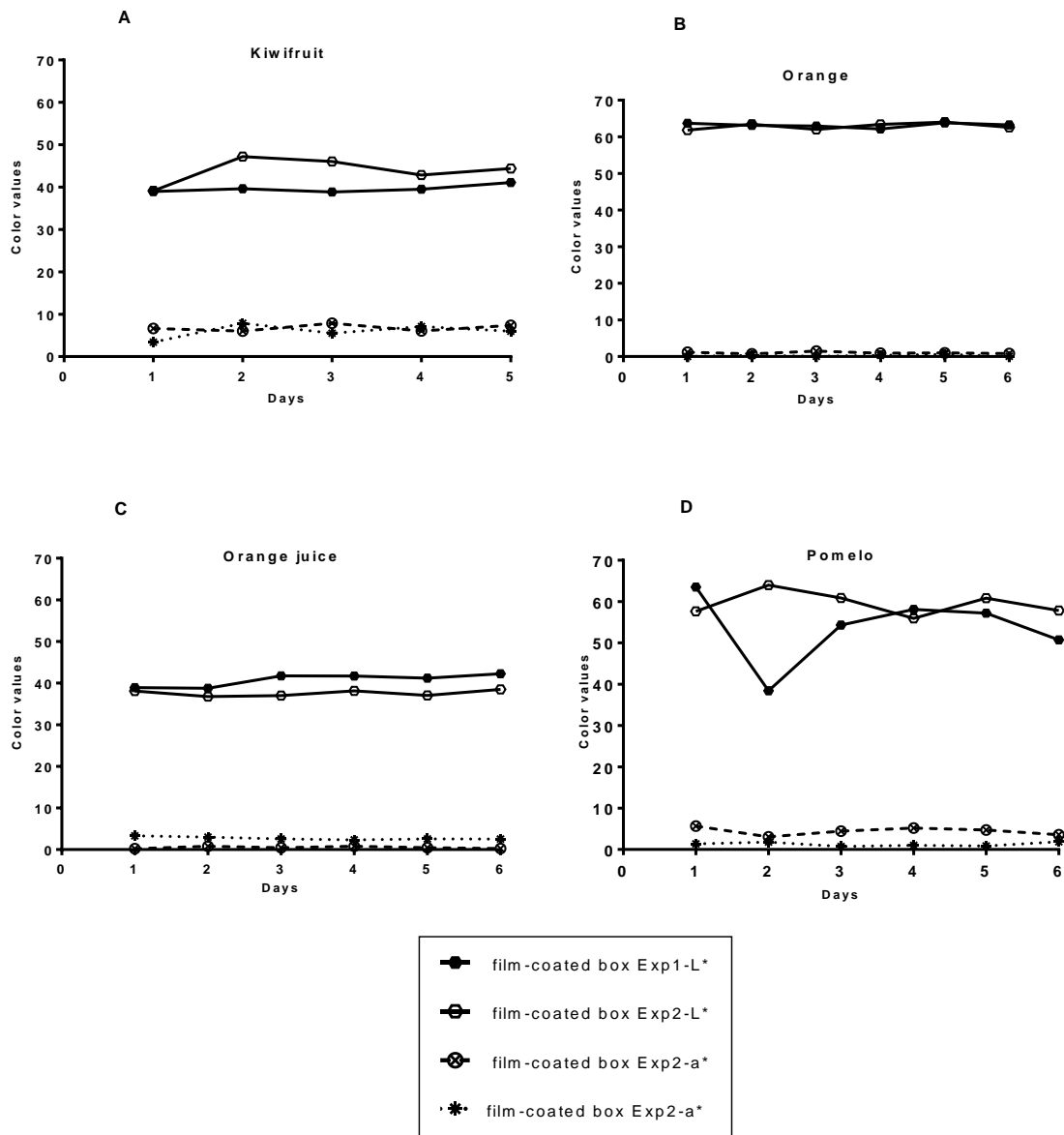


Figure 6. Color time variation (L^* and a^* values) of fresh-cut fruits in film-coated containers.

respectively) and apple slices in the plastic-lidded containers ($p < 0.05$). In contrast, orange juice did not show any pH variation during the storage in all the types of package. Previous studies reported color changes in fresh-cut fruits induced by acidic pH of additives (Gomes et al., 2012, 2014). In our study, we used orange juice as a natural additive. The pH of orange juice varied between 3 and 3.5, and did not lead to fresh-cut fruits browning, as observed in plastic-lidded containers.

The carbohydrates content did not significantly change in the fresh-cut fruits slices, except in orange juice packaged in plastic-lidded containers where a significant loss ($p < 0.01$) was found in sucrose, glucose and fructose content. The changes in glucose and fructose content

could be associated with sucrose polymer degradation, as shown in previous studies (Soliva-Fortuny et al., 2004). In fresh-cut fruits, carbohydrates generally undergo oxidation as a consequence of mechanical injury of their tissues (Watada et al., 1996). In our study, the slight and not significant variations in sucrose, glucose and fructose levels observed in all fruit slices suggest low oxidation of carbohydrates during the storage of these fruits probably due to low oxygen and low temperature of storage which prevent or reduce enzymes activity. Such hypothesis is supported by respiration measurements that showed oxygen decrease followed by an increase production of carbon dioxide and ethylene rates (data not shown).

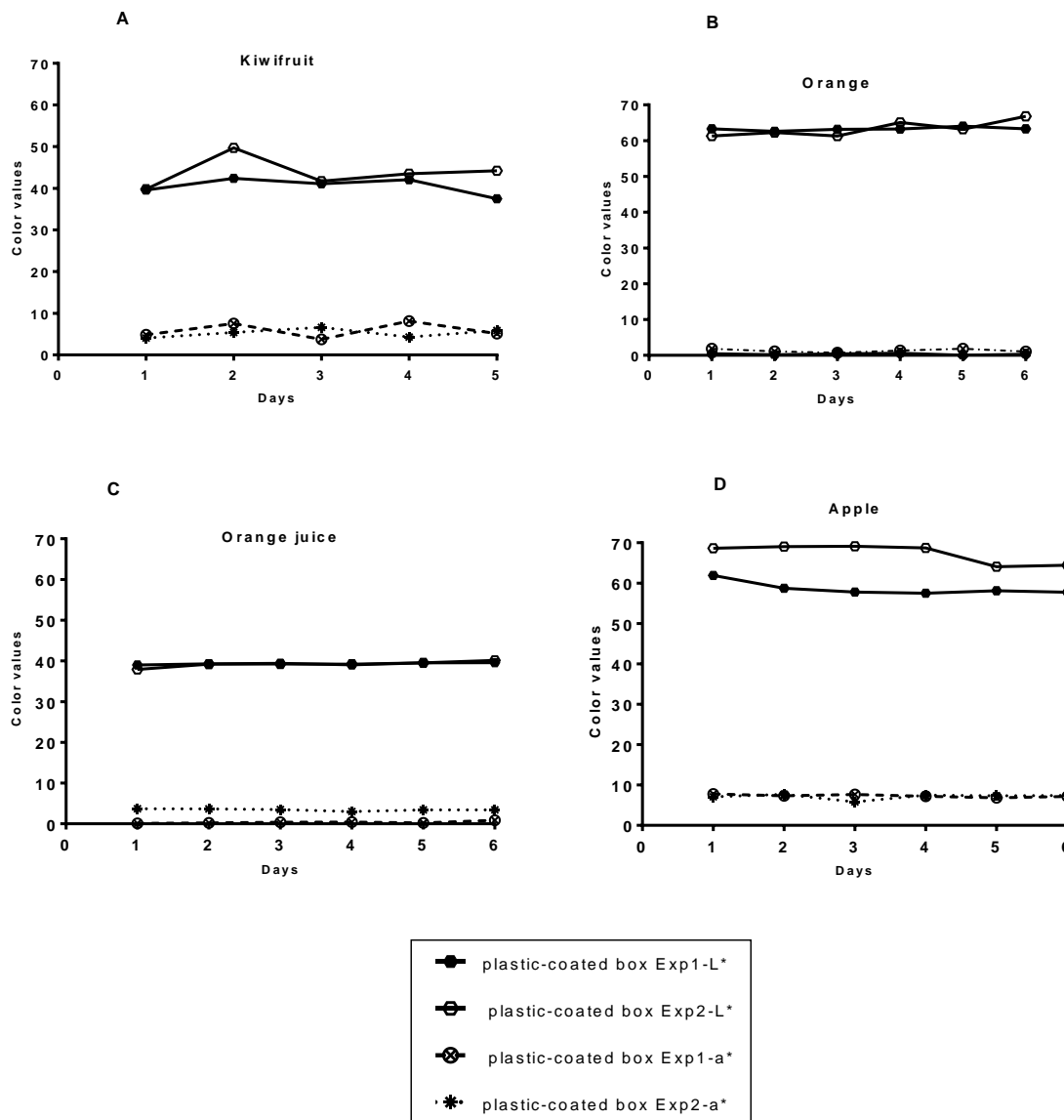


Figure 7. Color time variation (L* and a* values) of fresh-cut fruits in plastic-coated containers.

The obtained results indicate overall, a relative good stability of nutrients and well preserved organoleptic qualities of that fresh-cut fruits over the storage period.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Enzyme profiles of potential starter cultures for the fermentation of baobab seeds

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The extracellular enzymatic activity of ten (10) strains of predominant bacteria involved in Maari process has been investigated using the APIZYM (BioMérieux, France) commercial system, with the objective of determining the differences in the enzymatic profiles of the various species. Variable enzymatic activity was recorded showing the specific activity of each species during the fermentation of baobab seeds. Almost all isolates possessed phosphatase activity. All aerobic mesophilic bacteria (AMB) lacked trypsin. No lactic acid bacterium (LAB) was able to produce α -galactosidase. Naphthol-AS-BI-phosphohydrolase was produced by all isolates. The enzymatic pattern of these potential starter cultures can be used for predicting their suitability for baobab seeds fermentation and for monitoring their stability.

Key words: Enzymatic activity, baobab seeds, fermentation, starter cultures.

INTRODUCTION

Spontaneous fermentation of baobab seeds is a processing technique applied in Burkina Faso and in other countries in West Africa including Benin, Mali and Nigeria for the production of Maari, an indigenous condiment (Parkouda et al., 2010). The spontaneous nature of the process results in varying product quality which invariably depends heavily on the producer skill and processing conditions. Several microorganisms are known to be involved in the fermentation of baobab seeds. The dominance of *Bacillus* species, especially *Bacillus subtilis* is reported by Parkouda et al. (2010). Other microorganisms including *Enterococcus* sp. and *Pediococcus* sp. have also been reported to be

associated with the fermentation of baobab seeds. In our previous work, we reported that during the processing of the baobab seeds, carbohydrates and lipids content decrease but not protein content (Parkouda, 2010). These changes are likely to be explained by the cooking and loss in the cooking water; and the metabolic activities of the microorganisms involved in the fermentation (Kpikpi et al., 2009; Yagoub et al., 2004). Indeed, the raw materials used to produce the alkaline condiments are substrates in which *Bacillus* spp. grow and produce metabolites and enzymes recognized to have beneficial effects on health (Achi, 2005; Dahal et al., 2005; Wang and Fung, 1996). Mbajunwa et al. (1998) and Njoku et al.

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(1990) demonstrated the ability of *B. subtilis* to soften the tissue of African oil beans leading to the desired texture of *ugba* and suggested that the strain may possess pectic and proteolytic enzymes that readily hydrolyzed the pectin and protein components of the beans. The APIZYM system (bioMérieux, France) is a semi-quantitative micro-method for analysing enzymatic activities of microorganisms, which has been used to characterize many microorganisms (Gruner et al., 1992). For the purposes of quality control and standardization of Maari, there is the need to exploit starter cultures in Maari production. One of the technological properties that could be used to select microorganisms to be used as starter cultures, is the enzymatic activity. The objective of this study was then to assess the enzymatic profiles of the potential starter cultures for Maari production. The information would contribute to the development of starter cultures with predictable characteristics, which could be used in small-scale and commercial production of Maari for improved and consistent quality.

MATERIALS AND METHODS

Ten microorganisms isolated from fermented baobab seeds and previously characterized (Parkouda et al., 2010), were assessed of their extracellular enzymatic activities using APIZYM system kits (APIZYM BioMérieux, France) following the manufacturer's instructions. The microorganisms had been previously identified as *Bacillus coagulans* BL174, *Bacillus licheniformis* B6, *Bacillus subtilis* B3, *B. subtilis* B122, *B. subtilis* B222, *Staphylococcus sciuri* AB41, *Enterococcus avium* LB70, *Pediococcus acidilactici* L74, *Enterococcus casseliflavus* L142 and *Enterococcus faecium* L9. This APIZYM system consisted of a strip composed of 20 micro-cupules, which contain 19 substrates and one control, to detect 19 different enzymatic activities: Alkaline phosphatase, Esterase C4, Esterase lipase C8, Lipase C14, Leucine arylamidase, Valine arylamidase, Cystine arylamidase, Trypsin, α -Chemotrypsin, Acid phosphatase, Naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase.

The aerobic mesophilic bacteria consisting of *B. coagulans* BL174, *B. licheniformis* B6, *B. subtilis* B3, *B. subtilis* B122, *B. subtilis* B222, and *S. sciuri* AB41, were grown in Brain Heart Infusion broth (Fluka, Steinheim, Germany) while the lactic acid bacteria, made up of *E. avium* LB70, *P. acidilactici* L74, *E. casseliflavus* L142 and *E. faecium* L9 were propagated in MRS broth (Merck, Darmstadt, Germany). Cells were harvested by centrifugation at 5000 \times g for 10 min and washed 3 times in 5 ml of sterile saline solution containing 8.5 g l^{-1} NaCl and 1.5 g l^{-1} Bactopeptone (DIFCO), pH 7. Following the last washing step, an initial suspension of each culture was made and adjusted to McFarland turbidity standard (5 to 6) using API suspension medium (API, Biomerieux, France). Aliquots of 65 μL of each culture suspension were added to one of the 20 reaction cupules in the APIZYM strip. The strips were incubated at 37°C overnight after which the reactions were terminated by addition of one drop each of the APIZYM reagents A and B according to the manufacturer's instructions. The experiments were performed in duplicate. Enzymatic activity was recorded as positive if a score of 1 or greater was obtained after assessment of the colour intensity using the manufacturer's colour chart.

RESULTS AND DISCUSSION

The APIZYM profile of the representative strains previously isolated from Maari is shown in Table 1. Generally, the enzymatic activities varied significantly among the strains and species. *B. coagulans* seemed to produce 18 of the tested enzymes, as against 16, 7 and 6 for *B. subtilis* B222, B122 and B3, respectively; and 8 and 10 for *B. licheniformis* and *S. sciuri*. *E. faecium* L9 produced 18 from the 19 tested enzymes. *E. casseliflavus* L142, *E. avium* LB70 and *P. acidilactici* L74, produced comparatively few. Alkaline phosphatase, which cleaves orthophosphoric monoesters to orthophosphates and alcohols in alkaline conditions, was present in all tested isolates except for *P. acidilactici*. The isolates (exception for *B. subtilis* B3) produced both alkaline and acid phosphatases, two enzymes that have similar functions but have pH optima of 8.0 to 9.0 and 5.0, respectively, thus indicating an extensive phosphatase activity by these isolates (Waltman II et al., 1982). All the *Bacillus*, *Enterococcus* and *Staphylococcus* strains tested produced esterase (C4) and esterase lipase (C8), while *P. acidilactici* displayed negative results for these activities. Lipase (C14) activity was only observed for *E. faecium* L9, *B. coagulans* BL174, *B. subtilis* B222 and *S. sciuri* AB41. In a previous study, Antai and Ibrahim (1986) attributed oil degradation in African locust beans during fermentation to enzymes produced by *Staphylococcus* or *Leuconostoc* species.

Variability between species and within *B. subtilis* species in lipase and esterase activity was also previously reported (Ouoba et al., 2003b). Lipase activity has been identified as a property that may not be desirable during the production since it may result in rapid rancidity of the product (Wagenknecht et al., 1961). However, adequate lipolytic activity could probably be a good characteristic, because liberation of free fatty acids was required for the development of desired aroma characteristics (Beaumont, 2002; Odufa and Adesomoju, 1986).

All *Bacillus* isolates produced α -glucosidase, which hydrolyzes α (1-6) linkages at branch points of dextrin. Production of β -glucosidase, which hydrolyzes the β (1-4) linkages of glucosides such as cellulose or plant starch, was species and strains dependent. These results are in line with previous studies which reported that *Bacillus* spp. are producers of amylase, galactanase, galactosidase, glucosidase and fructofuranosidase, enzymes involved in degradation of carbohydrates during alkaline fermentation (Aderibigbe et al., 1990; Kiers et al., 2000; Omafuvbe et al., 2000; Sarkar et al., 1997). None of the *Bacillus* strains produced trypsin; trypsin activity was observed for *E. casseliflavus* L142 and *E. faecium* strains. No tested lactic acid bacteria was able to produce α -galactosidase.

Table 1. Enzyme activity of *Bacillus*, *Enterococcus*, *Staphylococcus* and *Pediococcus* strains isolated from *Maari* (potentially starter culture) determined with the APIZYM test.

Parameter	<i>Bacillus coagulans</i> BL174	<i>Bacillus licheniformis</i> B6	<i>Bacillus subtilis</i> B3	<i>Bacillus subtilis</i> B122	<i>Bacillus subtilis</i> B222	<i>Staphylococcus sciuri</i> AB41	<i>Enterococcus avium</i> LB70	<i>Pediococcus acidilactici</i> L74	<i>Enterococcus casseliflavus</i> L142	<i>Enterococcus faecium</i> L9
Esterase (C4)	+	+	+	+	+	+	+	-	+	+
Esterase Lipase (C8)	+	+	+	+	+	+	+	-	+	+
Lipase (C14)	+	-	-	-	+	+	-	-	-	+
Leucinearylamidase	+	+	+	-	+	+	-	+	+	+
Valinearylamidase	+	-	-	-	+	-	-	+	-	+
Cystinearylamidase	+	-	-	+	+	-	-	+	+	+
Trypsin	-	-	-	-	-	-	-	-	+	+
α-Chymotrypsin	+	-	-	-	+	-	-	-	+	+
Alkaline phosphatase	+	+	+	+	+	+	+	-	+	+
Acid phosphatase	+	+	-	+	+	+	+	+	+	+
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	+	+	+	+	+	+
α-Galactosidase	+	-	-	-	+	-	-	-	-	-
β-Galactosidase	+	-	-	-	+	-	-	-	+	+
β-Glucuronidase	+	-	-	-	+	+	-	-	-	+
α-Glucosidase	+	+	+	+	+	+	-	-	-	+
β-Glucosidase	+	+	-	-	+	+	-	+	+	+
N-Acetyl- β-glucosaminidase	+	-	-	-	-	-	-	-	+	+
α-Mannosidase	+	-	-	-	-	-	-	-	-	+
α-Fucosidase	+	-	-	-	+	-	-	-	-	+

NB: '+' refers to positive reaction and indicates presence of enzyme in concentrations of >5 nmol, '-' refers to negative reaction

The need to select the most appropriate starter cultures for the production of condiment is important in order to obtain the most desirable product and achieve the much needed product consistency to aid acceptability and industrialization of the traditional fermented foods in West Africa (Sanni, 1993). Isolate showing an interesting enzymatic activity profile would be important in developing a starter culture. The present observations seem to provide the basis for selecting isolates for the development of a starter culture, which could improve product

quality and consistency. *Bacillus* species, especially *B. subtilis*, were found to dominate the alkaline fermentation of seeds for food condiments production and to show interesting technological properties as reviewed by Parkouda et al. (2009). The possible role of *Enterococcus* strains that were repeatedly isolated during Kinema production, a similar condiment produced by fermentation of soybeans, was also investigated (Sarkar et al., 1994).

The usefulness of the enzymatic profile to differentiate bacteria, together with its possible

interest for the bacteria ability to degrade some substrates were previously evaluated (Ouoba et al., 2003a, b, 2007; Azokpota et al., 2006; Kpikpi et al., 2009).

The observations in this study show variability in the enzymatic capabilities among the different isolates, indicating contributions of each of the isolates to the fermentation of Baobab seeds in Maari production. This suggests that, an appropriate starter culture for Maari production would be made up of a consortium of species. Based on their predominance and their ability to

produce different extracellular enzymes, *B. subtilis* B222, *S. sciuri* AB41 and *E. faecium* L9 could be selected as suitable starter cultures but their safety aspects need to be studied.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Effect of pretreatment on physicochemical quality characteristics of a dried tomato (*Lycopersicon esculentum*)

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Tomato is highly perishable and drying is a convenient method of extending its shelf life and minimizing postharvest losses. During drying, some nutrients may degrade and thus affect general quality characteristics of the dried tomato. The effect of pretreatment in enhancing drying and product quality of dried tomato was investigated in this study. Slices of tomato were treated by dipping in (a) A solution containing 0.5% sodium metabisulphite for 10 min and (b) 0.1% ascorbic acid + 0.1% citric acid solution for 10 min (1:1) and (c) distilled water for 10 min (control). Convection dehydration was carried out on tomato slices using an electric dehydrator at 55°C for 6 h. Pretreatment of tomato affected some quality attributes such as total solids, lycopene, dehydration ratio, rehydration ratio and colour. Pretreatment with sodium metabisulphite recorded the least lycopene degradation, highest dehydration ratio (19.40 ± 1.03) and also facilitated the drying of tomato better than the other treatments. All the pretreated dried tomato samples produced good visual and exhibited a desirable red colour (a* values ranging between 24.49 ± 0.44-28.34 ± 0.03) which is characteristic of dried tomato products. Pretreatment with sodium metabisulphite before convection drying can enhance the lycopene content which is a desirable quality attribute for dried tomato.

Key words: Convection drying, dried tomato, sodium metabisulphite, pretreatment, drying rate, lycopene.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most widely consumed vegetable used in the preparation of many dishes in Ghana (Tambo and Gbemu, 2010).

However because of its short shelf life, poor handling, storage and the lack of proper processing, there is considerable damage and wastage of this seasonal crop

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in Ghana and other many tropical countries where up to fifty percent (50%) of post-harvest losses is recorded for tomato (Kitinoja and Gorny, 2009). One promising method of preventing or minimizing post-harvest losses is by using drying technology to preserve tomato. Drying decreases the water content of the raw product to levels that minimizes its biochemical, chemical and micro-biological deterioration. Drying is an attractive technology because it is very simple and can easily be adopted by the farmers, with minimal capital investments.

Tomato can be dried using various methods and the quality of dehydrated tomato product depends on factors such as tomato variety, total soluble solid content (Brix) of the fresh tomato, the rate of drying, the air humidity, the size of the tomato segments, the air temperature and velocity and the efficiency of the drying system (Gowen et al., 2008; Lewicki, 2006). More sophisticated and high capital cost drying technologies such as infrared radiation heating and freeze drying can also be used to obtain dehydrated tomato products.

In determining the method for dehydration, the quality attributes of the final product form is considered. Preservation of the nutritional quality, flavor and visual characteristics significantly influences the operational parameters of the drying method. A criterion such as maximum product temperature and environmental humidity during drying affects the final product quality. Convective drying can be carried out at high temperatures for short times or at lower temperatures for longer times; the former option being usually preferred since it produces less thermal damage and consumes less energy (Velic et al., 2004). In this process, hot air may cause a series of chemical, physico-chemical, physical and biological alterations that can affect the final quality of the dehydrated product.

Lycopene is the primary natural pigment responsible for the red orange colouration in tomato and serves as a biological antioxidant (Ibitoye et al., 2009). The antioxidant activity of lycopene, the most abundant carotenoid in tomato, has been the subject of several studies on fresh tomato and tomato products. Lycopene may degrade during the drying process, reducing the characteristic red colour of tomato. During the drying process and also during storage periods, oxidative damage takes place in tomato (Zanoni et al., 1999; Toor and Savage, 2006; Sharma and Maguer, 1996; Zanoni et al., 2000). In a study by Shi and Maguer (2000), they indicated that the main causes of lycopene degradation during processing and storage are isomerization and oxidation. Pre-treatment of tomato can enhance certain drying characteristics of tomato. Results show that the pre-treatment with CaCl_2 and NaCl increased water mobility in tomato slices during drying and influenced drying kinetics and texture of the dried product (Davoodi et al., 2007).

The objective of this study was to determine the influence of pre-treatments and convection dehydration

on the physicochemical properties of dried tomato.

MATERIALS AND METHODS

Sample preparation

Fresh tomatoes (Roma variety from Mexico) were sorted and washed under running tap water. They were cut into slices of 3/16" (inch) thickness, using a Nemco 56600-3 3/16 Easy Tomato Slicer II, (301 Meuse Argonne Hicksville, OH, USA). This size was selected based on results from preliminary studies.

Pretreatments prior to dehydration process

Sliced tomatoes were divided into three batches, and randomly assigned to three treatments as follows: dipping in (a) a solution of 0.5% sodium metabisulphite for 10 min, (b) a 0.1% ascorbic acid + 0.1% citric acid solution for 10 min (1:1) and (c) distilled water for 10 min at room temperature (served as control).

Dehydration processes

Hot/convection air dehydration

The samples were placed in a hot air dehydrator (Excalibur 3926T 9 tray food dehydrator, IL, USA) and set at 55°C for six hours. The weights of the samples were recorded every hour during the drying period. After drying, the samples were cooled to room temperature and packed in zip lock bags prior to analysis.

Physicochemical analysis

Moisture content and total solids of tomato samples were determined in triplicates (AOAC, 1999). Water activity (a_w) was determined using a water activity meter (Paw kit, Model Series 3 TE, Decagon Devices, Inc., Pullman, WA, USA). Colour of the samples was determined using the chroma meter (LABSCAN XE Hunterlab, VA, USA) and reported in CIELAB colour scales. L^* value being the degree of lightness to darkness, a^* value of the degree of redness to greenness, and b^* value, is degree of yellowness to blueness. The chromameter was calibrated against a white tile ($L^*=100$). The total soluble solids (TSS) of tomato juice were measured in triplicate by a digital Refractometer (AR 200, Reichert Analytical instrument, NY, USA). pH of the tomato juice was determined by a pH meter (Symphony SB70P VWR, Radnor, PA, USA). Total solids was estimated by subtracting moisture content from 100%; Total solids = 100% - moisture content.

Dehydration rate

Twenty grams of sliced tomatoes (pretreated and control) were pre-weighed and placed in a dehydrator adjusted to 50°C. The weight of the samples was checked and recorded every hour. After drying, the samples were placed in a desiccator and packaged into high density polyethylene bags. Dehydration ratio (DR) was calculated as mass of sliced tomato before loading to the dryer to mass of dehydrated tomato at the time of removal from dryer.

$$DR = \frac{\text{Weight of sample before drying}}{\text{Weight of sample after drying}}$$

Table 1. Effect of pretreatment on quality characteristics of fresh tomato samples before drying.

Pretreated fresh tomato	pH	Brix	Moisture (%)	TS (%)	aw	Lycopene (mg/100g)	Colour		
							L*	a*	b*
TC	4.45 ± 0.03 ^a	3.87 ± 0.06 ^c	95.13 ± 0.05 ^a	4.88 ± 0.05 ^b	0.94 ± 0.01 ^a	152.01 ± 1.62 ^a	41.42 ± 0.11 ^a	28.18 ± 0.06 ^a	27.57 ± 0.27 ^a
TAC	4.18 ± 0.03 ^b	4.10 ± 0.01 ^b	94.78 ± 0.4 ^a	5.22 ± 0.40 ^a	0.94 ± 0.00 ^a	145.41 ± 1.95 ^b	41.68 ± 0.02 ^a	26.67 ± 0.02 ^a	27.50 ± 0.07 ^a
TSM	4.40 ± 0.04 ^a	4.20 ± 0.01 ^{ab}	94.9 ± 0.01 ^a	5.10 ± 0.01 ^a	0.92 ± 0.01 ^b	158.45 ± 1.08 ^a	43.00 ± 0.01 ^a	27.49 ± 0.41 ^a	28.59 ± 0.55 ^a

^{a,b}Values expressed are mean values of 3 replicates ± SD. All mean scores, bearing different superscripts in columns differ significantly (p<0.05). TSM- tomato pretreated with 0.5% sodium metabisulphite, TC- control tomato samples pretreated with water, TAC- tomato pre-treated with 0.1% ascorbic acid + 0.1% citric acid.

The rehydration test

This was conducted as recommended by McMinn and Magee (1997a) and Prabhanjan et al. (1995). Five grams sample of the dried tomato was placed in 150 ml of distilled water in a beaker. The beaker was placed on a hot plate and covered with a watch glass. The water was brought to boiling point, taking approximately 3 min, and kept for 5 min. At the end of the rehydration period, the sample was transferred to a Buchner funnel, covered with No. 4 Whatman filter paper, and the excess water removed by applying a slight vacuum. The sample was removed and weighed. The data was calculated in terms of RR as follows:

$$RR = \frac{M_{rh}}{M_{dh}}$$

Where M_{rh} is the mass of the rehydrated sample (kg) and M_{dh} the mass of the sample dried for rehydrated test (kg).

Moisture loss

Three sets of seven samples of fresh pretreated tomato slices and control were weighed into small aluminum dishes (one slice each, weighing about 5 g) and placed in a dehydrator set at 55°C. One dish was removed every hour weighed and placed in a gravity oven (VWR scientific 1350G, VWR company USA) set at 105°C for 24 h to evaluate the moisture loss over time.

Lycopene analysis

The lycopene content (mg/100g total solids) was spectro-

photometrically determined on extracts in petroleum ether in triplicate at 505 nm (Gould and Gould, 1988) using a Helios UV-Visible spectrophotometer (Helios gamma, Thermo Spectronic, Madison, USA). Determinations were done in triplicate and the averages of these triplicate measurements were used.

Scanning electron microscopy (SEM)

Dried tomato slices were cut and subsequently fixed in 2.5% (w/v) glutaraldehyde overnight. It was rinsed extensively with distilled water and dehydrated in ethanol series (30-100%) for 30 min for each sample. Dehydrated fragments were dried at critical-point and mounted onto metal studs, coated with colloidal platinum with EMS 550x sputter coater machine and viewed using a scanning electron microscope (JSM -6610LV, JEOL INC, Peabody MA).

Statistical analysis

Statistical analysis was performed using Minitab Statistical Software Version 15. Analysis of variance (ANOVA) was done to separate differences between means of treatments with Duncan's multiple range test.

RESULTS AND DISCUSSION

Physicochemical characteristics of fresh pre-treated tomato slices

pH of the fresh pre-treated tomato ranged from 4.18 ± 0.03 - 4.45 ± 0.03 (Table 1). There were

significant differences (p = 0.02) in pH within the pretreated fresh tomato. However, the pH of tomatoes that were pre-treated with sodium metabisulphite (TSM) was not significantly different than the control sample (TC). However samples treated with ascorbic acid + citric acid (TAC) showed significantly lower pH, due to the acid nature of the ascorbic and citric acids.

Total soluble solids varied from 3.87 ± 0.06 - 4.20 ± 0.01. Brix for the control, TC (3.87) was significantly lower (p = 0.03) than the pretreated (TAC and TSM) samples. Moisture ranged from 94.78 ± 0.4% - 95.13 ± 0.05% for TAC and TC, respectively. Even though there was a marginal decrease in moisture content for the pre-treated TAC and TSM samples, probably due to osmotic dehydration, the differences in the moisture content of the control and TAC and TSM samples previous dehydration were not significant (p = 0.11).

Significant differences (p= 0.01) in the total soluble solids (TSS) was observed for fresh pre-treated tomato samples. TSS for TC (4.88) was significantly lower (p= 0.025) than TAC and TC. Water activity was significantly lower (p = 0.03) for TSM (0.92±0.01) as compared to TC (0.94 ± 0.01) and TAC (0.94 ± 0.00). High water activity values indicate a short shelf life for fresh tomato samples as bacteria, moulds, and yeast can grow in water activities above 0.9 (Damodaran et al., 2008).

Table 2. Effect of pretreatment on quality characteristics of dried tomato slices.

Pretreated dried tomato	TS (%)	Moisture (%)	aw	Dehydration ratio (DR)	Rehydration ratio (RR)	Lycopene mg/100g	Colour		
							L*	a*	b*
TC	84.32 ± 0.45 ^{bc}	15.68 ± 0.45 ^{bc}	0.40 ± 0.02 ^b	18.33 ± 0.91 ^{ab}	5.10 ± 1.45 ^{ab}	87.52 ± 1.94 ^{bc}	58.03 ± 0.73 ^a	24.49 ± 0.44 ^b	31.76 ± 0.06 ^a
TAC	84.81 ± 0.81 ^b	15.19 ± 0.81 ^b	0.43 ± 0.03 ^{ab}	17.81 ± 0.82 ^b	5.22 ± 1.34 ^a	84.89 ± 1.04 ^c	50.76 ± 0.13 ^b	26.98 ± 0.13 ^{ab}	29.46 ± 0.09 ^b
TSM	85.52 ± 0.65 ^a	14.48 ± 0.65 ^a	0.39 ± 0.03 ^b	19.40 ± 1.03 ^a	5.35 ± 0.93 ^a	92.29 ± 1.68 ^a	54.87 ± 0.01 ^{ab}	28.34 ± 0.03 ^a	32.55 ± 0.04 ^a

^{a,b,c}Values expressed are mean values of 3 replicates ± SD. All mean scores, bearing different superscripts in columns differ significantly ($p < 0.05$). TSM- tomato pretreated with 0.5% sodium metabisulphite, TC- control tomato samples pretreated with water, TAC- tomato pre-treated with 0.1% ascorbic acid +0.1% citric acid.

Lycopene ranged from 145.41 ± 1.95 - $158.45 \pm 1.08/100$ g (dry weight basis) for the fresh tomato samples. The level of lycopene is directly related to ripeness and increased pH (Thompson et al., 2000) and these factors may explain the wide variability of reported lycopene content in raw tomato. In fresh tomato, the content of lycopene was reported to range from 2.5 – 200 mg/100 g on wet weight basis (Takeoka et al., 2001). In this study, control fresh tomato samples had the lowest lycopene content (6.658 ± 0.53 mg/100g) which was significantly ($p = 0.15$) different from the fresh pretreated tomato samples. Colour L* a* and b* values did not significantly ($p > 0.05$) differ for both control and pretreated tomato.

Physicochemical characteristics of pretreated dried tomato

Results in Table 2 show that pre-treatment influenced some quality characteristics of tomato. Control samples TC ($15.68 \pm 0.45\%$) had the highest moisture content. Total solids content was higher in the pre-treated samples as compared to the control sample. The lowest total solids were recorded by TC ($84.32 \pm 0.45\%$). TSM ($14.48 \pm 0.65\%$) showed lowest moisture content and this may be due to partial effect of sodium metabisulphite in enhancing removal of water through osmotic dehydration. Similar observations

were reported by Gierschner and Philippos (1995b) and Olorunda et al., (1990).

The water activity of samples ranged from 0.39 ± 0.03 - 0.43 ± 0.03 and the relatively low water activity in the samples is a good indicator of a more shelf stable product. Water activity (aw) affects the storage stability of foods because some deteriorative processes in foods are mediated by water. The higher the water activity, the more susceptible the product is to microbial spoilage. The lowest water activity was recorded for samples treated with sodium metabisulphite, TSM (0.39). Subsequently, the dehydration ratio (DR) was significantly higher ($p = 0.021$) for TSM than for TAC (17.81 ± 0.82).

Rehydration can be considered as a measure of the injury to the material caused by drying and treatment preceding dehydration (McMinn and Magee, 1997a; Okos et al., 1992). In this study, rehydration ratio (RR) of dehydrated tomato varied from 5.10 ± 1.45 - 5.35 ± 0.93 and was not significantly affected ($p = 0.04$) by pre-treatments. Davoodi et al. (2007) found significant differences between RR of tomato when pretreated with CaCl_2 and NaCl.

Lycopene content varied significantly ($p = 0.04$) among the dehydrated tomato samples (Table 2). Data show that pretreatment with sodium metabisulphite (TSM) preserved lycopene better than treatment with ascorbic acid + citric acid

(TAC) which was not significantly different from the control, TC (87.52 ± 1.94 mg/100 g). TSM samples had the lowest lycopene degradation (92.29 ± 1.68 mg/100 g on dry weight basis) and this may be due to protective effect of sodium metabisulphite for lycopene pigments against heat damage. Similar protective effect has been reported (Davoodi et al., 2007) for lycopene in tunnel dried tomato pretreated with potassium metabisulphite (93.0 ± 0.07 mg/100g) and calcium chloride (91.0 ± 0.8 mg/100g). In this study by (Davoodi et al., 2007), lycopene content of control samples (no pretreatment) was the lowest (89.06 ± 0.6 mg/100g). The main role of bisulphite in dehydration of food products is to inactivate the enzymes that cause enzymatic browning in food products. According to Pizzocaro et al. (1993) bisulphites react with the o-quinones forming colourless complex compounds; additionally, bisulphite act as competitive inhibitors by binding a sulphhydryl group at the active site of the enzyme; thus, the polyphenoloxidase is irreversibly inhibited (Ferrer et al., 1989).

During dehydration and subsequent storage, the typical red colour characteristic of tomato gradually changes to brick-red and then to brown (visual appreciation). This phenomenon which is known as non-enzymatic browning or Millard reaction produces dark pigments and destroys the natural colour of products (Portta and Sandei, 1990).

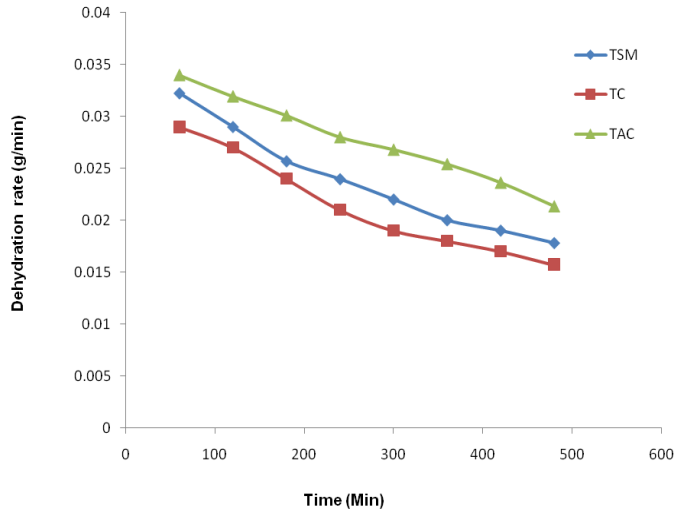


Figure 1. Dehydration rate curves for pre-treated tomato dried at 55°C in hot air convection dehydrator.

Tristimulus colour a^* which measures the degree of redness to greenness in the tomato samples was significantly ($p= 0.035$) higher for pretreated samples TSM (28.34 ± 0.03) and TAC (26.98 ± 0.13) than control samples (24.49 ± 0.44). Lycopene was better protected in TSM than TAC because sulfites blocked the formation of brown pigments in the Maillard reaction pathway (Taylor et al., 1986; Sulaeman et al., 2001).

Dehydration rate

Existing literature (Van Arsdell and Copley, 1963; Mujumdar, 1987) has defined a generalized drying curve that includes a constant drying rate region and falling rate regions. However, not all materials follow this pattern. A constant rate period was not observed during the drying process (Figure 1). However, falling rates were observed in all samples. A substance undergoes a constant drying rate when a film of water is freely available at the drying surface for evaporation into the drying medium. The falling rate regions are indicative of an increased resistance to both heat and mass transfer and occur when the surface water no longer exists and water to be evaporated comes from within the structure and must be transported to the surface (Hawladar et al., 1991).

The moisture content as a function of time is presented in Figure 2. The TC samples recorded the highest final moisture content (7.52%) and TSM the lowest (6.21%) after 11 h of drying. The stationary phase was observed after 10 h. Pretreatment with sulphites act by plasmolyzing cells (Gould and Russel, 1991), which facilitate the drying process unlike the control.

Microstructure of dehydrated tomato

Tomato is considered to be rather complex with an inner

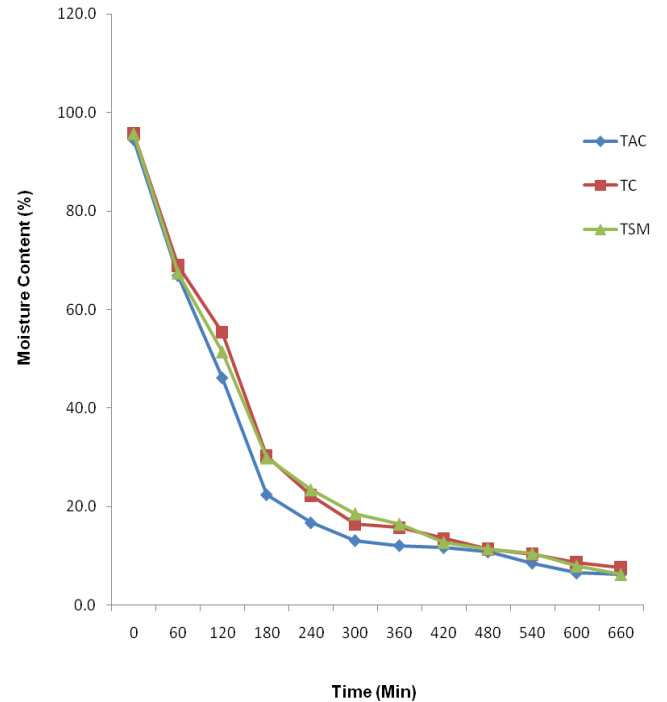


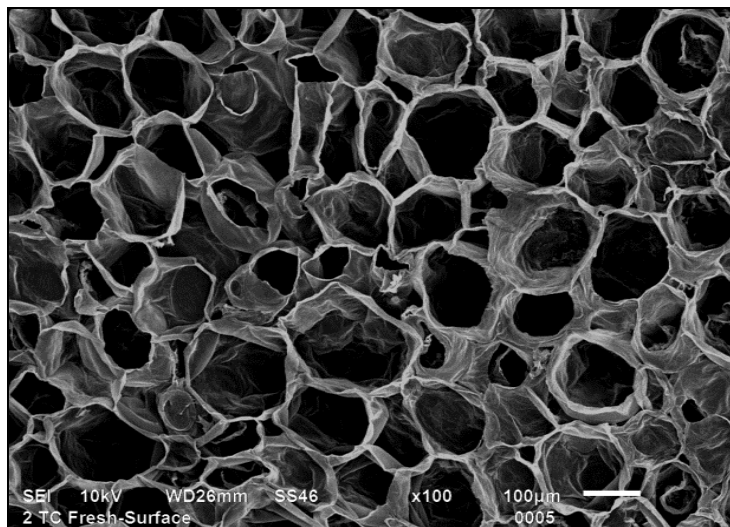
Figure 2. Change in moisture content with time for pretreated tomato dried at 55°C in a hot air convection dehydrator.

wall structure resembling a fibrous material while the pulposus areas which contain the seeds resemble a non-porous material; it is considered to be hygroscopic (Hawladar et al., 1991).

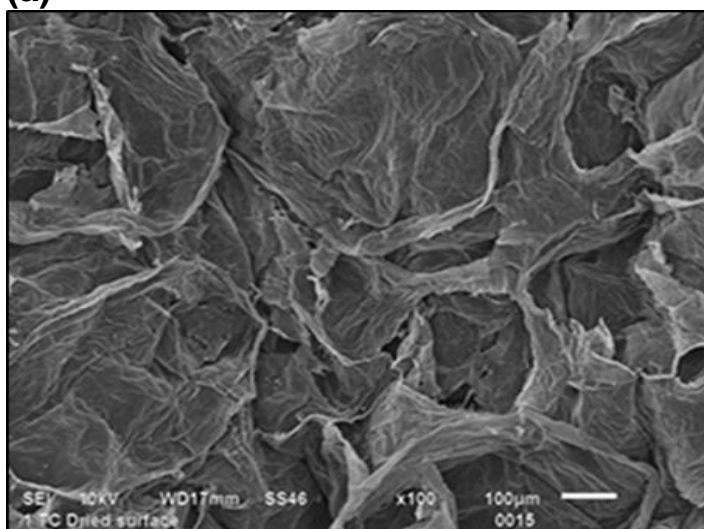
SEM examination of the surface of fresh and dried tomato cell walls revealed pit fields and associated radiating ridges of cross section of the cell walls. SEM of fresh tomato cells (Figure 3a) look firm and intact showing the cell structure. It is clear that in the dried samples (Figure 3b) the cell walls have collapsed due to removal of water. These observations have been explained by Lewicki and Jakubczyk (2004) to be due to shrinkage and creation of internal tensions. Zogzas et al. (1994) also confirmed that, the amount of collapse was proportional to the amount of moisture lost during the hot air drying process.

Conclusion

Pretreatment of tomato had effect on physicochemical quality parameters such as moisture, total solids, lycopene and colour. Pretreatment with sodium metabisulphite facilitated the drying rate of tomato and had the least effect on the reduction of lycopene. However, all the pretreated dried tomato samples produced had good visual appeal and exhibited a desirable red colour which is characteristic of dried tomato products.



(a)



(b)

Figure 3. Scanning electron microscopy (SEM) showing microstructure of pretreated fresh and dried tomato slice. The microstructure of the surface of (a) fresh tomato (b) dried tomato slices is shown in a and b at SEM magnification of 100x.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Analysis of quality attributes of banana drinks blended with aqueous extracts of ginger

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This study aimed to investigate the effect of ginger (*Zingiber officinale*) on the microbiological, physicochemical and sensory quality of banana (*Musa spp.*) drink. Production of banana juice was achieved with hot and unheated water extraction. The various juices obtained were supplemented with aqueous extracts of ginger in concentrations of 2.5 and 10%, stored and analyzed periodically for microbiological and physicochemical (pH, vitamin C, proteins, total sugars, Mg, k, Ca, Fe, Na, Zn) characteristics. The results showed that ginger addition to either hot or unheated banana juice extraction treatment significantly reduced growth of microorganisms as reflected in total viable counts from day 4 of storage. Vitamin C, proteins and mineral contents increased as compared to control samples. The results of this study suggest that the incorporation of ginger extracts into banana drink could be an effective means of preserving banana drinks.

Key words: Quality, banana drink, blended, ginger.

INTRODUCTION

Plantain is one of the primary sources of food product intended for consumption in Côte d'Ivoire. It is the second food crop in Côte d'Ivoire with an annual production of 1.6 million tons. Its main production area is the midwest (ANADER, 1998).

Although it occupies a prominent place in the diet of Ivorian, production of plantain remains seasonal. This seasonality is characterized by a period of relative abundance (October to March) where post-harvest losses are high. During this period, the sales prices are low and

another period of shortage in urban markets (April-September) marked by the high cost of banana and inaccessibility of the product for low income households.

Thirty to forty percent of this production is unfortunately lost before consumption, mainly due to poor harvest, transport and storage (Lépengué, 1999). Indeed, these losses are due to storage of bananas at room temperature (28-35°C) than the recommended 8-12°C (Varoquaux et al., 2002) temperature, the remoteness of production and lack of accessible roads to urban areas.

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Moreover, not processing or very low level of transformation of plantain is an obstacle to the development of the plantain sector in Africa in general and in Côte d'Ivoire in particular.

Thus, "the production of plantain juice clarified and stabilized" will not only help reduce post-harvest losses but also offer a new product that will improve the income of the plantain industry. However, the juice of plantain like that of *Hibiscus sabdariffa* (zobo) (Omemu et al., 2006) has a shelf life of not more than 24 h if not refrigerated.

To solve this problem of conservation, Dougheri et al. (2007) have used benzoic acid thus extending the shelf life of zobo (a drink made from the calyces of *Hibiscus sabdariffa*) to 14 days. They were able to preserve the same the organoleptic qualities beyond 14 days. However, the use of these chemicals has various negative effects on the health of consumers (Adesokan et al., 2010). In this regard, Kolapo et al. (2007) preferred natural plant extracts with antimicrobial properties. The aim of the present study was to determine the effect of ginger (*Zingiber officinale*) on microbiological, physicochemical and sensory quality of fruit juice of plantain (*Musa spp.*)

MATERIALS AND METHODS

Sampling

Plantain variety "Corne" and the roots of ginger (*Z. officinale*), were purchased at market Guro Adjamé (Abidjan) in Côte d'Ivoire. These samples were carefully sorted and manually washed with bleach, then rinsed thoroughly with distilled water before use.

Ginger juice extraction

One hundred grams of ginger roots were cut into small pieces with a sterile knife.

The ground material obtained is then mixed in 50 ml of distilled water using a "Kenwood blender" having cutting blades until a fine paste. The paste is dissolved in 100 ml of distilled water and filtered through a muslin cloth. The filtrate is stored in sterile bottles in a refrigerator at 5°C until use (Adesokan et al., 2010).

Banana juice production

A cold and hot water extraction methods were performed. For both methods, 500 g banana pulp were dispersed in 1 L of distilled water in a blender brand "Phillips". The resulting mash was filtered using a sieve of 0.250 mm diameter. The filtrate was then centrifuged at a speed of 4000 rev/min for 30 min with a centrifuge brand Heraeus Christ, Bofuge III. Residues separated juice was placed in a sterile bottle and ginger juice added at different concentrations (2.5 and 10%). Juice of plantain non-supplemented ginger served as controls.

Physicochemical analyses

Measurement of pH at 25°C was done for each day for 15 days using a pH meter, according to AOAC (1990). Proteins were determined by Kjédhal method (BIPEA 1976). The dosage of

vitamin C was produced according to the AFNOR method (1995). Mineral elements: iron, potassium, calcium, magnesium, sodium, copper, zinc, manganese were determined by atomic absorption spectrophotometer according to the method of AOAC (1990) and Abulude et al. (2007).

Microbiological analyses

The various banana juices were carefully diluted in sterile distilled water. Appropriate dilutions were poured onto agar PDA (potato dextrose agar) and MacConkey respectively for the enumeration of total coliforms and flora. Boxes cast in MacConkey medium were incubated at 35°C for 24 h while those of PDA medium were incubated at 28°C for 72 h and supplemented with streptomycin to prevent bacterial growth (Adesokan, 2005).

Sensory analysis

Different extracts of banana juice were analyzed by a panel of 17 people previously formed by the Ivorian society of Tropical Technology (I2t). The samples were evaluated on a scale of 0 to 10.

Statistical analysis

STATISTICA 10 software was used to perform statistical analyses. Comparisons between the dependent variables were determined by analysis of variance and Duncan's test and Newman Keuls. Statistical significance was defined at 5%. The results for sensory tests were subjected to analysis of variance using the software Tastel +

RESULTS

The physicochemical analyzes of the various plantain drinks are shown in Table 1. The results show that the vitamin C content (mg/100 g) increased from 102.61 to 135.01 in hot extracts samples (C) and from 108.01 to 264.46 in cold extracts samples (F). The lowest value, 102.61 is the ascorbic acid content of hut sample treatment with no ginger (C0) and the highest value, 264.46 is that of cold sample treatment with 10% ginger (F10). The pH values vary slightly from 4.75 to 4.99 in samples F0, F10 and C10. Total sugars in turn have values ranging between 51.4 mg/100 ml in the C0 sample and 67.2 mg/100ml in the F2,5.

Moreover the results indicate that the lowest protein content (0.25%) is that of the sample C0 while the highest content 0.65% is that of cold extraction treatment supplemented with 10% ginger. Plantain drinks contain varying concentrations of Mg^{2+} , K^+ , Ca^{2+} , Fe^{2+} , Na^+ and Zn. The sample F10, that is, the cold water extract treatment containing 10% ginger has a higher concentration than the other juice samples. The enumeration of the total flora, expressed in cfu/ml of various banana juice samples are summarized in Table 2.

These results show that there is a steady increase in the total flora with time of storage. However, the samples

Table 1. Contents of proteins, total sugars, minerals, vitamin C and pH of banana juice supplemented with ginger extracts.

Parameters	C0	C2,5	C10	F0	F2,5	F10
Vitamine C (mg/100 ml)	102.61 ± 0.602 ^d	108.01 ± 0.13 ^c	135.01 ± 0.25 ^b	108.01 ± 0.01 ^c	135.01 ± 0.04 ^b	264.46 ± 1.25 ^a
pH	4.93 ± 0.01 ^b	4.96 ± 0.23 ^a	4.99 ± 0.15 ^a	4.75 ± 0.26 ^c	4.97 ± 0.20 ^a	4.99 ± 0.01 ^a
Total sugars (mg/100 ml)	51.4 ± 0.772 ^f	54.9 ± 1.01 ^c	57 ± 1.5 ^b	54.2 ± 0.54 ^d	67.2 ± 1.2 ^a	55.3 ± 0.02 ^e
Proteins ((%)	0.25 ± 1.03 ^d	0.56 ± 0.01 ^b	0.59 ± 0.56 ^b	0.53 ± 1.6 ^c	0.56 ± 0.83 ^b	0.65 ± 1.5 ^a
Mg ²⁺ (mg/100 ml)	143.01 ± 0.25 ^d	152.56 ± 1.54 ^b	148.34 ± 1.02 ^c	137.11 ± 1.03 ^e	177.41 ± 2.03 ^a	130.29 ± 0.97 ^f
K ⁺ (mg/100 ml)	155.33 ± 1.01 ^b	124.13 ± 0.21 ^e	115.83 ± 0.56 ^f	132.41 ± 0.40 ^d	138.33 ± 1.21 ^c	191.66 ± 1.01 ^a
Ca ²⁺ (mg/100 ml)	411.85 ± 0.45 ^e	1397.32 ± 0.74 ^d	3954.2 ± 1.47 ^a	255.82 ± 0.98 ^f	1620.05 ± 0.46 ^c	3523.42 ± 1.51 ^b
Fe ²⁺ (mg/100 ml)	10.98 ± 0.77 ^f	12.23 ± 0.68 ^e	14.01 ± 1.57 ^c	12.63 ± 0.01 ^d	14.18 ± 0.13 ^b	15.67 ± 0.58 ^a
Na ⁺ (mg/100 ml)	25.8 ± 0.04 ^f	27.43 ± 0.01 ^e	27.6 ± 0.25 ^d	59.75 ± 0.77 ^a	38.3 ± 0.77 ^b	27.93 ± 1.24 ^c
Zn ²⁺ (mg/100 ml)	0.112 ± 0.41 ^c	0.113 ± 0.21 ^c	0.167 ± 0.15 ^a	0.131 ± 0.05 ^b	0.132 ± 1.21 ^b	0.171 ± 0.65 ^a

Means with different letters in the same row indicate significant differences ($p \leq 0.05$) ($n = 3$). C0: juices extracted with hot water with 0% ginger; C2,5: extracted juice in hot water with 2.5% ginger; C10: juices extracted with hot water with 10% ginger; F0: juices extracted in cold water with 0% ginger; F2,5: juice extracted in cold water with 2.5% ginger; F10: juices extracted with cold water with 10% ginger.

Table 2. Total viable counts (cfu/ml) of the different banana drinks during storage.

	Day 1	Day 4	Day 7
C0	9 × 10 ⁴ ^b	2.02 × 10 ⁶ ^b	3 × 10 ⁷ ^b
C2,5	3.3 × 10 ³ ^d	2.78 × 10 ⁴ ^d	2.9 × 10 ⁶ ^e
C10	7.7 × 10 ² ^f	1.1 × 10 ⁴ ^f	4.8 × 10 ⁵ ^f
F0	3 × 10 ⁵ ^a	4.9 × 10 ⁶ ^a	7 × 10 ⁷ ^a
F2,5	3.4 × 10 ³ ^c	4.66 × 10 ⁴ ^c	3.3 × 10 ⁷ ^c
F10	3.9 × 10 ³ ^e	3 × 10 ⁴ ^e	3.5 × 10 ⁶ ^d

Sample codes are as stated in Table 1. Values are means of three replicates.

Table 3. Sensory evaluation of different banana drinks.

Sample	Color		Texture		Taste		Appearance		Aroma		Acceptability	
	D1	D7	D1	D7	D1	D7	D1	D7	D1	D7	D1	D7
C0	7.2 ^a	6.6 ^a	3.4 ^c	3.7 ^c	4.4 ^{ba}	4.7 ^a	3.3 ^d	3.4 ^c	5.2 ^a	5.8 ^b	5.3 ^a	5.2 ^a
C2,5	6.6 ^{ba}	4.9 ^b	4.6 ^{cb}	4.1 ^{cb}	3.9 ^{cba}	3.4 ^b	5.3 ^c	5.6 ^b	3.7 ^b	3.9 ^c	4.9 ^a	4.4 ^b
C10	4.1 ^c	4.3 ^{cb}	4.4 ^{cb}	4.4 ^{cba}	3.3 ^c	2.2 ^c	5.9 ^{cb}	5.8 ^{ba}	2.5 ^c	1.9 ^d	4.7 ^a	3.4 ^c
F0	4.6 ^c	3.5 ^c	6.5 ^{ba}	5.4 ^{cba}	4.7 ^a	4.6 ^a	5.3 ^c	5.6 ^b	7 ^a	6.5 ^a	5.4 ^a	5.2 ^{ba}
F2,5	5.4 ^{cb}	5.0 ^b	6.1 ^a	5.7 ^a	4.5 ^{cba}	4.2 ^a	6.8 ^{ba}	7.3 ^{aba}	5.5 ^a	6.4 ^{ba}	4.8 ^a	4.7 ^{ba}
F10	5.6 ^{cb}	5.1 ^{ba}	5.4 ^{ba}	5.2 ^{ba}	3.8 ^{cb}	2.3 ^{cb}	6.4 ^a	7.9	3.3 ^{cb}	3.6 ^c	4.7 ^a	3.2 ^c

Values are means of three replicates.

fortified with ginger contain less germs than the controls. After seven days of storage, the sample F0 contain the highest flora (7.10 cfu/ml), while the lowest (4, 9.10 cfu/ml) quantity is contained in the C10 sample.

The organoleptic properties of plantain juice are shown in Table 3 and these results indicate that the cold extract F2 has the highest overall acceptability of 5.4, while the

cold extract sample F10 has the lowest acceptability (3.2).

DISCUSSION

At the end of this study, it is clear that vitamin C content is higher in the different juices supplemented with 2.5 and 10% ginger extracts. This shows that the vitamin C level can be improved by ginger addition. This assertion

seems justified by research conducted by Adesokan et al. in 2005 who indeed improved the ascorbic acid content of the juice "ZOBO" through the use of mixtures of ginger

and garlic in fresh or powder forms. The pH value of banana juice was increased with increasing ginger concentration. This implies that plantain juice with more acid should not be used when the stomach is empty. Proteins content also increases with the content of ginger. These show that ginger improves protein content as in the study of Adesokan et al. (2005) and Olayemi et al. (2011). Different plantain drinks characterized in this study have varying contents of metal ions such as Mg^{2+} , K^+ , Ca^{2+} , Fe^{2+} , Na^+ and Zn^{2+} . A study by Olayemi et al. (2011) on three different "zobo" drinks showed variations in their mineral contents following spices incorporation. Similar results were revealed by Adesokan et al. (2005) study in the "zobo" drinks supplemented ginger.

In addition, we found that ginger extract can reduce the total flora of plantain juice during 7 days of storage. The presence of mono and sesquiterpenoids within the ginger extracts is considered main cause of their antimicrobial activity (Bajpal et al., 2009). The mode of action of these compounds involves cell walls and cell membranes attacks affecting their permeability and functions (electron transport, nutrient uptake and enzyme activity).

These results are in agreement with those of Adesokan et al. (2005) and Ogiehor et al. (2008) who were able to maintain the quality of "zobo" drink for 42 days by combining refrigeration and ginger (0.2%) addition.

Conclusion

From this work, it can be concluded that ginger provides mineral fortification and antimicrobial benefits to banana drink during storage for 7 days. Therefore, it is suggested that ginger could be used to extend the shelf life of fruit juices, which seems more healthful than synthetic additives. The ginger extracts showed an interesting antimicrobial activity and could therefore be used to preserve various food products. The extracts may find application in antibacterial and antifungal therapies. Additional research is needed to elucidate mechanisms of action of bioactive molecules in the extracts.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of extrudate from sweetpotato flour and tomato pomace blend by extrusion processing

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Sweet potato flour and tomato pomace blend were used for the development of extruded products. The response surface methodology was adopted in the experimental design to investigate the effect of feed proportion (5-25% tomato waste powder), moisture content (13-17%), screw speed (275-325 rpm) and barrel temperature (120-140°C) on the quality of the extruded products. Regression equation describing the effect of each variable on the system parameters and product responses were obtained. In all the experiments, the responses were almost equally affected by changes in tomato pomace level, feed moisture, extrusion temperature and screw speed. Increase in barrel temperature results in maximum expansion, minimum hardness and maximum water absorption index (WAI). Higher tomato pomace proportion in feed composition showed minimum expansion, maximum bulk density, minimum WAI and maximum water solubility index (WSI). The compromised optimum condition obtained by numerical optimization were: barrel temperature, 137.01°C, screw speed 343.48 rpm, feed moisture 13.86% and tomato pomace 21.31%. The findings of this study demonstrate the feasibility in development of value added extruded products from tomato pomace and sweet potato flour.

Key words: Twin screw extrusion, sweetpotato flour, tomato pomace, response surface methodology.

INTRODUCTION

Sweetpotato (*Ipomea batatas*) also known as Shakharkhand, is a very important crop in the developing world. This tuber is an efficient producer of calories which make significant nutritional contributions to the diet. Apart from being a staple crop for some parts of the world (Papua New Guinea, some parts of the Philippines, Tonga and Solomon Islands), sweetpotato plays a multitude of varied roles in the human diets, being either supplemental or a luxury food. Sweetpotato is one of the

seven crops in the world which produce over 135 hundred million metric tons of edible food products in the world annually. China alone produces 80-85% of the total world production (FAO, 1984). The remaining countries in Asia have the next highest production and then followed by Africa and Latin America (Wanda, 1987). The nutritional qualities of sweetpotato which are important in meeting human nutritional needs include carbohydrates, vitamins, high quality protein, fiber and minerals like

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potassium and iron. Sweet potato contains as much as 44% dry matter (Moorthy, 2002; Hoover, 2001). However, most commercial cultivars, especially in the US, contains 20-30% dry matter. The major components of dry matter are carbohydrates which make up 90% of dry matter in most cultivars. In sweetpotato, the protein content is generally low, ranging from 1.0 to 14.2% dry weight basis (dwb) (Bradbury and Howard, 1989). Sweetpotato protein is good quality and contains excess amounts of essential amino acids except tryptophan and total sulfur amino acids (Wanda, 1987). The possibility of utilization of sweet potato and cassava flour in bread has been investigated by several researcher. The good quality bread can be produce by replacing 10-15% wheat flour with sweetpotato flour (Greene and Bovell-Benjamin, 2004).

Tomato (*lycopersicon esculentum*) is a nutritious fruit and an important part of human diet worldwide. A waste (tomato pomace) at the rate of 4-7% is produced from the tomato processing industries. Tomato pomace consists of dried and crushed skins and seeds of the fruits. The dried pomace contains 44% seed and 56% pulp and skin (Sogi et al., 1998). Tomato pomace is a rich source of bioactive compounds such as high-valuable oils, dietary fibre, vitamins and secondary plant metabolites (Bildstein et al., 2009). The skin (an important component of pomace) is a good source of lycopene. It has attracted attention due to its biological and physiochemical properties, especially related to its effects as a natural antioxidant. Although it has no pro vitamin A activity, it does exhibit the singlet oxygen quenching capacity twice higher than of beta-carotene. This makes its presence in the diet of considerable interest. Increasing clinical evidence supports the role of lycopene as a micronutrient with important health benefits, because it appears to provide protection against a broad range of epithelial cancers. The use of pomace could provide valuable substance and at the same time reduce the waste disposal problems.

Extrusion technology is very useful because nutrient losses are lower than other thermal processing methods (Moscicki et al., 2003). In the extruder, food mix is thermo-mechanically cooked at high temperature, pressure and shear stress which is generated in the screw-barrel assembly. The cooked melt is then texturized and shaped in the die (Arhaliass et al., 2003). It offers several advantages over other types of cooking processes, such as faster processing time and significant reduction in energy consumed; which consequently results in a lower price for the final product. Extrusion cooking technology is used increasingly in the food industries for the development of new products (Sebio and Chang, 2000). Extrusion cooking of food ingredients brings gelatinization of starch, denaturation of protein, modification of lipid and inactivation of enzymes, microbes and many anti nutritional factors (Bhattacharya and Prakash, 1994). Some attempts have been made to

produce extruded product either by partial substitution or using sweetpotato flour alone. Extrusion processing is a very viable method used for preparation of valuable products from fruits and vegetables waste due to its versatility, relative low cost and high productivity. Therefore, the objective of this research was to investigate the possibility of utilization of tomato pomace with sweetpotato flour to produce the nutritionally rich extruded product. Response surface methodology was used to study the effect of various process variables on the various properties of extrudates.

MATERIALS AND METHODS

Fully matured sweetpotato was purchased from a local market. Hand peeling was done with knife. The peeled sweetpotato was dipped into 2% salt solution in order to prevent initial browning. Sweetpotato slices of 2-3 mm thickness were dipped in a solution containing 0.5% potassium metabisulphite (KMS) and 0.5% citric acid for 30 min. Thus, treated slices were dried in cabinet dryer at 55°C. Slices were ground and flour was passed through 2 mm sieve to get uniform particle size. Flour was then packed in HDPE bag and sealed tightly. Tomato pomace was taken from Nigger Agro Pvt. Ltd. The pomace was dried to moisture content of about 3.5±.5% (wet basis) in cabinet dryer at 55°C. Dried pomace was ground and stored at refrigeration temperature for future use.

Sample preparation

Samples were conditioned by sprinkling the calculated amount of distilled water in all the dry ingredients. The ingredients were weighed and then mixed in the Food Processor (Make: Maharaja Whiteline, Asiatic Engineers Pvt. Ltd., New Delhi 600W) with mixer attachment for 20 min. This mixture was then passed through a 2 mm sieve to reduce the lumps formed due to addition of moisture. After mixing, samples were stored in polyethylene bags at room temperature for 24 h (Stojceska et al., 2008). The moisture content of all the samples was estimated using the hot air oven method (Ranganna, 2003). All the samples were kept in high precision (±0.1°C) incubator (Macro Scientific works, New Delhi) at 60°C for 12 h duration for the stabilization of moisture.

Extrusion experiments

Extrusion trials were performed using a co-rotating twin-screw extruder (Basic Technology Pvt. Ltd. Kolkata, India). The main drive is provided with 7.5 HP motor (400 V, 3 ph, 50 cycles). The output shaft of worm reduction gear was provided with a torque limiter coupling. The twin screw extruder was kept running for 30 min to stabilize the set temperatures. The samples were then poured into feed hopper and the feed rate was adjusted to 4 kg/h for easy and non choking operation with 4 mm die. The product (approximately 30-35 mm long cylindrical shape) was collected at the die end and kept at 55± 0.5°C in an incubator (Macro Scientific Works, New Delhi) for 12 h duration to remove extra moisture from the product. The product was then packed in already numbered zipped lock packs and kept in proper storage.

Experimental design

Response surface methodology (RSM) was adopted in the

Table 1. Values of independent process variables at five levels of the central composite design arrangement.

Independent variables	Code	± Increment	Levels in coded form				
			-2	-1	0	+1	+2
Tomato pomace (%)	X ₁	5	5	10	15	20	25
Feed moisture (%)	X ₂	01	13	14	15	16	17
Screw speed (rpm)	X ₃	05	120	125	130	135	140
Die temperature (°C)	X ₄	25	275	300	325	350	375

Table 2. CCRD (coded and actual levels) employed for development of sweet potato flour and tomato pomace flour based extruded snacks.

S/N	Coded variables				Actual variables			
	X1	X2	X3	X4	X1 TomatoPomace (%)	X2 Moisture content (%)	X3 Screw speed (rpm)	X4 Temperature (°C)
1	-1	-1	-1	-1	10	14	300	125
2	1	-1	-1	-1	20	14	300	125
3	-1	1	-1	-1	10	16	300	125
4	1	1	-1	-1	20	16	300	125
5	-1	-1	1	-1	10	14	300	140
6	1	-1	1	-1	20	14	300	140
7	-1	1	1	-1	10	16	300	140
8	1	1	1	-1	20	16	300	140
9	-1	-1	-1	1	10	14	350	125
10	1	-1	-1	1	20	14	350	125
11	-1	1	-1	1	10	16	350	125
12	1	1	-1	1	20	16	350	125
13	-1	-1	1	1	10	14	350	135
14	1	-1	1	1	20	14	350	135
15	-1	1	1	1	10	16	350	135
16	1	1	1	1	20	16	350	135
17	-2	0	0	0	5	13	325	130
18	2	0	0	0	25	15	325	130
19	0	-2	0	0	15	13	325	130
20	0	2	0	0	15	17	325	130
21	0	0	-2	0	15	15	325	120
22	0	0	2	0	15	15	325	140
23	0	0	0	-2	15	15	275	130
24	0	0	0	2	15	15	375	130
25	0	0	0	0	15	15	325	130
26	0	0	0	0	15	15	325	130
27	0	0	0	0	15	15	325	130
28	0	0	0	0	15	15	325	130
29	0	0	0	0	15	15	325	130
30	0	0	0	0	15	15	325	130

experimental design as it emphasizes the modeling and analysis of the problem in which response of interest is influenced by several variables (Montgomery, 2001). The independent variables selected for the experiments were: Tomato pomace (x₁), moisture content (x₂), screw speed (x₃), and barrel temperature (x₄). The five levels of the process variables were coded as -2, -1, 0, +1 and +2

(Montgomery, 2001) and design in coded and at the actual levels (X) is given in Table 1. The experimental design with coded and actual level is shown in Table 2. The levels were also selected based on the conclusion of previous experiments. The dependent variables were lateral expansion, bulk density (BD, g/cm³), color (L*, a*, b*), water absorption index (WAI), water solubility index

(WSI), texture, lycopene content, reducing sugar content and sensory characteristics as product responses.

Product analysis

Lateral expansion

The ratio of the diameter of extrudate and the diameter of the die end was used to express the expansion of extrudate (Ainsworth et al., 2006). Lateral expansion (LE, %) was calculated using the mean of the measured diameters. The values were calculated as the average of three replicate.

$$LE = (\text{diameter of product} - \text{diameter of die hole}) / \text{diameter of die hole} \times 100 \quad (1)$$

Bulk density

The individual cylindrical extruded rod was weighed individually, the diameter and length were measured by using a digital vernier caliper. Density of extrudate was calculated according to the method of (Stojceska et al., 2008).

$$\text{Density (g cm}^3\text{)} = 4m/\pi d^2 L \quad (2)$$

Where m is the mass (g) of a length L (cm) of extrudate with diameter d (cm). The values were calculated as the average of three replicate.

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

WAI and WSI were determined according to the method developed for cereals (Stojceska et al., 2008). The ground extrudate was suspended in water at room temperature for 30 min, gently stirred during this period, and then centrifuged at 3000 xg for 15 min. The supernatant was decanted into an evaporating dish of known weight. The WAI was the weight of gel obtained after removal of the supernatant per unit weight of original dry solids. The WSI was the weight of dry solids in the supernatant expressed as a percentage of the original weight of sample.

$$WAI \text{ (g/g)} = \frac{\text{Weight gain by gel}}{\text{Dry weight of extrudate}} \quad (2)$$

$$WSI \text{ (\%)} = \frac{\text{Weight of dry solid in supernatant}}{\text{Dry weight of extrudate}} \times 100 \quad (3)$$

Texture characteristics

Texture characteristics of the extrudates were measured by using a TA – XT2 Texture analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a 500 kg load cell. An extrudate 35 mm long was compressed with a probe SMS – P/75 – 75mm diameter at a crosshead speed 5 mm/s to 3 mm of 90% of the diameter of the extrudate. The compression generates a curve with the force over distance. The highest first peak value was recorded as this value indicated the first rupture of snack at one point and this value of force was taken as a measurement for hardness (Stojceska., 2008). Five randomly collected samples of each replicate were selected for texture measurement and the values are reported in an average.

Lycopene content

The lycopene content was estimated by spectrophotometric method (Ranganna, 2003).

Reducing sugar (Maltose) content

Reducing sugar was estimated by Di-nitrosalicylic Acid (DNS) method (Caraban et al., 2005). DNS reagent was prepared by dissolving DNS- 10 g, sodium sulfite- 0.5 g, and sodium hydroxide- 10 g in one liter distilled water. The DNS reagent (3 ml) was mixed with 3 ml of maltose solution. The mixture was heated at 90°C for 10 min to develop the red-brown color. Then 1 ml of 40% potassium sodium tartrate (Rochelle Salt) solution was added to the above mixture to stabilize the color. After cooling to room temperature in a cold water bath, absorbance was recorded at 546 nm. A standard curve of Malthus was prepared using the same procedure as above, taking known amount of maltose instead of samples.

Sensory characteristics of extrudates

Sensory analysis was conducted on five samples with tomato pomace levels of 5, 10, 15, 20 and 25%. Fifteen panelists were asked to assess the expanded snacks for flavor acceptability, and to mark on a Hedonic Rating Test (1– Dislike extremely, 5– Neither like nor dislike and 9– Like extremely) in accordance with their opinion of taste, texture, color and overall acceptability.

Statistical analysis of responses

The responses for different experimental combinations were related to the coded variables (x_i , $i = 1, 2, 3$ and 4) by a second degree polynomial equation:

$$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_4 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{33}x_3^2 + \beta_{44}x_4^2 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{14}x_1x_4 + \beta_{23}x_2x_3 + \beta_{24}x_2x_4 + \beta_{34}x_3x_4 + \varepsilon \quad (5)$$

The coefficients of the polynomial were represented by β_0 (constant), $\beta_1, \beta_2, \beta_3, \beta_4$ (linear effects); $\beta_{12}, \beta_{13}, \beta_{14}, \beta_{23}, \beta_{24}, \beta_{34}$ (interaction effects); $\beta_{11}, \beta_{22}, \beta_{33}, \beta_{44}$ (quadratic effects); and ε (random error). Data were modeled by multiple regression analysis and the statistical significance of the terms was examined by analysis of variance for each response.

Analysis of data

A complete second order quadratic model employed to fit the data and adequacy of the model was tested considering R^2 (the coefficient of multiple determination, a measure of the amount of variation around the mean explained by the model), Adj R^2 (a measure of the amount of variation around the mean explained by the model, adjusted for the number of terms in the model), predicted R^2 (a measure of how good the model predicts a response value) and Fischer's F-test. Coefficient of determination R^2 is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fit. It is also the proportion of the variability in the response variables, which account for the regression analysis. When R^2 approaches unity, the better the empirical model fits the actual data. The smaller the value of R^2 , the less relevance the dependent variables in the model have in explaining the behavior variation. The models were then used to interpret the effect of various predictors (terms) on the response. The analysis of variance (ANOVA) tables was generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significances of all terms in the polynomial were judged statistically by computing the F-value at probability (p) of 0.01 or 0.05. The regression coefficients were then used to make statistical calculations to generate contour maps from the regression models. Optimization of process parameters was

Table 3. Central composite design arrangement and experimental results for each test run.

Std. No	Independent variables				Responses							
	X1	X2	X3	X4	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8
1	-1	-1	-1	-1	128.28	0.41	12.55	5.65	26.49	11.83	1.29	6.97
2	1	-1	-1	-1	118.28	0.35	19.75	5.61	36.97	8.53	2.31	6.72
3	-1	1	-1	-1	111.95	0.47	18.20	5.95	31.23	6.37	1.90	6.55
4	1	1	-1	-1	105.00	0.48	17.91	5.79	39.33	5.20	2.65	6.45
5	-1	-1	1	-1	82.81	0.48	16.24	5.15	27.01	7.12	2.05	6.57
6	1	-1	1	-1	83.88	0.43	25.36	4.81	35.98	5.16	3.54	6.35
7	-1	1	1	-1	93.59	0.48	17.56	5.91	34.95	12.29	1.90	6.25
8	1	1	1	-1	87.58	0.45	19.64	5.13	41.53	9.72	3.36	6.33
9	-1	-1	-1	1	127.66	0.40	15.28	5.65	30.40	16.60	1.70	7.12
10	1	-1	-1	1	118.83	0.33	17.66	5.33	34.56	7.04	2.25	6.91
11	-1	1	-1	1	104.84	0.50	18.36	5.86	33.12	21.58	1.50	6.61
12	1	1	-1	1	94.06	0.48	8.21	5.46	35.82	11.43	2.46	6.51
13	-1	-1	1	1	115.16	0.46	18.48	5.31	29.57	16.55	2.00	6.55
14	1	-1	1	1	121.80	0.39	20.12	4.54	29.64	10.66	3.04	7.05
15	-1	1	1	1	107.66	0.45	17.64	5.48	35.59	18.02	1.58	6.25
16	1	1	1	1	99.95	0.46	9.09	4.74	35.94	14.75	2.73	6.42
17	-2	0	0	0	113.75	0.46	13.55	6.27	27.09	16.49	0.91	6.77
18	2	0	0	0	112.81	0.41	18.25	5.80	35.53	3.55	3.57	6.53
19	0	-2	0	0	127.50	0.37	20.80	5.22	34.42	14.49	2.27	7.12
20	0	2	0	0	105.47	0.49	18.55	5.98	39.90	13.00	2.71	6.35
21	0	0	-2	0	105.63	0.51	14.48	6.22	27.49	11.52	1.34	6.95
22	0	0	2	0	80.63	0.56	19.36	4.96	29.49	10.06	2.71	6.12
23	0	0	0	-2	105.64	0.43	16.28	5.25	38.30	8.60	2.78	6.77
24	0	0	0	2	124.00	0.33	8.99	4.45	30.25	16.80	1.75	7.25
25	0	0	0	0	110.78	0.47	16.20	5.34	34.92	6.97	2.55	7.00
26	0	0	0	0	114.82	0.43	16.16	5.30	35.72	8.35	2.46	7.24
27	0	0	0	0	108.53	0.50	16.80	5.89	35.35	4.46	2.39	7.19
28	0	0	0	0	112.50	0.45	18.64	5.14	33.02	8.16	2.46	7.12
29	0	0	0	0	111.09	0.46	16.35	5.35	33.44	6.88	2.73	7.20
30	0	0	0	0	112.03	0.47	16.54	5.30	35.02	6.51	2.64	7.10

Y1, Lateral expansion; Y2, Bulk density; Y3, Water solubility index; Y4, Water adsorption index; Y5, Texture hardness; Y6, Maltose; Y7, Lycopene; Y8, Overall Acceptability.

done by partially differentiating the model with respect to each parameter, equating to zero and simultaneously solving the resulting functions. Design Expert 6.0 was used for this purpose and contour plots were developed for selected parameters. Design Expert (Statease Inc, version 6.01, 2001, Minneapolis, MN, USA) was used for the analysis of the data.

RESULTS AND DISCUSSION

Proximate analysis of raw materials

Proximate analysis of sweet potato flour shows that the tomato pomace powder was very high in protein (18.1%) and fat (13.5%). The experiment arrangements and

variation of responses of extruded products are shown in Table 3.

Effect of process variables on product lateral expansion ratio

Lateral expansion is the most important physical property of the snack food. Starch, the main component of cereal plays major role in the expansion process (Kokini et al., 1992). The measured expansion ratio of the extrudate varied from 80.625 to 128.28%. The following regression equation was selected to represent the variation of expansion ratio with independent variables:

Table 4. Analysis of variance results for fitted models.

Response	Source	Sum of squares	df	Mean squares	F-value	P-value
LE	Regression	4758.953	14	339.9252	25.93626	< 0.0001*
	Lack of fit	174.8758	10	17.48758	4.026263	0.0688
	Pure error	21.71689	5	4.343378		
	Residual	196.5927	15	13.10618		
	Total	4955.546	29			
BD	Regression	0.075189	14	0.005371	12.64846	< 0.0001*
	Lack of fit	0.003796	10	0.00038	0.737647	0.6818
	Pure error	0.002573	5	0.000515		
	Residual	0.006369	15	0.000425		
	Total	0.081558	29			
WSI	Regression	353.363	14	25.24021	17.68127	< 0.0001*
	Lack of fit	16.98939	10	1.698939	1.92045	0.2442
	Pure error	4.423283	5	0.884657		
	Residual	21.41268	15	1.427512		
	Total	374.7757	29			
WAI	Regression	5.556187856	14	0.396871	9.823989	< 0.0001*
	Lack of fit	0.266685953	10	0.026669	0.393011	0.9021
	Pure error	0.339285633	5	0.067857		
	Residual	0.605971586	15	0.040398		
	Total	6.16	29			
Hardnes (H)	Regression	428.0071	14	30.57193	15.5089	< 0.0001*
	Lack of fit	23.63475	10	2.363475	1.991461	0.2314
	Pure error	5.934021	5	1.186804		
	Residual	29.56877	15	1.971251		
	Total	457.5758	29			

* significant at P < 0.05, df: degrees of freedom.

$$LE = 111.63 - 1.85x_1 - 5.67x_2 - 6.94x_3 + 4.80x_4 - 5.14x_3^2 + 1.91x_1x_3 + 3.90x_2x_3 - 3.86x_2x_4 + 7.18x_3x_4 \quad (6)$$

It was observed that tomato pomace (x_1), moisture (x_2) and screw speed (x_3) had highly significant negative linear effect ($P < 0.001$) and the temperature had highly positive significant effect on expansion followed by a negative quadratic effect of screw speed (Table 4). The interaction of moisture and screw speed (x_2x_3) and screw speed and temperature (x_3x_4) had strong significant positive effect ($P < 0.001$) on expansion of extrudates (Equation 6). The expansion of extrudates decreased with increase in the level of tomato pomace. This may be attributed to the dilution effect of pomace on starch and results are in agreement with the work of Anton (2008). It appears that expansion increased with increasing barrel temperature. The increase in temperature will increase the degree of superheating of the water in the extruder and would increase at higher temperature, leading to the slightly greater expansion. Similar finding has been reported by several workers (Ding et al., 2005; Camire and King, 1991; Lerrea et al., 2005). The increase in die temperature will decrease the melt viscosity. The reduced

viscosity effect would favor the bubble growth during extrusion which leads to increased expansion of extrudates (Mercier and Feillet, 1975). However, increased feed moisture leads to a decrease in expansion. Increased feed moisture content during extrusion would change the amylopectin molecular structure of the material reducing the melt density thus decreasing the expansion of the extrudate. Foods with lower moisture tend to be more viscous than those with higher moisture and, therefore, the pressure difference would be smaller for higher moisture foods, leading to a less expanded product. These observations are in agreement with the work reported by Ding et al. (2005), Baik et al. (2004), Gujral et al. (2001) and Singh et al. (2007). Response surface plot showed that lateral expansion decreased with increasing moisture content whereas increased with a decrease in screw speed as shown in Figure 1.

Effect of process variables on product bulk density

The bulk density, which considers expansion in all

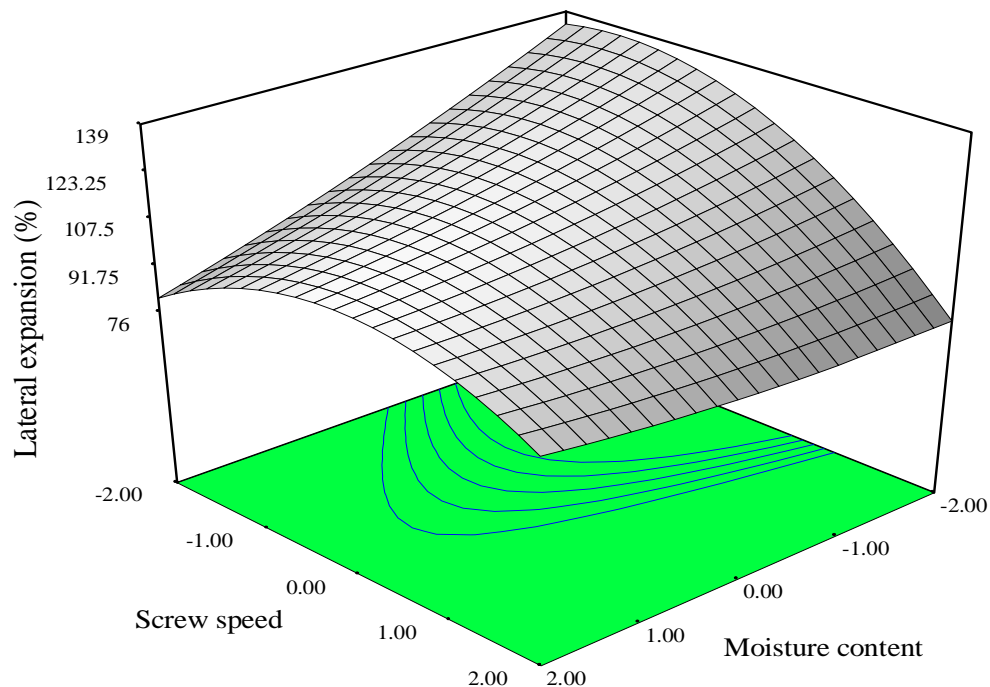


Figure 1. Response surface plot for lateral expansion ratio as a function of feed moisture and screw speed.

direction, ranged from 0.33 to 0.56 g/cm³ for the extrudate. The following regression equation was selected to represent the variation of bulk density (BD) (g/cm³) with independent variables:

$$\text{Bulk density} = 0.46 - 0.016.x_1 + 0.031.x_2 + 0.011.x_3 - 0.012.x_4 - 0.00989.x_2^2 + 0.017.x_3^2 - 0.021.x_4^2 + 0.013.x_1.x_2 - 0.022.x_2.x_3 \quad (7)$$

It was observed that tomato pomace (x_1) and temperature (x_4) had highly significant negative linear effect ($P < 0.05$) whereas moisture content and screw speed had highly positive significant ($P < 0.001$) effect on bulk density.

The quadratic term of feed moisture (x_2^2) and barrel temperature (x_4^2) had highly negatively significant ($P < 0.001$) effect on product bulk density. The other quadratic terms of and screw speed (x_3^2) had significant positive effect on product bulk density at 95% confidence level. The interaction term of tomato pomace and moisture content (x_1x_2) had a significant effect at 95% confidence level. ANOVA for bulk density of quadratic models (Equation 7) is given in Table 4. The regression model fitted to experimental results of bulk density showed a good coefficient ($R^2 = 0.9219$), whereas lack of fit was not significant.

The dependence of bulk density and expansion on feed moisture would reflect its influence on elasticity characteristics of the starch based material. Increased feed moisture content during extrusion would change the amylopectin molecular structure of the material reducing the melt elasticity thus decreasing the expansion, but

increasing the density of extrudate (Faubion and Hosney, 1982; Fletcher et al., 1985; Ilo et al., 1999; Launay and Lisch, 1983). The coefficient of tomato pomace (x_1) was negative. It reveals that the increase in tomato pomace level decreases product bulk density (Figure 2). This may be due to the large difference between densities of sweet potato flour and tomato pomace. Increase in temperature decreased bulk density as in agreement with Ilo et al. (1999) and Singh et al. (2007). Higher temperature provides higher potential energy for flash-off of super-heated water from extrudates as they leave the die. With higher barrel temperatures, the extrudates exiting the die lose more moisture and become lighter in weight (Koksel et al., 2004). This result is consistent with our result on the effect of temperature on lateral expansion. Quadratic terms of feed moisture (x_2^2) and barrel temperature (x_4^2) had a negative coefficient showing convex shaped variation in bulk density with these variables. Quadratic terms of screw speed (x_3^2) had a positive coefficient showing concave shaped effect on bulk density. The sign of all the significant square terms is opposite to the expansion terms as expected. In this study, interaction term of tomato pomace and moisture (x_1x_2) was found to be positively significant ($P < 0.05$). Therefore, we can assume that bulk density will show convex shape variation with the change in value of variables. It reveals that higher product bulk density will obtain increase tomato pomace and feed moisture. Other significant ($P < 0.05$) interaction term of feed moisture and screw speed had negative coefficient. This indicates a concave

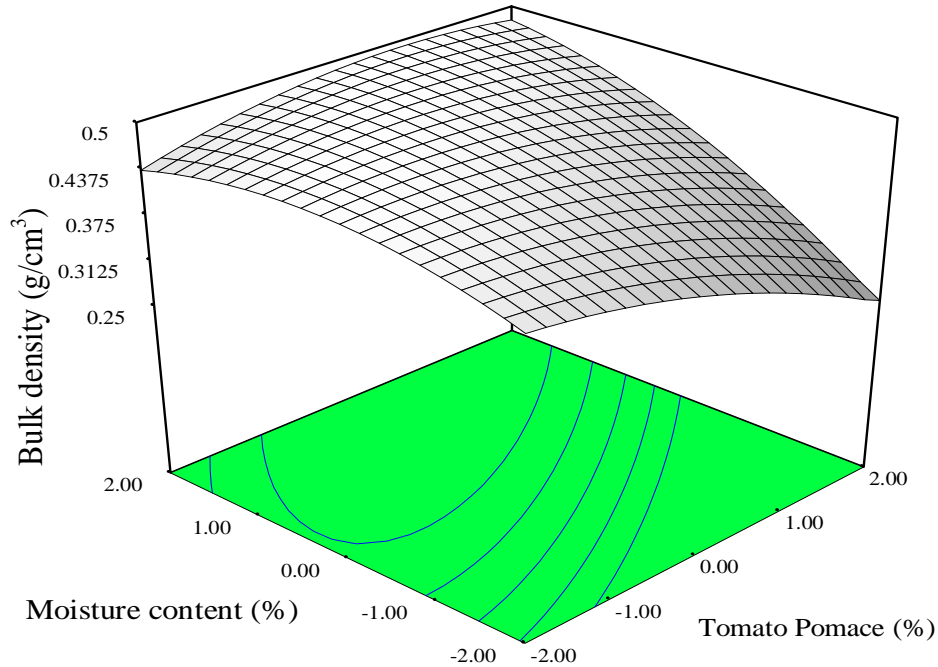


Figure 2. Response surface plots for the variation of bulk density as function of feed moisture and tomato pomace.

shaped variation in bulk density. So there will be low product bulk density with decrease in both screw speed and feed moisture.

Effect of process variables on product water solubility index (WSI)

The WSI values ranged from 8.21 to 25.36% for the sweet potato flour and tomato pomace extrudates. A multiple regression equation was generated relating to water solubility index (WSI) to coded levels of variability.

$$WSI = 16.78 + 0.53x_1 - 0.97x_2 + 1.08x_3 - 1.54x_4 + 0.84x_2^2 - 0.92x_4^2 - 2.33x_1x_2 - 2.05x_1x_4 - 0.86x_2x_3 - 1.10x_2x_4 \quad (8)$$

The negative coefficient of moisture content (x_2) of WSI model supports that the value will decrease significantly ($P < 0.05$) with increasing feed moisture content. The result is consistent with previous studies on extrudate from rice (Ding et al., 2005) and from maize and finger millet (Onyango et al., 2004). It suggested that increasing WSI is caused by greater shear degradation of starch during extrusion at low moisture conditions. The WSI increased significantly ($P < 0.05$) with increasing screw speed (Figure 3). The increase in WSI with increasing screw speed was consistent with the results reported for corn meal and corn and wheat extrudates (Jin et al., 1995; Mezreb et al., 2003). Mezreb et al. (2003) reported that the increase of screw speed induced a sharp increase of specific mechanical energy, the high mechanical shear degraded macromolecules, and so the molecular weight

of starch granules decreased and hence increased WSI (Equation 8).

WSI decreased significantly ($P < 0.05$) with increasing sweet potato flour proportion in the extrudate. WSI is a parameter that reflects the degradation suffered by the components of the fiber Larrea et al. (2005). Tomato pomace is high in fiber content which disrupts the continuous structure of the melt in the extruder, impeding elastic deformation during extrusion (Moraru and Kokini, 2003). So, the highest WSI values may be due to the disintegration of starch granules and low molecular compounds from extrudate melt during extrusion. This may cause an increase in soluble material. WSI determines the amount of free polysaccharide or polysaccharides released from the granule after addition of excess water (Sriburi and Hill, 2000; Aylin et al., 2008). Increase in feed moisture content significantly ($P < 0.050$) decreases WSI. Increase in feed moisture increases the plasticity of feed thus minimizes the chance of the formation of small fraction polymer. WSI increased significantly ($P < 0.05$) with an increase in screw speed (x_3). In this experiment, the WSI decreased significantly with increasing barrel temperature (Figure 3). In this study coefficient of the interaction term of x_1x_2 , x_1x_4 , x_2x_3 and x_2x_4 were found to be negatively significant therefore; they will show convex shape variation with the change in value of variables.

Effect of process variables on product WAI

The WAI ranged from 4.45 to 6.27 g/g for sweetpotato

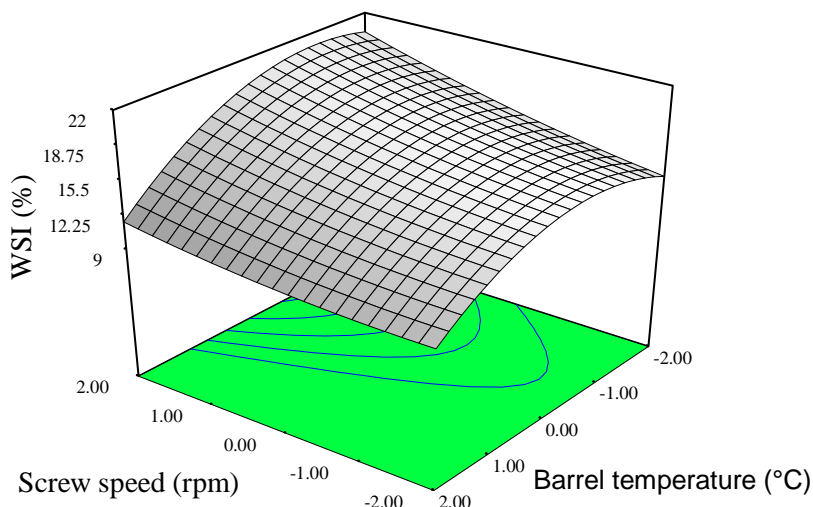


Figure 3. Response surface plot for water solubility index as a function of screw speed and temperature.

flour and tomato pomace extrudate. WAI measures the amount of water absorbed by starch that can be used as an index of gelatinization. A multiple regression equation between water absorption index (g/g) of extrudate and coded levels of independent variables was as follows:

$$\text{WAI} = 5.39 - 0.19x_1 + 0.16x_2 - 0.28x_3 - 0.14x_4 + 0.144x_1^2 - 0.15x_4^2 - 0.11x_1x_3 \quad (9)$$

The negative coefficient of linear terms of feed moisture (x_1), screw speed (x_3) and temperature (x_4) indicated that the content of WAI decreased with the increase of these variables. A decrease in WAI with increasing temperature was probably due to decomposition or degradation of starch (Pelembé et al., 2002). Ding et al. (2005) also stated that the WAI decreases with increasing temperature if dextrinization or starch melting prevails over the gelatinization phenomenon. Similar findings were reported in extrusion of corn fiber and corn starch blend (Artz et al., 1990). Singh et al. (2007) observed decrease in WAI with the addition of pea grits in extrusion of rice. Screw speed had a significant negative effect on WAI which shows that increase in screw speed will decrease WAI (Equation 9). The coefficient for interaction term of feed composition and screw speed (x_1x_3) is significant and negative thereby it will show the concave shape (Figure 4) variation with the change in value of variables. ANOVA for WAI in Table 4 showed that the model was significant ($P < 0.05$), however the lack of fit was not significant. Figures also shows that there will be higher value of WAI at low level of tomato pomace irrespective of screw speed level. In our study, sweetpotato flour is only one source of starch, so there is higher amount of starch for gelatinization. In Figure 4, an increase in feed composition (tomato pomace) decrease

WAI and increase in feed moisture increases WAI. The figures shows that higher values of WAI will be obtained at a higher level of moisture and lower level of tomato pomace.

Effect of process variables on texture of extrudate (H)

Hardness of the extrudate varied between 26.50 and 41.53 N. The quadratic model for hardness (H) in terms of coded levels of variables was developed as follows:

$$H = 34.58 + 2.43x_1 + 1.99x_2 - 1.04x_4 - 0.68x_1^2 + 0.78x_2^2 - 1.38x_3^2 - 1.68x_1x_4 + 0.92x_2x_3 \quad (10)$$

Hardness of extrudates was found to increase with increased feed composition. The positive effect of feed composition indicates that the higher tomato pomace content causes less crispy extrudate. This might be the result of the effect of fiber in the tomato pomace. Fiber reduces the cell size, probably by causing premature rupture of gas cells, which reduces the overall expansion and results in a less porous structure (Lue et al., 2000). Concave shaped variation resulted in hardness due to increase in moisture as a quadratic term of moisture was significant, indicating that hardness increases more sharply at higher moisture level than at lower level. It might be due to the reduced expansion caused by the increased moisture content (Ding et al., 2005). Increase in temperature resulted in a decrease in product hardness. Therefore, a crispy texture was obtained by increasing temperature due to decrease in hardness.

This result is in agreement with Bhattacharya and Prakash (1994), Ryu and Walker (1995) and Duizer and Winger (2006).

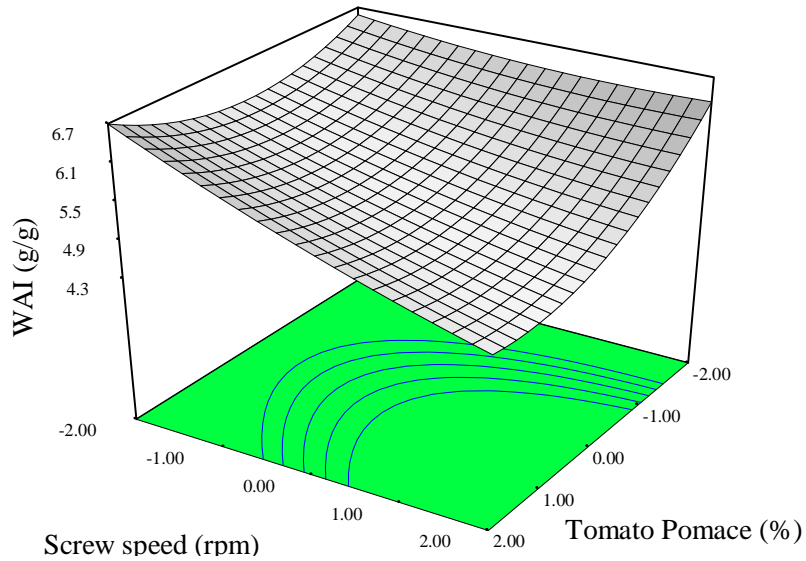


Figure 4. Response surface plot for water absorption index as a function of tomato pomace and screw speed.

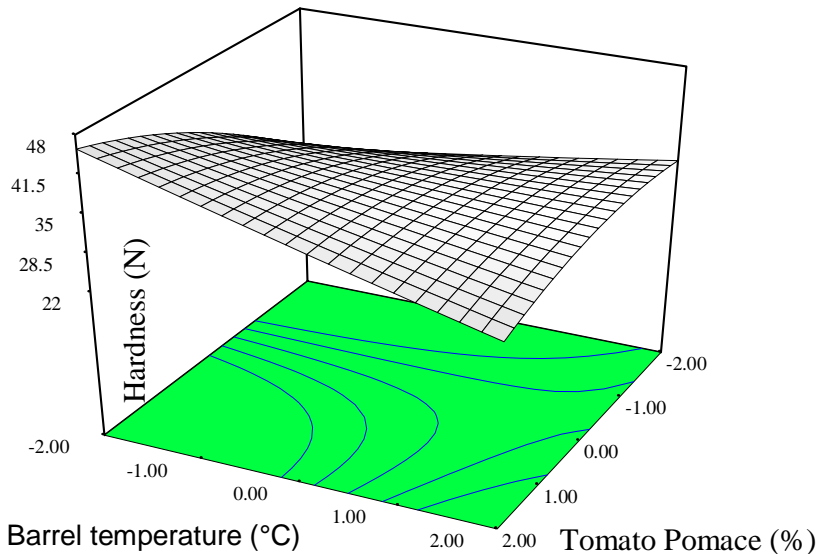


Figure 5. Response surface plot for hardness as a function of tomato pomace and barrel temperature.

F-value for interaction term of feed composition and barrel temperature (x_1x_4) is 22.87 and p value 0.0002 predicting the term is significant which is in agreement with the finding of Aylin et al. (2008). Since the coefficient of a term is negative, it will show convex shape variation with the change in value of variables. Response surface plot (Figure 5), predicted that hardness increased with increasing feed composition and barrel temperature (equation 10).

The interaction term of feed moisture and screw speed (x_2x_3) was significant at 95% confidence level (F-value

6.88 and P-value 0.0192). Coefficient of x_2x_3 was positive so causes concave shaped variation in product hardness. Response surface plot (Figure 5) show variation in hardness with an increase in feed moisture and screw speed.

Effect of process variables on product maltose content (MC)

Extrusion cooking is one processing method that has

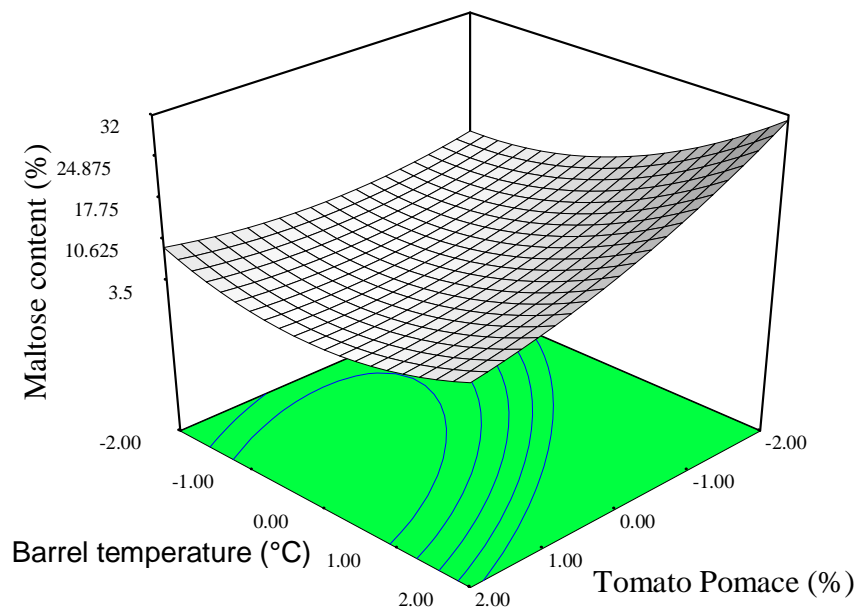


Figure 6. Response surface plot for maltose content as a function of tomato pomace and barrel temperature.

been used to modify the digestible characteristics of starchy materials. Maltose content (MC) of extrudate varied from 5.16 to 21.57. The quadratic model for maltose content (MC) in terms of coded levels of variables was developed as follows:

$$MC = 6.89 - 2.66x_1 + 2.78x_4 + 1.65x_2^2 + 0.91x_3^2 + 1.39x_4^2 - 1.24x_1x_4 \dots (11)$$

The maltose content of extrudates was found to decrease with increase in tomato pomace. Increase in tomato pomace limits the availability of starch. Maltose content is directly related to the amount of starch present in the raw material. The negative effect of feed composition indicates that the highest tomato content causes the less gelatinized extrudate (Equation 11). This result is supported by trends in the expansion ratio with respect to feed composition. Increase in tomato pomace caused a decrease in expansion ratio. The expansion ratio is also a degree of gelatinization dependent factor. This might be the result of the effect of fiber in the tomato pomace. Fiber reduces the cell size, probably by causing premature rupture of gas cells, which reduces the overall expansion and results in a less porous structure (Lue et al., 2000; Aylin et al., 2008). The study showed that starch digestion (maltose content) increases with increase in temperature. Among the significant terms, barrel temperature had the highest F-value, 38.07 indicating the most influencing factor for starch digestion. The quadratic term of barrel temperature was also significant at 95% confidence level. It indicates that as extrusion temperature increases, extent of starch

conversion will be higher. Higher temperature might have favored hydrolytic breakage of starch chains along with the formation of shorter chain during gelatinization.

Study of analysis of variance of the data showed that maltose content had a significant effect of quadratic terms of feed moisture and screw speed. Positive coefficient of both quadratic terms indicated that there will be concave shaped variation in maltose content with increase in values of feed moisture and screw speed. Higher expansion was obtained at lower feed moisture and screw speed. Figure 6 shows that increasing feed moisture up to middle value decreases maltose content. There may be dominant effect of water as plasticizer for that range of moisture. However, further increase in feed moisture to positive extreme value caused increased maltose content. In this situation, there may be significant hydrolytic cleavage of the starch at elevated temperature (120 to 140°C).

The interaction terms, x_1x_4 was found to be negatively significant. It indicates that lower amounts of maltose will be obtained at higher tomato pomace level depending upon range temperature. Figure 6 shows the variation in maltose content as a function of feed composition and barrel temperature.

Effect of process variables on product lycopene content

The lycopene content of the extrudate ranged from 0.91 to 3.56 mg/100g (db). The model represents the variation of lycopene content and for further analysis.

Table 5. Analysis of variance results for fitted models.

Response	Source	Sum of squares	df	Mean squares	F-value	P-value
Maltose Content	Regression	542.8975	14	38.7784	7.838053	0.0001*
	Lack of fit	64.39502	10	6.439502	3.27985	0.1010
	Pure error	9.816763	5	1.963353		
	Residual	74.21179	15	4.947452		
	Total	617.1093	29			
Lycopene Content	Regression	11.79661	14	0.842615	25.09101	< 0.0001
	Lack of fit	0.421448	10	0.042145	2.560826	0.1555
	Pure error	0.082288	5	0.016458	0.082288	
	Residual	0.503735	15	0.033582	0.503735	
	Total	12.30035	29			
Overall acceptability (OA)	Regression	3.228657	14	0.230618	11.98093	< 0.0001
	Lack of fit	0.251175	10	0.025118	3.343932	0.0975
	Pure error	0.037557	5	0.007511		
	Residual	0.503735	15	0.033582		
	Total	3.517389	29			

* significant at P < 0.05, df: degrees of freedom.

$$\text{Lycopene content} = 2.54 + 0.57x_1 + 0.29x_3 - 0.16x_4 - 0.073x_1^2 - 0.13x_3^2 - 0.067x_4^2 + 0.12x_1x_3 - 0.13x_2x_3 - 0.085x_2x_4 \dots(12)$$

Feed composition was found to be the main factor affecting product lycopene content (Table 5). It is obvious that the tomato pomace is the only source of lycopene in the extrudate. The lycopene content of the sweet potato flour and tomato pomace extrudate increased with increase in screw speed. This may be due to lower residence time for lycopene degradation. However, significant negative effect of a quadratic term of screw speed (x_3^2) indicate a convex shaped variation in lycopene content to increase in screw speed. It means the lycopene content of the extrudate increases initially and again decreases with increase in screw speed. Decrease in lycopene content at higher screw speed might be due to input of higher amount of mechanical energy. Increase in screw speed might cause even mixing and disruption of tomato pomace exposing more lycopene to heat. Study of the data reveals that the increase in barrel temperature caused a decrease in the lycopene content of the extrudate. Although the quadratic term of barrel temperature (x_4^2) was significant only at the 90% confidence level, however, it could be said that as temperature increases the rate of lycopene destruction increases showing a convex shaped variation in lycopene content (Equation 12).

The interaction term of feed composition and screw speed (x_1x_3) had a significant positive effect on lycopene content. A positive coefficient indicates that higher

lycopene content will result to an increase in tomato pomace level and screw speed. Figure 7 shows the change in lycopene content as a function of feed composition and screw speed. Coefficient of the interaction term of feed moisture and screw speed (x_2x_3) was negative indicating that the lower lycopene content will result to lower feed moisture depending up on screw speed. Figure 7 shows the change in lycopene content as a function of feed moisture and screw speed.

Effect of process variables on product overall acceptability (OA)

The overall acceptability of the product ranges from 6.12 to 7.25 in the extrudates prepared from sweet potato flour and tomato pomace powder (Table 3). The coefficient of the model and other statistical attributes of overall acceptability are shown in Table 5. The Model F-value of 11.98 implies the model is significant. In this case x_2 , x_3 , x_4 , x_1^2 , x_2^2 , x_3^2 , x_4^2 , and x_1x_3 are significant model terms at P<0.05. Considering all the above criteria, the following model was selected for further analysis.

$$OA = 7.14 - 0.18x_2 - 0.16x_3 + 0.92x_4 - 0.15x_1^2 - 0.13x_2^2 - 0.17x_3^2 - 0.06x_4^2 + 0.07x_1x_3 \dots(13)$$

The coefficients of the linear terms x_2 and x_3 are negative which indicates that increase in feed moisture and screw speed will decrease the overall acceptability. The term x_4 positive indicate that increase in temperature will increase the overall acceptability. All the quadratic terms are significant at 95% confidence level. Of the

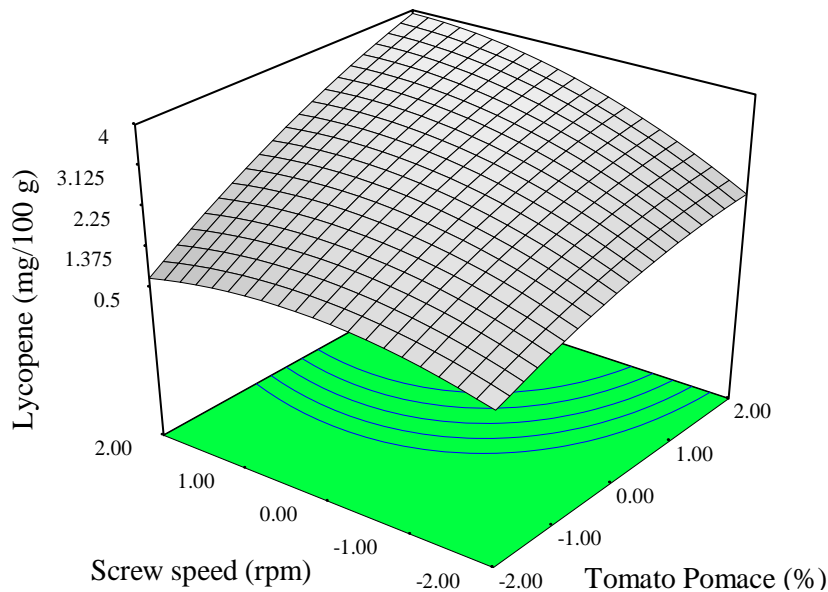


Figure 7. Response surface plot for lycopene content as a function of tomato pomace and screw speed.

quadratic terms x_1^2 , x_2^2 , x_3^2 and x_4^2 have a negative coefficient. The interaction term of tomato pomace and screw speed (x_1x_3) had a significant positive effect at 95% confidence level. Increase in feed moisture caused a decrease in the overall acceptability of the extrudate. As the quadratic term of x_3^2 is also significant with negative coefficient, it indicates that product OA will be very low with further increase in screw speed. The temperature had significant positive linear effect on product OA. It indicates that increase in barrel temperature increases product OA. This may be due to higher expansion of the extrudate. However, further increase in temperature caused slight decrease in product OA. Although there is no decrease in the expansion ratio at higher temperature, decrease in OA may be due to degradation of color pigment (lycopene). Feed composition had a significant effect only at quadratic level showing convex shaped variations on product OA. Initially, increase in overall acceptability may be due to increase in redness of product due to increase in tomato pomace level. But a further increase in tomato pomace level decreased product OA. This may be due to low expansion and increased hardness at higher pomace level (Table 5).

Optimization

A numerical multi-response optimization technique was applied to determine the optimum combination of sweet potato flour and tomato pomace in feed composition, feed moisture, screw speed and barrel temperature for the production of extrudate containing sweet potato flour and

tomato pomace. The assumptions were to develop a product which would have maximum score in sensory acceptability so as to get market acceptability, maximum expansion, minimum bulk density, and minimum hardness. Lycopene content was set as target. This is done to get 6.5 mg lycopene from 200 g extrudate. According to American Dietetic Association, incorporation of 6.5 mg lycopene per day has a protective effect over chronic diseases. Therefore, among responses, these parameters were attempted to be maintained whereas other parameters were kept within range. Under these criteria, the uncoded optimum operating conditions for development of sweet potato flour and tomato pomace extrudate were 137.01°C of barrel temperature, 343.48 rpm of screw speed, 13.86% of feed moisture and 21.31% of tomato pomace. The responses predicted for these optimum process conditions resulted to lateral expansion of 129.90%, bulk density, 0.32 g/cm³; water solubility index, 18.71; water absorption index, 4.44 g/g; hardness, 29.52; maltose content, 9.44; lycopene content, 3.01 and overall acceptability, 6.96 with desirability, 0.880. The suitability of the model developed for predicting the optimum response values was tested using the recommended optimum conditions of the variables and was also used to validate experimental and predicted values of the responses.

Conclusion

In the experiment the product responses were almost equally affected by changes in tomato pomace level, feed moisture, extrusion temperature and by screw speed.

Increase in barrel temperature resulted in maximum expansion, minimum hardness and maximum WAI. Higher tomato pomace proportion in feed composition showed minimum L value of color, minimum expansion, maximum bulk density, minimum WAI and maximum WSI whereas minimum moisture content is sought for higher crispiness, lower hardness, higher expansion and lower bulk density of developed extrudates. In this experiment, numerical optimization studies resulted in 137.01°C of barrel temperature, 343.48 rpm of screw speed, 13.86% of feed moisture and 21.31% of tomato pomace as optimum variables to produce acceptable extrudates.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Quality assessment of acha-based biscuit improved with bambara nut and unripe plantain

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Five value added biscuit products were produced from three different raw materials, namely acha (*Digitaria exilis* Staph), bambara nut and unripe plantain, at different proportions: 100:0:0% (ACH105), 80:10:10% (ACH801), 70:20:10% (ACH702), 60:30:10% (ACH603) and 50:40:10% (ACH504), respectively. The raw materials were cleaned, sorted, dried and milled into flour and used to produce biscuits. Sensory parameters evaluated were taste, texture, colour, flavour, crispness and general acceptability. Result showed that biscuit products ACH801 and ACH702 were acceptable ($p < 0.05$). Physical parameters determined were spread ratio, weight and break strength. The proximate composition for 80:10:10% acha, bambara nut and unripe plantain flour biscuit are 6.20% protein, 2.04% moisture, 20.12% fat, 2.28% ash, 2.27% crude fibre, 69.36% carbohydrate. The energy content is 483.32 Kcal/100 g. Shelf life studies on the bacterial, mould and yeast were also carried out and the results obtained showed a snack product containing nutrients required for diabetic patient with a projected shelf life of one year.

Key words: Quality, biscuit, acha, *Digitaria exilis*, bambara, unripe plantain.

INTRODUCTION

Biscuit may be regarded as a form of confectionary, dried to very low moisture content. Consumption of whole grains is an excellent source of dietary fibre and nutraceutical that are of benefit in the management of obesity and diseases such as diabetes (Jideani and Jideani, 2011). The consumption of cereal foods such as biscuit has become very popular in Nigeria especially among children. Most of these cereal foods are poor in protein content and protein quality (Alobo, 2001). Enrichment of cereal-based foods with other protein

sources such as oil seeds and has received considerable attention (Ayo et al., 2007; Dhingra and Jood, 2002; Elkhailifa and El-Tinay, 2002; Ayo and Gaffa, 2002; Ayo and Olawale, 2003). Legumes are high in lysine, an essential limiting amino acid in most cereals (Alobo, 2001).

The production of good quality biscuit would depend on selecting the correct flour for each type and appropriate processes involving steps such as mixing, aeration and fermentation, machining including laminating, baking,

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cooling and packaging (Okaka and Okaka, 2005). The introduction of composite flour into the bakery world has brought about different changes into baked products

Some of the oldest popular cereal grains of *Digitaria* spp., *Digitaria exilis* (acha) and *Digitaria iburua*, (iburu), also known as fonio or hungry rice are indigenous grains of West Africa (Jideani, 2012). Acha and iburu proteins have composition similar to that of white rice (Temple and Bassa, 1991; Jideani and Jideani, 2011), but having relatively higher sulphur amino acids (methionine and cystine) content (de Lumen et al., 1993; Lasekan, 1994; Jideani et al., 1994). These and other attributes of acha and iburu show the uniqueness of the grains and their potential in contributing significantly to whole grain diets. Acha grain can also be grounded into flour to produce biscuit (Jideani, 2012).

However, there is sufficient evidence showing that higher whole grains diet when compared with refined grain diet are beneficial for treating or managing several health problems (Jones, 2009). Recent research has shown that acha helps diabetic patients to recover due to its low glycemic index (Balde et al., 2008). The *in vitro* starch digestibility and glycemic property of acha, iburu and maize porridge has been investigated (Jideani and Podgorski, 2009). The study showed that the total starch (TS) for maize, acha and iburu flours were 45.3, 43.6 and 41.5%, respectively. The resistant starch (RS) was 2.9, 2.1 and 1.2, respectively for maize, acha and iburu flours and the digestible starches (DS) 43.7, 41.4 and 40.0%. The authors concluded that acha and iburu may have potential in a low GI food as porridge from both grains had low estimated value of 40. Low-GI diets may improve both glycemic control and cardiovascular risk factors for patients with type 2 diabetes (Jenkins et al., 2008).

Bambara nut (*Vigna subterranea*) is an important legume grain in semi arid Africa. It is resistant to high temperature and suitable for marginal soil where other leguminous crops cannot be grown (Baryeh, 2001). Bambara nut is an important source of high protein value for poorer people in Africa who cannot afford expensive animal protein (Baryeh, 2001; Stading, 2006).

Plantain is the common name for herbaceous plants of the genus *Musa*. Plantains are classified formally as *Musa acuminata* and *Musa balbisiana* depending on their genomic constitution. It provides more than 25% of the carbohydrate requirements for over 70 million people and tends to be firmer and lower in sugar content. Plantains are commonly cooked or otherwise processed and are used either when green or unripe (starchy) or over ripe (sweet) (Oke et al., 1998). An average plantain has about 220 calories and is a good source of potassium and dietary fiber (Randy et al., 2007). It is rich in carbohydrate, dietary fiber, iron, vitamins and minerals. It is ideal for diabetics, children and pregnant women and can also be a good supplement for marasmus patients. Plantain contain small amount of serotonin which has the ability to dilate the arteries and improve blood circulation.

Its regular consumption helps to cure anemia and maintain a healthy heart (USDA, 2010). A diet of unripe plantain is filling and can also be a good inclusion in a weight loss diet plan (Oke et al., 1998).

Many countries have produced composite product using other cereals like rice, sorghum, acha, bambara nut, maize and starchy root. Biscuit may be classified either by the degree of enrichment and processing or by the method adopted in shaping them (Okaka and Okaka, 2005). Wheat flour blended with acha flour up to 30% has shown no significant difference in terms of sensory qualities (Ayo and Nkama, 2003). Acha-wheat flour has been fortified with soybean flour to improve the nutritional value when used for production of biscuit (Ayo et al., 2007). Acha-malted soybean has been used for the production of bread and biscuits (Ayo et al., 2014). Acha-based biscuit improved with bambara nut and unripe plantain is a good and cheap source of nutrient than wheat flour used by the baking industry.

Despite the inherent potentials of acha, bambara and unripe plantain, little has been done to incorporate them in most food formulations. The objectives of this research work are (i) production and packaging of acha biscuit, improved with bambara nut and unripe plantain at different proportions, (ii) evaluation of the physical properties of the biscuits, (iii) determination of acceptability of the biscuits and (iv) evaluation of the chemical composition of the acha-based biscuits and microbiological properties of the best two biscuits.

MATERIALS AND METHOD

Source of material

Acha grain (*D. exilis*), bambara nut (*V. subterranea*), unripe plantain (*M. paradisiaca*), Royal baking powder, evaporated peak milk, Simans baking fat, Dangote granulated sugar, and salt were purchased from Wunti Market Bauchi, Nigeria. The equipment and chemicals used were of analytical grade.

Preparation of acha and bambara nut flours

Acha (*D. exilis*) grains were washed with clean water several times in a container in order to remove tiny stones and dust as well as foreign materials. The bambara nut (*V. subterranea*) were dry clean to remove shells and other foreign material such as stones in the grain. Both acha and bambara nut were drained and sun-dried, milled using hammer mill (CD1155-3-1317) and sieved with the aid of a 425 µm sieve (Endecotts Ltd, London, England). The flour was packaged in polyethylene bag and stored at low temperature as acha or bambara flour

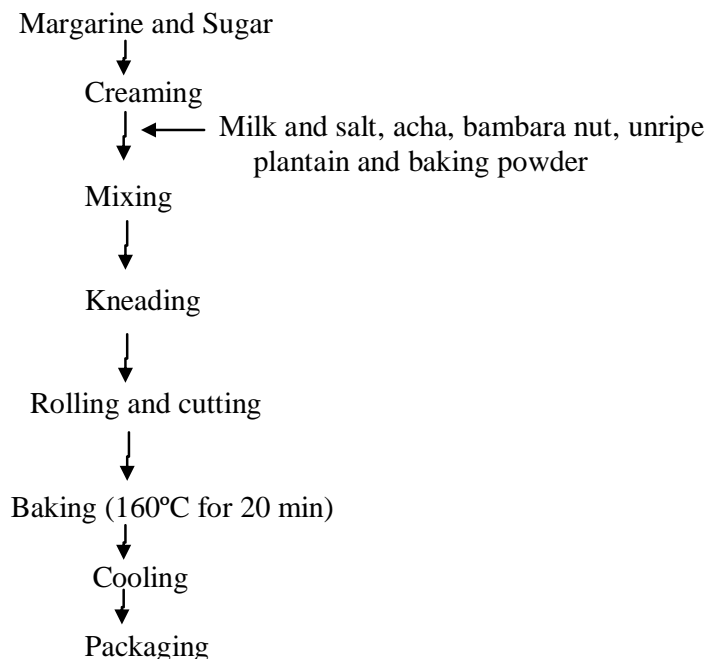
Preparation of unripe plantain flour

Unripe matured plantain (*M. paradisiaca*) was washed with clean water and peeled with stain less knife to separate skin from fruit. The peeled fruit was sliced to equal size and dried under the sun. The drying, milling, sieving and storage were as in acha and bambara nut flours.

Table 1. Recipe (%) for acha-based biscuit samples.

Sample code	Acha flour	Bambara nut flour	Unripe plantain flour	Fat	Egg	Sugar	Salt	Baking powder
ACH105	100	0	0	49	30	45	0.6	3.6
ACH801	80	10	10	49	30	45	0.6	3.6
ACH702	70	20	10	49	30	45	0.6	3.6
ACH603	60	30	10	49	30	45	0.6	3.6
ACH504	50	40	10	49	30	45	0.6	3.6

Source: Department of Food Technology, Kaduna Polytechnic, Kaduna, Nigeria (2004).

**Figure 1.** Production of acha, bambara nut and unripe plantain biscuit.

Production of acha, bambara and unripe plantain flour biscuit

The essential ingredients for the production of composite biscuit and their various proportions are shown in Table 1. The acha, bambara nut and unripe plantain flours were used at various proportions while the milk, fat, baking powder, sugar and salt were the same for all the proportions. The recipe for the composite biscuit was chosen based on the Department of Food Technology Kaduna Polytechnic, Kaduna, Nigeria (2004) manual with slight moderations. The flow chart for production of composite biscuit from acha, bambara nut and unripe plantain is shown in Figure 1. After weighing with an electric weighing balance (Sauter, RC 8021 model), the fat was manually mixed vigorously with sugar for 10 min to form a cream. The acha, bambara nut and unripe plantain flours at different levels as shown in the recipe in Table 1 was added with the other ingredients like salt, baking powder. The mixing was done properly and the method of Okaka (1997) was used to produce the biscuits.

Physical analysis

For spread ratio, two rows of five well formed biscuits were made and the height measured. They were also arranged horizontally

edge to edge and the sum of the diameters measured. The spread ratio was calculated as diameter divided by height (Gomez et al., 1997). Break strength was determined using break strength device (Okaka and Isieh, 1990). Biscuit sample of 0.4 cm thickness was placed centrally between two parallel metal bars 2 cm apart and weights were applied until the biscuit snapped. The least weight that caused the breaking of the biscuit was regarded as the break strength of the biscuit.

Sensory evaluation of the biscuit samples

The biscuits were subjected to sensory evaluation using twenty panelists from the Federal Polytechnic, Bauchi, Nigeria based on their familiarity with the product. The products, appropriately coded and of the same size and temperature ($29 \pm 3^\circ\text{C}$) were placed in white plastic plates. The panelist rinsed their mouths with bottled water after tasting each sample (Larmond, 1977) served to the in different boots under the florescent light. A nine-point Hedonic scale with one (1) representing "extremely dislike" and nine (9) "extremely like" was used. The qualities assessed were color, texture, taste, flavor, crispness and general acceptability (Akinjayeju, 2009).

Although, the panelists were not trained, their selection was based on basic requirements of a panelist, such as availability for

Table 2. Physical quality of acha-based biscuit samples.

Sample code	Acha flour (%)	Bambara nut flour (%)	Unripe plantain flour (%)	Weight (g)	Spread ratio	Break strength (g)
ACH105	100	0	0	10.0	9.15	900
ACH801	80	10	10	10.0	9.30	600
ACH702	70	20	10	9.50	9.50	700
ACH603	60	30	10	9.50	10.32	700
ACH504	50	40	10	9.20	11.22	850

the entire period of evaluation, interest, willingness to serve, good health (not suffering from colds), not allergic or sensitive to the products evaluated (Penfield and Campbell, 1990).

Proximate composition

The moisture, protein, fat, ash and crude fibre contents were determined according to AOAC (2000). The total carbohydrate (CHO) was determined by difference: CHO = 100 – (% moisture + % protein + % fat + % ash). Food energy (kcal/100 g) was calculated according to the method of Marero et al. (1988) using the factor: [(4 x % Protein) + (4 x % Carbohydrate) + (9 x % Fat)]

Microbiological analysis

The pour plate method was adopted as described by Jideani and Jideani (2006). Nutrient agar and potato dextrose agar were prepared as specified by the manufacture for bacteria and fungi respectively. Serial dilution was carried out on suspension of the biscuit samples using quarter strength peptone water before plating on the media. Incubation was done for 24 h at 37°C for bacteria, and 3 to 4 days for yeast and mould at room temperature of 25°C. The colonies were counted using an electronic colony counter (Gallenkamp, 443 300 66087, UK).

Statistical analysis

The results obtained were subjected to analysis of variance (ANOVA) and Duncan multiple range test (Duncan, 1955) was used to separate means where significant differences existed. The software used for the statistical analysis was MINITAB ver. 16.

RESULTS AND DISCUSSION

The physical analysis of the biscuits is shown in Table 2. The weight of the biscuits ranged from 9.2 - 10.0 g. The control sample ACH105 (100:0:0% acha, bambara nut and unripe plantain flour biscuit) had the highest weight. The weight of the biscuits decreased with increase in bambara nut flour with sample ACH801 (80:10:10% acha, bambara nut and unripe plantain flour biscuit) having the highest weight and sample ACH504 (50:40:10% acha, bambara nut and unripe plantain flour biscuit) having the lowest. The decrease in weight could be due to the increase in the fat content of the blended bambara nut flour, as fat is lighter in weight (Ayo et al.,

2007). These results were similar to those for bambara groundnut-maize flour (Akpapunam and Darbe, 1994), millet-sesame flour (Alobo, 2001) and amaranth-wheat flour (Ayo, 2001).

The spread ratio of the biscuit increased from 9.30 - 11.22. The increase is an indication of the binding properties of the flour and of the texture of the biscuits. The increase in the fats content (Table 4) could also affect the spread ratio (Ayo et al., 2007). The break strength decreased from 900 - 600 g with increase in percentage of bambara nut flour. The decrease could be due to the increase in the percentage of fats (19.70-20.12%) with increase in percentage of bambara nut flour added, diluting the protein and carbohydrate level which are the principal compounds responsible for hardness in biscuits (Okaka and Isieh, 1990).

The mean scores for the sensory evaluation of the biscuits are shown in Table 3. There was no significant difference ($p < 0.05$) in crispness and flavour of the biscuits while significant differences ($p < 0.05$) existed in taste, texture, colour and overall acceptability. The biscuits compared favourably with 100% acha biscuit which significantly differed ($p < 0.05$) from the other biscuits except in flavor and crispness.

There was a general decrease in the mean scores of all the parameters with increase in bambara nut and unripe plantain flours except for texture which increased. Sample ACH801 (80:10:10% acha, bambara nut and unripe plantain flour biscuit) had the highest mean value in most of the parameters monitored while sample ACH105 (100:0:0% acha, bambara nut and unripe plantain flour biscuit) had the lowest mean value in all the parameters with the exception of texture. The crust texture was related to the external appearance of the biscuit top, which is the smoothness or roughness of the crust (Ayo et al., 2007). The texture reduced from 7.30 for 100% acha flour biscuit to 6.85 for 80:10:10% acha, bambara nut and unripe plantain flour biscuit and gradually increased as the proportion of bambara nut and unripe plantain flour increased.

There was a general decrease in the overall acceptability of the biscuits with decrease in acha flour and increase in bambara nut flour and unripe plantain flour. Sample ACH801 (80:10:10% acha, bambara nut and unripe plantain flour biscuit) had the highest mean

Table 3. Sensory evaluation of the biscuit samples.

Sample code	Proportion of flours (%)			Taste	Crispness	Flavour	Texture	Colour	Overall Acceptability
	Acha	Bambara nut	Unripe plantain						
ACH 105	100	0	0	6.80 ^a ± 1.39	7.15 ^a ± 1.53	6.35 ^a ± 1.63	7.30 ^a ± 1.46	6.95 ^a ± 1.43	6.90 ^{ab} ± 1.58
ACH 801	80	10	10	7.30 ^a ± 1.63	7.20 ^a ± 1.80	7.25 ^a ± 1.48	6.85 ^a ± 1.95	7.50 ^a ± 1.61	8.00 ^a ± 0.85
ACH 702	70	20	10	7.00 ^a ± 1.65	7.05 ^a ± 2.01	6.85 ^a ± 1.42	7.05 ^a ± 1.28	7.40 ^a ± 1.67	7.50 ^{ab} ± 1.49
ACH 603	60	30	10	6.95 ^a ± 1.82	7.50 ^a ± 1.64	6.85 ^a ± 1.63	7.25 ^a ± 1.21	7.30 ^a ± 1.56	6.40 ^b ± 1.78
ACH 504	50	40	10	6.35 ^a ± 1.60	7.05 ^a ± 2.04	6.65 ^a ± 1.76	7.35 ^a ± 1.46	7.25 ^a ± 1.83	6.80 ^{ab} ± 1.79

Values are mean ± standard deviation of twenty panelists. Means within each column not followed by the same superscript are significantly different ($p < 0.05$) from each other using Duncan multiple range test.

Table 4. Proximate (%) and energy compositions of acha based biscuit improved with bambara nut and unripe plantain.

Parameter	Samples				
	ACH 105	ACH 801	ACH 702	ACH 603	ACH 504
Acha	100	80	70	60	50
Bambara nut	0	10	20	30	40
Unripe Plantain	0	10	10	10	10
Moisture	2.06 ^a ± 0.05	2.04 ^a ± 0.10	2.03 ^a ± 0.00	2.08 ^a ± 0.01	2.07 ^a ± 0.09
Protein	5.30 ^d ± 0.13	6.20 ^c ± 0.02	6.43 ^c ± 0.11	7.00 ^b ± 0.15	7.51 ^a ± 0.03
Fat	19.86 ^a ± 0.14	20.12 ^a ± 0.02	20.10 ^a ± 0.07	20.08 ^a ± 0.06	19.70 ^a ± 0.57
Ash	2.31 ^d ± 0.02	2.28 ^d ± 0.14	2.67 ^c ± 0.06	3.41 ^a ± 0.02	3.09 ^b ± 0.05
Crude fibre	3.56 ^a ± 0.05	2.27 ^{ab} ± 0.02	2.43 ^a ± 0.06	2.08 ^b ± 0.04	2.40 ^a ± 0.13
Carbohydrate	71.47 ^a ± 0.15	69.36 ^{ab} ± 0.15	68.77 ^{abc} ± 0.06	67.43 ^{bc} ± 0.06	67.63 ^{bc} ± 1.02
Energy (Kcal/100 g)	485.82 ^a ± 1.22	483.32 ^a ± 0.56	481.70 ^{ab} ± 0.83	478.44 ^b ± 0.21	477.86 ^b ± 1.75

Values are mean ± standard deviation. Any mean not followed by the same letter on each column are significantly different ($p < 0.05$).

value of 8.0 in overall acceptability which made the biscuit most acceptable (Figure 2). This is an indication that the improvement was acceptable to the consumers. Therefore, the two best biscuits based on the overall acceptability of sensory properties were ACH801 and ACH702 (Table 3).

The proximate and energy compositions of acha

biscuit improved with bambara nut and unripe plantain flours are shown in Table 4. The moisture content (%) of the acha based biscuit increased from 2.03-2.08 with addition of bambara nut and unripe plantain. The low moisture content of the biscuit will require a unique packaging material to prevent reabsorption of moisture. The protein

content (%) of the biscuit increased from 5.30 - 7.51 with increase in bambara nut and unripe plantain. Sample ACH105 had no addition of bambara nut hence had lowest protein content. Bambara nut is a rich source of protein. Legume was used and they are known to be high in both fat and protein (Dhringa and Jood, 2002). The fat



Figure 2. Acha based biscuit (80:10:10% acha, bambara nut and unripe plantain flours) in polyethylene bag and paperboard.

content (%) ranged from 19.70 - 20.12 with increase in bambara nut and because of added baking fat. The baking fat could be reduced in subsequent baking as to elongate the shelf life of the biscuits. The ash content (%) increased from 2.28 - 3.09 with increase in bambara nut and added unripe plantain flour which is a rich source of mineral.

Ash content indicates the presence of mineral matter in food. Ash is a non organic compound containing the mineral content of food. It aids in the metabolism of other compound such as protein fat and carbohydrate (Okaka and Ene, 2005). The crude fibre (%) decreased from 3.56-2.08 with increase in bambara nut and unripe plantain flour. Also, decrease was observed in the carbohydrate and energy contents.

The microbial analysis for first week showed no growth in bacteria, mould and yeast. This could be because the

product is freshly produced and packaged but growth began at the second week with 1.0×10^4 cfu/g for sample ACH 801 and at fourth week it increased to 3.5×10^4 cfu/g (Table 5). This increase could be due to make up of the product which contained protein. From the result, it could be concluded that mould and yeast are the possible spoilage organism associated with dry product. Bacteria growth in the product was as a result of handling during processing.

Conclusion

High quality biscuit was produced from acha, bambara nut and unripe plantain. The addition of bambara nut and unripe plantain improved the physical, proximate and sensory quality of acha biscuit. Nutritionally, the protein

Table 5. Microbial count of the two acceptable biscuit.

Sample	No. of viable count		
	Weeks	Bacteria (cfu/g)	Mould/yeast (cfu/g)
ACH801 (80% acha, 10% bambara nut & 10% unripe plantain)	1 st	Nil	Nil
	2 nd	1.0 x 10 ⁴	1.0 x 10 ⁴
	3 rd	2.0 x 10 ⁴	3.0 x 10 ⁴
	4 th	2.1 x 10 ⁴	4.0 x 10 ⁴
ACH702 (70% acha, 20% bambara nut & 10% unripe plantain)	1 st	Nil	Nil
	2 nd	1.0 x 10 ⁴	2.0 x 10 ⁴
	3 rd	2.0 x 10 ⁴	3.0 x 10 ⁴
	4 th	3.5 x 10 ⁴	5.0 x 10 ⁴

content increased from 5.30 - 7.51%, fat from 19.86 - 20.12%, ash 2.28 - 3.09%, crude fibre 2.27 - 2.39% at different proportions. The sensory qualities were significantly different with addition of unripe plantain and bambara nut flours. For ACH801 (80: 10: 10% acha, bambara nut and unripe plantain flours), the biscuit was generally acceptable. The production of biscuit from acha could reduce the use of wheat importation for baking. The authors recommend acha based biscuit improved with bambara nut and unripe plantain for both children and diabetic patients.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Effect of three processing methods on some nutrient and anti-nutritional factor constituent of *Colocasia esculenta* (Amadumbe)

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Colocasia esculenta L. Schott (Amadumbe) is a major starchy food crop used in the rural areas in Kwazulu-Natal province, South Africa. Like most root crops, Amadumbe is rich in carbohydrate content with low protein and lipids. Preliminary screening of Amadumbe revealed the presence of some anti-nutrients (amylase inhibitor, trypsin inhibitor, oxalate, alkaloids, saponin, phytate and total phenols). The effect of various domestic processing (boiling, roasting and frying) on the levels of the anti-nutrients in Amadumbe tubers was investigated. A 'white' and 'purple' (local denomination) Amadumbe variety was used. Anti-nutrients were reduced (6-90%) by the domestic processing techniques. Of the three different treatments, boiling appeared to be the most effective in reducing levels of all the investigated anti-nutrients in both Amadumbe varieties. It is therefore concluded from the results of this study that the anti-nutritional factors, though present in raw tubers, should not pose a problem with regard to human consumption if the tubers are properly processed.

Key words: Anti-nutrients, processing, *Colocasia esculenta*.

INTRODUCTION

Vegetables synthesize and store certain biologically active substances named anti-nutritional factors (Bhandari and Kawabata, 2004). Anti-nutrients of plant foods like legumes (Soetan and Oyewole, 2009; Mohamed et al., 2011), cassava (Montagnac et al., 2009; Ebuehi et al., 2005), wild yam (Shajeela et al., 2011), sweet potato and potato (Olayiwola et al., 2009; Burlingame et al., 2009) have been investigated. Their presence in food give rise to a genuine concern for human health in that they prevent digestion and absorption of essential nutrients (Mohamed et al., 2011;

Offor et al., 2011). Most anti-nutritional factors are heat labile and are partially inactivated during ordinary cooking (Prathibha et al., 1995). The residual anti-nutrients can, however, be responsible for the development of serious gastric distress (Brune et al., 1989). Prolonged eating of residual anti-nutrients have also been linked to the etiology of goiter (FAO, 2013), tropical ataxic neuropathy (FAO, 2013; Sarkiyayi and Agar, 2010), inactivating digestive enzymes (Akande et al., 2010), lowering the bioavailability of nutrients (Duranti, 2006; Hotz and Gibson, 2007; Barde et al., 2012), nausea,

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bloating and vomiting (Liener, 1986).

Amadumbe (zulu for *Colocasia esculenta*) is an introduced species widely grown in the sub-tropical parts of South Africa as a subsistence crop (Makgoba, 2004; Shange, 2004). It is commonly used in rural communities in Zululand as a source of dietary starch. The tubers are processed by baking, roasting, or boiling; and the leaves are processed like spinach by boiling them for about 15 min, the leaves are also used in making salad. An active α -amylase inhibitor in Amadumbe has been isolated and characterized (McEwan et al., 2010). Little information is available on the residual levels of anti-nutrients in Amadumbe. It is therefore pertinent that this under-utilized crop be investigated for the nutritional and anti-nutritional components.

The present study examined the effects of domestic processing on the anti-nutrient levels of Amadumbe. A 'white' and 'purple' (local denomination) Amadumbe variety was used (Ferrerres et al., 2012). Domestic cooking or roasting alters the nature and bioavailability of many food constituents (Suresh et al., 2006).

MATERIALS AND METHODS

Sample collection

Purple and white varieties of Amadumbe (*Colocasia esculenta*) tubers were obtained from the local market at Esikhawini, KZN province, South Africa. Tubers were washed under tap water, peeled and cut into 1cm³ pieces. These were then divided into four portions. One portion (the unprocessed sample) was dried at 55°C for 24 h, milled into a fine powder, and stored in brown bottles until use. The other three portions were separately processed (boiled, fried and roasted).

Processing techniques

500 g of the samples were separately subjected to the following processing techniques: i) boiling: 500 g of 1 cm³ Amadumbe pieces were boiled in distilled water on a stove for 30 min; ii) frying: 500 g of 1cm³ pieces were fried in 30 ml Sunflower vegetable cooking oil for 15 min; iii) roasting: 500 g of 1 cm³ Amadumbe pieces were roasted in a baking pan in an oven for 30 min at 180°C.

The processed samples were dried at 55°C for 24 h after which they were milled into a fine powder and stored in 500 ml glass laboratory bottles until use. The samples were then screened for the presence of nutrients and anti-nutrients.

Proximate composition

Protein, moisture, carbohydrate, ash and crude fat contents were determined as described in AOAC methods (AOAC, 1984).

Determination of anti-nutrients

Trypsin inhibitor

The method of Kakade et al. (1974) was used to determine the anti-

trypsin activity. Trypsin activity was measured by using benzoyl-DL-arginine-*p*-nitroaniline (BAPNA) as substrate in the presence and absence of sample extract. *p*-Nitroanilide released was measured spectrophotometrically at 410 nm. Trypsin inhibitor activity was therefore expressed as the decrease in trypsin activity per unit weight of sample, using the formula:

$$\text{TIA} = 2.632 \cdot D \cdot \Delta t/S \text{ mg pure trypsin inhibited g}^{-1} \text{ sample}$$

D is the dilution factor, Δt is the change in absorbance and S is the amount of sample weighed out.

Amylase Inhibitor

α -Amylase and α -amylase inhibitory activities were estimated according to the method utilized by Bernfeld (1955). Amylase inhibitor extracts were mixed with amylase and incubated for 30 min at 37°C. The reaction was started by adding extract-enzyme mixture to test tubes containing buffered starch solution (2 mg starch in 20 mM phosphate buffer of pH 6.9 containing 0.4 mM NaCl) and was incubated for 20 min. This reaction was terminated by adding 3,5-dinitrosalicylic acid (DNS) reagent to the assay mixture. The assay tubes were kept in a boiling water bath for 5 min, cooled under tap water and the colour formed by maltose oxidation was measured at 530 nm. Controls without inhibitor were run simultaneously. One α -amylase unit (1UI) was defined as the amount of enzyme that will liberate 1 μ mol of maltose from the starch under the assay conditions (10 min, 37°C, pH 6.9). The amylase inhibitor activity (AIA) was determined as the percentage decrease in α -amylase activity (at the stated conditions) in the presence of Amadumbe extracts.

Total polyphenols

Total polyphenols were determined according to Prussian blue spectrophotometric method (Price and Butler, 1977). The total phenols were extracted into 2 M HCl. Timed additions were done to the extracted 1 ml sample with 0.10 M FeNH₄(SO₄)₂ and 0.008 M K₃Fe(CN)₆ to develop colour. Absorbance was measured spectrophotometrically at 720nm. The total phenol concentration was estimated from the gallic acid standard curve.

Alkaloid

Amadumbe alkaloids were detected by the method of Harborne (1973). 1 ml Amadumbe sample were covered for 4 h in 10% acetic acid in ethanol. This was filtered and extract was concentrated. Precipitation was done by adding concentrated ammonium hydroxide and then it was washed with dilute ammonium hydroxide. The alkaloid residue was dried and weighed.

Oxalate

Oxalate was determined by the method of Munro and Basir (1969). Oxalate was extracted from the sample (1:5 w/v) with 0.15% citric acid and treated with tungstophosphoric acid. Precipitated oxalate was solubilized with 1% hot diluted H₂SO₄ and titrated against KMnO₄ as equivalent to 0.3 g/100 ml of calcium oxalate.

Phytate

The colorimetric assay of phytate was performed according to

Table 1. Nutritional composition (g/100%DM) of unprocessed as well as processed Amadumbe tubers from Esikhawini local market, Zululand.

Parameter	Moisture	Ash	Crude fat	Crude proteins	Carbohydrates	
					Starch	Soluble sugar
Esikhawini white	89	4.4	0.8	5.04	28	4.0
Boiled white (BW)	88	4.4	0.78	5.04	25	3.0
Roasted white (RW)	84	3.6	1.58	6.87	27	2.0
Fried white (FW)	85	4	10.58	4.2	18	2.7
Esikhawini purple	89	3.3	0.28	4.5	25	2.0
Boiled purple (BP)	88	3.2	0	4.56	22	3.1
Roasted purple (RP)	87	4	2.52	4.36	20	2.5
Fried purple (FP)	85	3.2	15.05	3.95	15	2.0

Mehlich N^o. 1 double acid extraction for metals (Mehlich, 1953). Double acid extraction (HCl and H₂SO₄) was done on sample materials for 3 h. Samples were filtered under vacuum through Whatman N^o. 1 filter paper. Colour was developed by adding ammonium molybdate, ascorbic acid and potassium antimonytartrate and absorbance was measured at 820 nm. The concentration of phytate was calculated from its phosphorus content.

Total cyanogens

Cyanogens were assayed enzymatically by the method described by O'Brien et al. (1991). Amadumbe samples were homogenized (1:5 w/v) with orthophosphoric acid/ethanol (1:1) medium. An aliquot of extract was added to buffer A prepared from 0.1 M H₃PO₄ and Na₃PO₄, and β-glycosidase with activity of 5 EU ml⁻¹. Colorimetric procedure for total cyanogens was followed with pyridine/pyralozone reagent. Absorbance was measured at 620 nm.

Saponin

Saponin content was determined by the method of Fenwick and Oakenfull (1981). Saponin was extracted from 10 g Amadumbe for 24 h in a reflux with acetone. Re-extraction with methanol in the Soxhlet apparatus was done for another 24 h. Colour development was done with vanillin in ethanol and sulphuric acid. Absorbance was measured at 500nm.

RESULTS

Proximate composition

The data for the processed and the unprocessed samples are presented in Table 1. Water content was high in the investigated starchy staples which on average ranges between 84-89%. The ash content was between 3.2 and 5.4% of the dry weight elements. The lipid content for these unprocessed corms ranged between 0.73 and 1.54%. The crude fat content for the fried Amadumbe tubers was understandably higher (10.58 and 15.05% respectively), than the mean crude fat value for the boiled and roasted samples (1.15%) as well as for the unprocessed samples (0.9%). The chemical composition

of the unprocessed *C. esculenta* tubers reveals high levels of starch (28-16%), the predominant component of dry matter. Processing decreased the total carbohydrate content of the Amadumbe tubers.

Anti-nutritional factors

The levels of anti-nutritional factors in the locally grown Amadumbe's are also very important in the assessment of their nutritional status. The results for unprocessed and processed Amadumbe tubers are presented in Table 2. Amadumbe was found to contain α-amylase and trypsin inhibitor, total phenols, alkaloids, oxalates, phytates, cyanogens and saponin.

The results reveal that there was a significant reduction in the anti-nutritional compounds (except for oxalate) during domestic processing. The highest losses of oxalate (0.13 mg.g⁻¹ decreased to 0.06 mg.g⁻¹ – 54%) occurred with boiling the white Amadumbe sample and there was an increase in oxalate content with roasting and frying. The highest losses of most of the anti-nutrients occurred with boiling the Amadumbe samples in water.

DISCUSSION

In general, variations were observed in the proximate composition values obtained between the two varieties as well as the processed samples. Such variations between the two varieties have been ascribed to differences in the genetic background as well as climate, season, and the agronomic factors (Onwueme, 1982). Apart from the soluble sugar, all the proximate indices measured in the Amadumbe corms were similar to most other taro or cocoyam species (Bradbury and Holloway, 1988; Pérez et al., 1998; Sefa-Dedeh and Agyir-Sackey, 2004). For each Amadumbe variety, processing methods showed no significant effects on the moisture, ash and protein contents. Fried tubers had a higher level of fat (10.58 and 15.05% respectively) than raw or cooked tubers in this

Table 2. The levels of some anti-nutritional factors in processed and unprocessed tubers (*Colocasia esculenta*) from Zululand.

Anti-nutrients (mg.g ⁻¹)	Esikhawini		Boiled		Roasted		Fried	
	White	Purple	White	Purple	White	Purple	White	Purple
Trysin Inhibitor	19.7	16.5	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0(100)
Amylase Inhibitor	21	25	11 (48)	9 (64)	5 (76)	7 (72)	6 (71)	6 (76)
Total phenols	11.5	13.0	7.1 (38)	9.8 (24)	10.0 (13)	12.2 (6)	10.8 (6)	14.0 (0)
Alkaloids	0.19	0.18	0.04 (79)	0.06 (67)	0.04 (79)	0.08 (56)	0.13 (32)	0.04 (78)
Oxalates	0.13	0.10	0.06 (54)	0.06 (40)	0.14 (0)	0.21 (0)	0.14 (0)	0.1 (0)
Phytates	3.1	2.8	1.3	1.4	1.1	1.8	0.8	0.8
Cyanogens	0.012	0.025	0.001 (92)	0.008 (68)	0.003 (75)	0.003 (88)	0.002 (83)	0.006 (76)
Saponin	0.136	0.145	0.056	0.052	0.079	0.10	0.123	0.995

^aFigures in parentheses indicate the percentage decrease over the values of the corresponding raw tuber.

investigation and the changes in fat content of the fried products were due to fat being absorbed from the frying medium (oil). The differences in processing may therefore be due to variations in other components (Afoakwa and Sefa-Dedeh, 2001). The high moisture and carbohydrate content and the low amounts of fat and protein are typical of most root crops (FAO, 2013). Tuber and root crops are generally rich in carbohydrates, and Amadumbe are mainly consumed as a cheap but good source of carbohydrate (Alcantara et al., 2013).

The nutritional importance of any food product depends not only on the nutrient composition but also on the presence of anti-nutritional factors. The higher the concentration of these metabolites the more dangerous they become to health. The essence of estimating the concentrations of these secondary plant metabolites is to establish the amount of anti-nutrient levels in Amadumbe's consumed.

When the values were compared with other works for taro, cocoyam and yam, the oxalate (Lewu, 2010; Offor et al., 2011; Alcantara et al., 2013) and cyanogen (FAO, 2013; Amanze, 2009) content were observed to be low, while total phenols (Offor et al., 2011; Alcantara et al., 2013) and phytate (Lewu, 2010; Polycarp et al., 2012; Alcantara et al., 2013) were considerably higher. It was difficult to compare the amylase inhibitor activities for taro, cocoyam and other tubers reported by different investigators, mainly because of the difference in method used. The initial trypsin inhibitor level in the Amadumbe tubers were completely inactivated with all three domestic processing methods. A similar observation of inactivation with cooking of the trypsin inhibitors of taro was reported (Sasi Kiran and Padmaja, 2003). The drastic reduction of trypsin inhibitor activity (TIA) values on cooking could possibly be due to high heat treatments during cooking of tubers and the well established heat labile nature of trypsin inhibitor (Prathibha et al., 1995; Bradbury et al., 2006). The reduction of TIA is expected to enhance the proteins digestibility of the Amadumbe tubers. The processing methods reduced on average 48-76% of the initial α -amylase inhibitors. It is apparent that these

inhibitors (α -amylase) were more heat stable than the trypsin inhibitors. It has been reported that amylase inhibitors persist through cooking temperatures despite their susceptibility to heat inactivation (Liener and Kakade, 1980).

The highest losses of oxalate (0.13 mg.g⁻¹ decreased to 0.06 mg.g⁻¹; 54%) occurred with boiling the white Amadumbe sample (Table 2). It is apparent that boiling caused considerable cell rupture and facilitated the leakage of soluble oxalate into cooking water (Albihn and Savage, 2001; Luma and Katongole, 2011). When the samples were roasted and fried, there was an increase in oxalate content. This apparent increase could be related to the relative increase in dry matter as Amadumbe is roasted and fried. Similar cooking studies on oca (*Oxalis tuberosa*) showed that boiling considerably reduced the oxalate concentration in the whole tuber, while baking increased the concentration of soluble oxalates in the cooked tissue (Albihn and Savage, 2001). The phytate content of the investigated tubers got reduced up to 74% when processed. The apparent decrease in content of phytic acid during cooking may be partly due to leaching into the cooking medium or degradation by heat or the formation of insoluble complexes between phytate and other components such as phytate-protein and phytate-protein-mineral complexes (Siddhuraju and Becker, 2001; Mohamed et al., 2011). Reduction of phytate is expected to enhance the bioavailability of proteins and dietary minerals of the Amadumbe tubers. The processing techniques were also highly effective in substantially reducing the cyanogens to low levels (0.1-0.8 mg HCN equivalent/100 g). Boiling for 30 min were found to be highly effective in reducing the HCN by up to 92 and 68% respectively. The reduction of hydrogen cyanide due to boiling may be as a result of the fact that free cyanide and bond cyanide are both water soluble and hence may be leached out during boiling (Udensi et al., 2007).

This study shows that the tuber of *C. esculenta*, (Amadumbe), could be used more as food material for human consumption, judging from the high carbohydrate, adequate protein and low lipid content. The results

indicate that the studied anti-nutritional factors, though showing a significant concentration in raw tubers, should not pose a problem in human consumption if the tubers are properly processed. The reduction of these anti-nutrient levels on processing is expected to enhance the nutritional value of these Amadumbe tubers.

Consumption of such properly cooked Amadumbe tubers may serve an additional dietary carbohydrate source for the rural people living in Zululand. However, the reductions in anti-nutrients which constitute the undesirable properties may imply possible reduction in the levels of nutrients and mineral elements. It is believed that supplementation from other sources such as vegetables usually eaten with the cooked Amadumbe, could improve the levels of these elements.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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