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African Journal of Food Science

Full Length Research Paper

Physicochemical characterization of epicuticular coating of apples sold in Abuja Nigeria

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Most of the apples consumed across Nigeria are imported from South Africa, Europe and United States of America. Despite the epicuticular hydrophobic wax naturally produced by the fruit which makes it resistant to external spoilage factors, extra coating is usually employed by producers for protection against biotic and abiotic attack during storage in order to extend shelf-life. Nowadays, Nigerians have raised concerns on the nature, level and safety of these wax coatings in apples available in Nigerian market. The results of organoleptic, physicochemical and Fourier transfer infrared spectroscopy (FTIR) analyses suggested that the epicuticular waxes of apples in Abuja markets are mainly from petrochemical sources with adulterants from waxes of vegetable sources or beeswax. The mineral concentration of Cu, K, Mg, Mn, Zn and Pb in waxes per average sized apples (mg/g) ranged from 1.73 to 15.66, 10.80 to 1357, 26.46 to 173.12, 0.11 to 0.22, 2.05 to 41.80 and 3.78 to 11.66 mg/g, respectively. The results indicated hundred (100%) of the samples contained copper (0.02 mg/g) and zinc (0.05 mg/g), and sixty percent (60%) contained lead above WHO permissible limit (0.01 mg/g). It is however recommended that the layer of coating should be to reduce apple growers or sellers to the barest minimum to avoid over-coating and the amount of waxes consumed per apple. Waxes can be removed from apple by dipping the apple in hot water for a few seconds to remove the wax or washing with pure white vinegar and rinsing in warm water.

Key words: Apples, waxes, beeswax, paraffin wax, mineral, Fourier transfer infrared spectroscopy (FTIR).

INTRODUCTION

The fruit of *Malus pumila* Mill, also known as *Malus sylvestris* L. (Mill.) *var. Domestica* Borkh (Rosaceae) commonly known as Apple, sold and consumed in FCT-Abuja and other parts of Nigeria are mostly not home-grown. The unfavourable tropical climatic conditions make their domestication in the country difficult. South Africa is a major supplier of apples particularly to the Federal Capital Territory, Abuja. Some sources are as far as the United States of America and New Zealand among others (Foraminifera Market Research, 2018). The outer layers of aerial morphological parts of plants especially fruits are coated with a hydrophobic layer called epicuticle

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wax (Müller and Riederer, 2005). The epicuticular wax served various functions particularly as a shield to provide water repellence, prevent moisture loss due to uncontrolled non-stomatal long-term storage, and loss of organic and inorganic compounds by leaching. It served as a protective covering against abiotic and biotic environmental stresses; mechanical damage, abrasions; attack and infection by plant pathogens such as fungi and insects (Bringe et al., 2006) and also against absorption of fertilizers, growth regulators, fungicides, insecticides and herbicides. Thus, making a significant influence on the production, storage and processing of the apples (Bajwa and Sandhu, 2014). Components of the fruit cuticle included a structural matrix known as cutin which is composed primarily of hydroxyl-and hydroxyepoxy-fatty acids and waxes, which consisted of homologous series of long-chain aliphatic compounds, including fatty acids, aldehydes, primary and secondary alcohols, ketones, alkanes and alkyl esters. Triterpenoids especially ursolic acid, have also been reported (Jetter and Schaffer, 2001; Buschhaus and Jetter, 2011).

The quantity and quality of the naturally produced epicuticular wax may be affected by environmental factors. Therefore, organic commercial waxes are sometimes added to augment the fruit natural waxing covering and to improve attractiveness. Such organic commercial waxes include shellac wax, carnauba wax or a combination of the two, resins, paraffin wax, bees wax, jojoba wax, soybean gum, and many others (Thompson, 2003; Syed et al., 2004; El-Anany et al*.,* 2009; Hassan et al., 2014). The tendency of overloading the body with some of these chemicals which are required by the body (K, Mg, Cu, Mn, and Zn) and the toxic one (Pb) through food consumption is certain if caution is not taken, knowing that the body requirement of the necessary minerals has been established (DRI, 2011; Shaffer, 2019). The need for continual check of every consumable food or other related products which might have a direct or indirect link to human health is therefore, important. According to the USA department of Agriculture, freshly plucked hard apples will barely last 2 weeks before rotting. Hence, the study aimed at evaluating the physicochemical properties, elemental profile and characteristics of the epicuticlar wax present on the outer layer of apple fruits present in Nigeria's' market using Abuja as a case study.

MATERIALS AND METHODS

All reagents used were of analytical grade and obtained from stock available at the National Institute for Pharmaceutical Research and Development (NIPRD) laboratory. Other materials used for the sample preparation were heating mantle (Model: Gallenhamp, Germany), glass funnel, volumetric flask (50 ml), digestion tube (100 ml), filter paper (Whatman; Size 110 mm), Micropipette (Model: perfect choice, sizes 200 µl and 0-1000 µl), sample bottles (50 ml) and Flame Atomic Absorption Spectrophotometer (Model: GBC Avanta, version 2.0, USA) at NIPRD**,** and FTIR (model Agilent carry 360, USA).

Sample collection and preparation

Apples sold in leading consumer stores and grocery markets in Abuja, the Federal Capital Territory, were randomly obtained and categorized into nine (9) groups based on brand name and source. Eight (8) of the groups were for the eight clearly branded apples and sourced from leading stores. The last group was for randomly selected unbranded apples from the local markets at Zuba, Kubwa, Karmo and Mararaba. The apples were packed in labelled sealable bags and coded "A" to "I" based on brand and/or source of purchase. A minimum of Ten (10) samples per group were obtained and immediately processed. Epicuticular coating from each apple was manually scrapped using hand protective coverings and new razor blades, into petri dishes.

Determination of physicochemical properties

Identification tests for solubility, taste and odour were carried out using standard methods as described by European Pharmacopoeia (2005). Commonly used and available waxes such as beeswax, white and yellow soft paraffin were used as references.

Beeswax

Isopropyl alcohol (50 ml) was added to 0.2 - 1 g of beeswax/ epicuticular waxes and dissolved by warming to 65°C in a water bath. 5 ml of warm water (35°C) was added under stirring (European Pharmacopoeia, 2005). The formation of white flocculent substances depicted the presence of beeswax.

White and yellow soft paraffin

Wax (2 g) was melted at 45°C to obtain a homogeneous phase after which 2 ml of distilled water and 0.2 ml of 0.05 M iodine was added. Mixture was shaken and allowed to cool (European Pharmacopoeia, 2005). The solid upper layer is violet-pink. Samples from apples were treated as above.

Fourier transfer infrared spectroscopy (FTIR)

A little quantity of each sample was directly analyzed using Agilent carry 360 FTIR Spectrometer by Attenuated Total Reflection (ATR) compressed KBr disk method. The samples were scanned within the range of 4000.00 to 650.00 cm^{-1} at resolution 8 cm^{-1}.

Atomic absorption spectroscopy (AAS)

Stock solutions of Cu, K, Mg, Mn, Pb and Zn at 1000 µg/ml in 5% nitric acid ($HNO_{3)}$ were used in the preparation of the standard curve. A concentrated solution of HNO_3 was used for the sample digestion. The instrument operating condition as stated in Table 1 was used for the elemental analysis and the data obtained were processed according to the methods described by Samali et al. (2017).

RESULTS AND DISCUSSION

Waxes are very similar in physicochemical characteristics and are usually very difficult to distinguish. However, based on the analytical study, some inferences could be

Table 1. Instrument operating condition for the analysis.

Element	Wavelength (nm)	Slit width	Lamp current
Cu	324.7	0.5	5
K	769.9	0.5	6
Mg	202.6	1	3
Mn	280.1	0.2	5
Pb	217	0.2	5
Zn	213.9	0.5	5

Table 2. Physicochemical properties of epicuticular waxes.

Characteristics	Average weight of waxes per apple (mg)	Colour/physical appearance	Taste	Odour	90 % v/v ethanol/ warming
Sample A	49.0	whitish/ flaky	Tasteless-Lard	Rancid	Partially soluble (with colloids)
Sample B	11.8	light brown/ flaky	Tasteless-Lard	fruity	Partially soluble
Sample C	12.9	whitish/ flaky	Tasteless-Lard	fruity	Partially soluble
Sample D	26.0	light brown/ flaky	Tasteless-Lard	Rancid	Partially soluble (with colloids)
Sample E	39.3	creamish/ flaky	Tasteless-Lard	Rancid	Partially soluble (with colloids)
Sample F	30.7	creamish/ flaky	Tasteless-Lard	Rancid	Partially soluble
Sample G	30.0	whitish /flaky	Tasteless-Lard	faint	Soluble
Sample H	16.0	creamish/ flaky	Tasteless-Lard	fruity	Soluble
Sample I	38.3	whitish/ flaky	Tasteless-Lard	faint	Partially soluble

All samples were soluble in xylene and glycerol (warm) while insoluble in distilled water (warm) and DCM.

Table 3. Identification tests for samples.

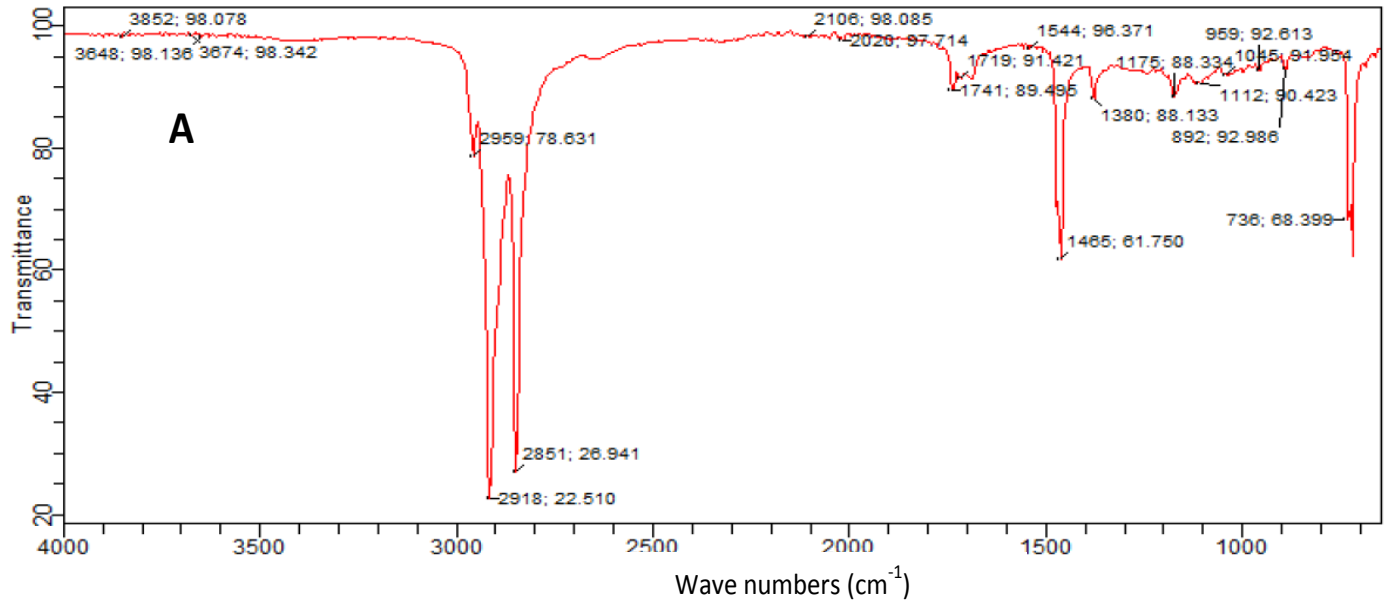
Sample	Beeswax	Inference	white and yellow soft paraffin	Inference
A	white flocculent substance formed but caked	Possible positive	Brown single layer	Negative
B	white flocculent substance formed and did not cake	Possible positive	Brown single layer	Negative
C	white flocculent substance formed turned cream and caked	Possible positive	Brown single layer	Negative
D	white flocculent substance formed but caked	Possible positive	Brown single layer	Negative
E	white flocculent substance formed and did not cake (thicker than bees wax)	Possible positive	Brown single layer	Negative
F	white flocculent substance formed and did not cake (thicker than bees wax)	Possible positive	Brown single layer	Negative
G	white flocculent substance formed turned cream and caked	Possible positive	Brown single layer	Negative
H	white flocculent substance formed turned cream and caked	Possible positive	Brown single layer	Negative
I	white flocculent substance formed and did not cake	Possible positive	Brown single layer	Negative

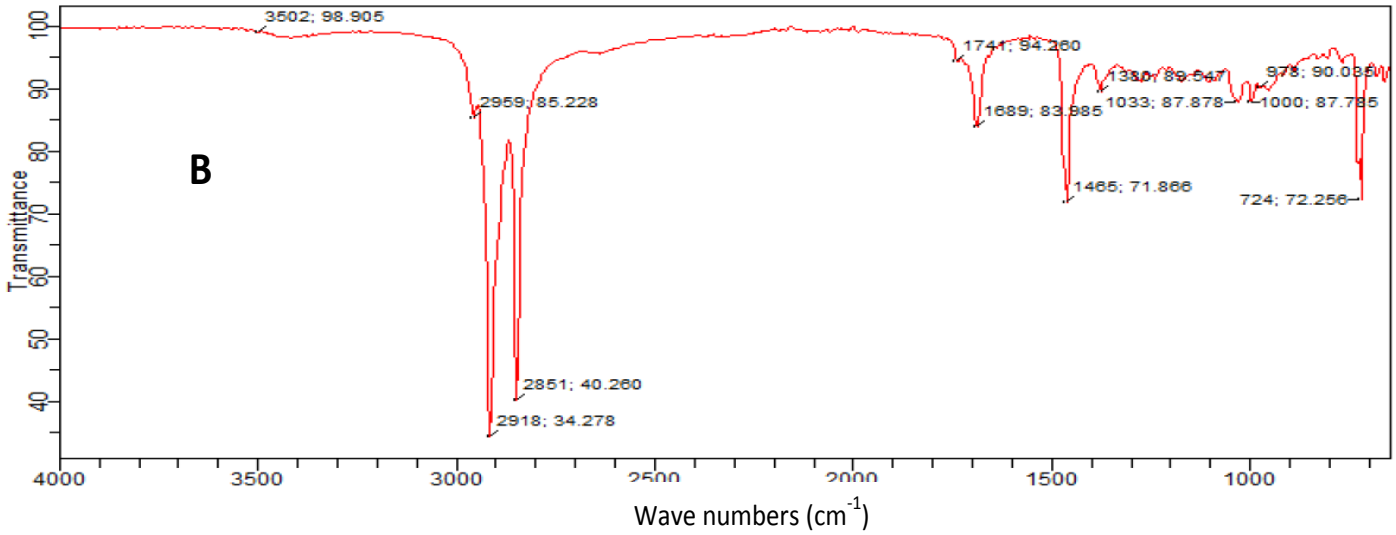
made as to the origin or the presence and level of non-edible minerals in them (Bajwa and Sandhu, 2014). Tables 2 and 3 depicted the results of physicochemical properties of the sample. An average of 0.22 mg of waxes per gram of apple was observed.

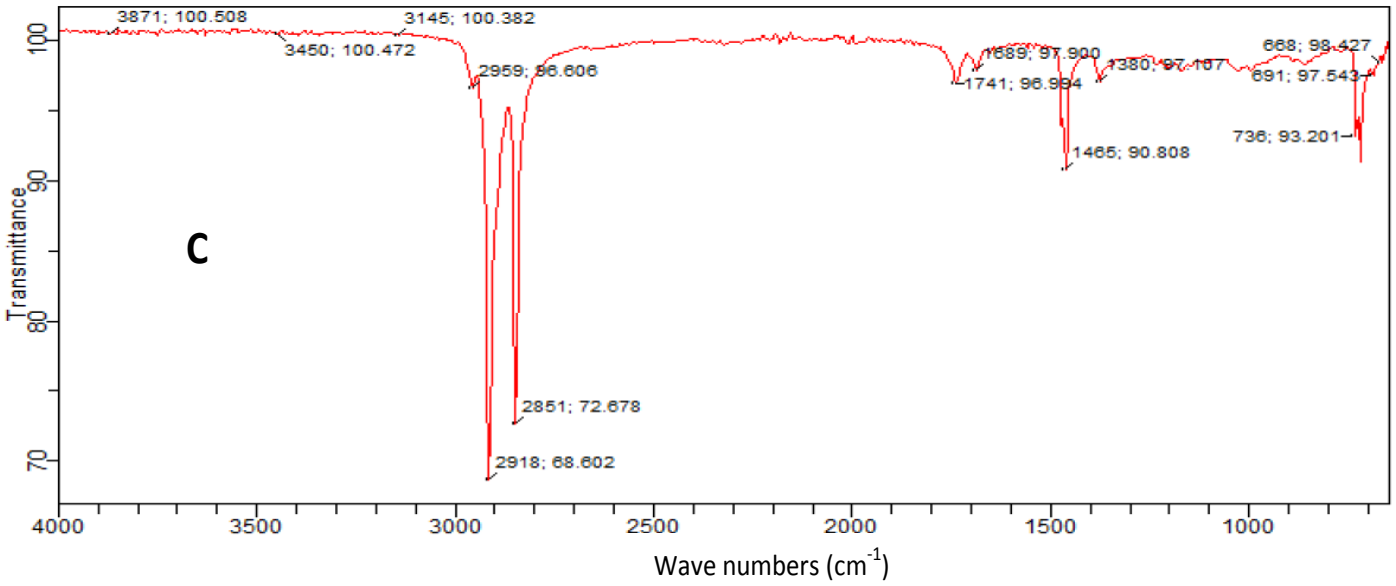
The physicochemical properties of the epicuticular wax samples from different sources A-I showed similar solubility properties depicting that they were insoluble in aqueous solvent and soluble in a more non polar solvent xylene than DCM and is partially soluble despite warming in alcohol as seen in Table 3. Identification tests showed more similarities with beeswax; however, results showed the absence of white and yellow soft paraffin. All the epicuticular waxes showed a positive result to the presence of terpenes (purple/blue colour). The plates also showed that the samples were neither beeswax nor white soft paraffin.

FTIR spectra analysis

The results of FTIR analysis (Figure 1) showed that all


A
3852; 98.078
3648; 98.136
3674; 98.342
2106; 98.085
2026; 97.714
1544; 96.371
1719; 91.421
1741; 89.495
1175; 88.334
959; 92.613
1045; 91.954
1112; 90.423
1380; 88.133
892; 92.986
736; 68.399
2959; 78.631
1465; 61.750
2851; 26.941
2918; 22.510
Transmittance
Wave numbers (cm-1)


B
3502; 98.905
1741; 94.260
1380; 89.547
978; 90.035
1033; 87.878
1000; 87.785
2959; 85.228
1689; 83.985
1465; 71.866
724; 72.256
2851; 40.260
2918; 34.278
Transmittance
Wave numbers (cm-1)


C
3871; 100.508
3145; 100.382
3450; 100.472
1689; 97.900
1380; 97.167
668; 98.427
2959; 96.606
1741; 96.994
691; 97.543
736; 93.201
1465; 90.808
2851; 72.678
2918; 68.602
Transmittance
Wave numbers (cm-1)

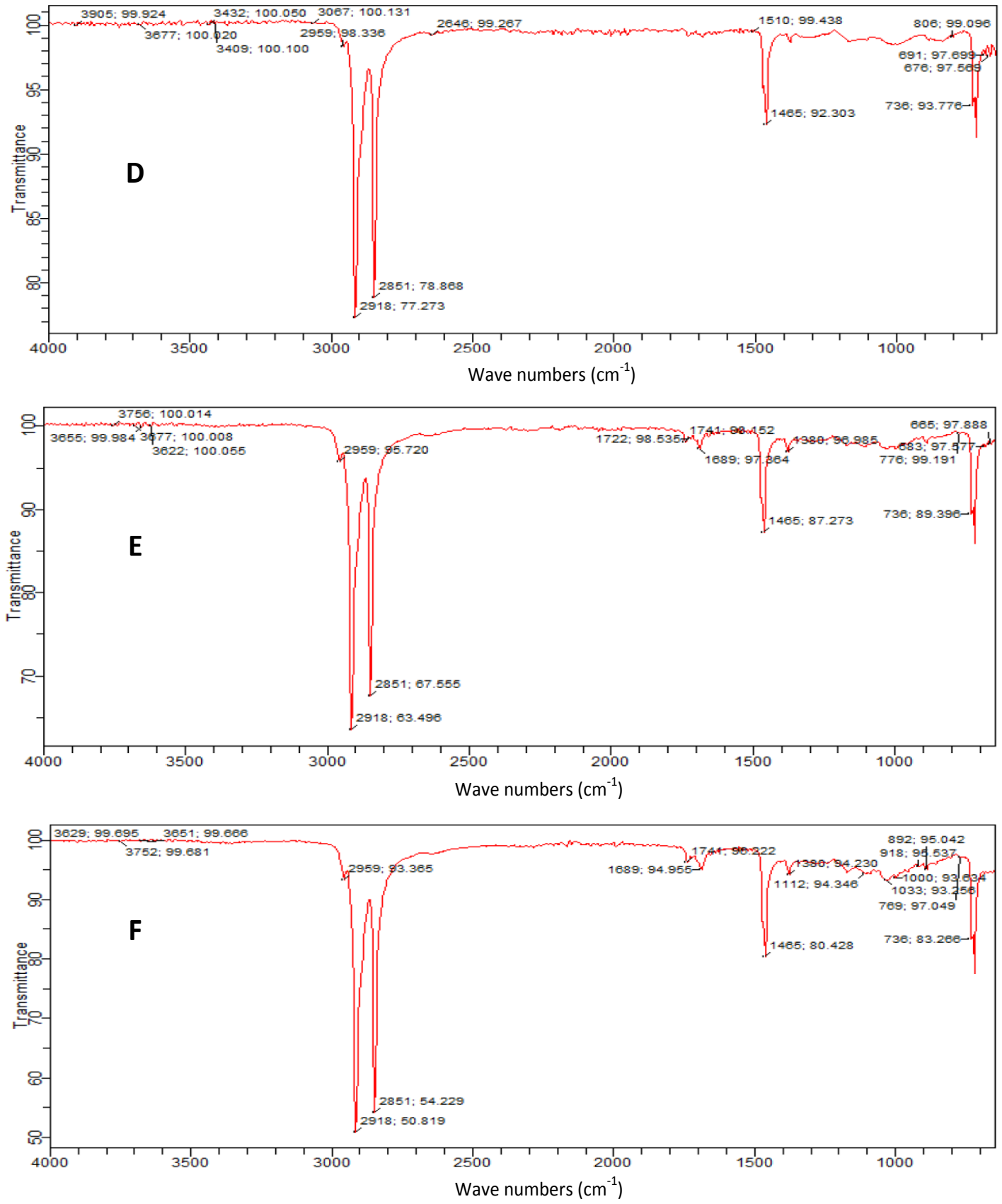


Figure 1. IR Spectra of epicuticular coating of apple samples. Key: A= sample A; B = sample B+D; D = Sample E; E = Sample F; F = Sample I+H.

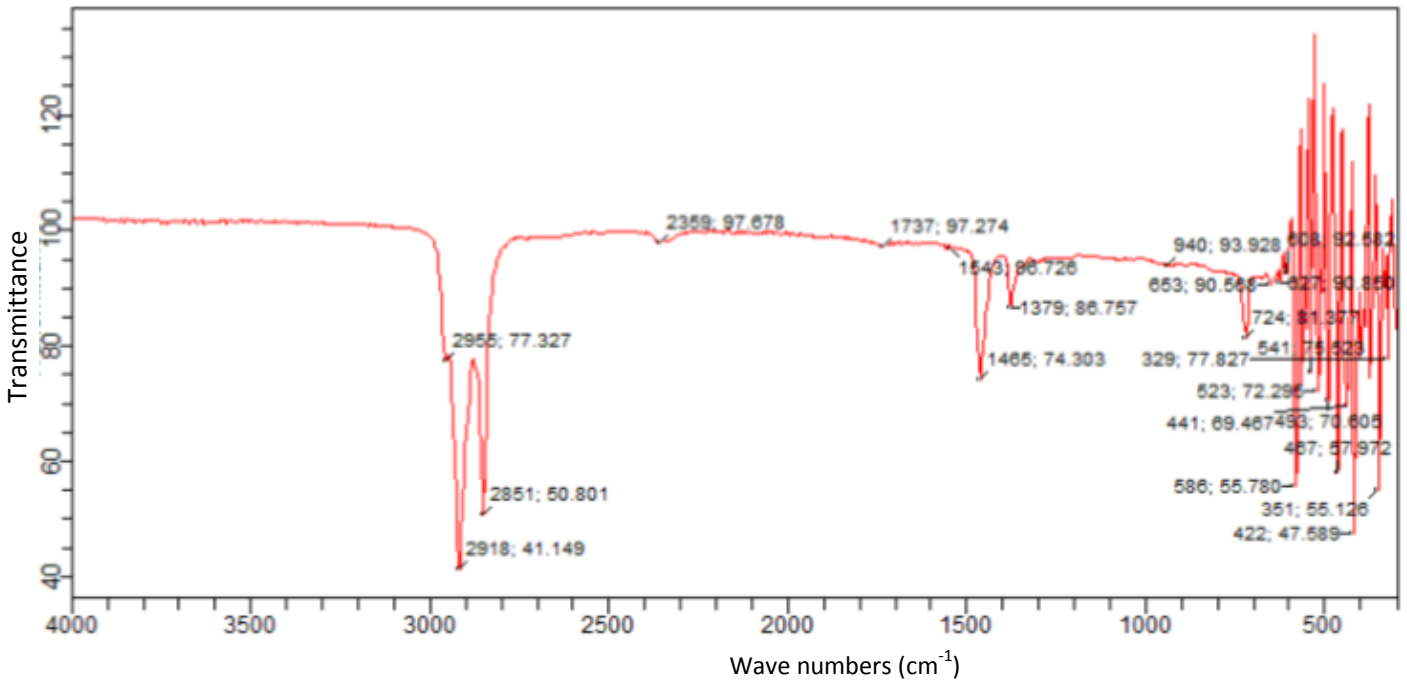


Figure 2. Characteristic FTIR spectrum of white soft paraffin.

the samples had major peaks at 2918, 2851, 1465 and 736 or 724 cm^{-1}. The major peak at 2918 and 2851 cm^{-1} corresponds to stretching vibration of sp^2 hybridized C-H bond or methylene C-H of saturated aliphatic or alicyclic compounds. The strong methylene/methyl bending vibration at 1465 cm^{-1} and the weak symmetric methyl C-H band at 1380 cm^{-1} in addition to the major band at 736 or 724 cm^{-1} (methylene rocking vibration, n ≥ 3) is indicative of a long-chain linear aliphatic structure. The very weak peaks at 1033-978 cm^{-1} are due to methylene C-H of cyclohexane ring, which may be from the solvent of extraction. Since splitting did not occur at 1465 and 724 cm^{-1}, as predictable for branched chains, the linearity and unbranched nature is further confirmed. There was no terminal vinyl or pendant vinylidene C-H stretching (3100-3000 cm^{-1}) or bending (1420-1290 cm^{-1}), and the absence of C=C stretch at 1680-1600 cm^{-1} confirms the absence of unsaturation. No aromatic CH usually at 890-820 cm^{-1} was observed.

The moderate peak or band at 1689 cm^{-1} (or 1719 cm^{-1} for sample D+B) is indicative of carbonyl of conjugated ketone, while the minor peak at 1741 cm^{-1} is indicative of a carbonyl C=O stretch of esters and/or alkyl carbonates (Coates, 2000). The very weak band at 1175 cm^{-1} for sample D+B (probably due to C=O and C-H bending of esters, or C-O of tertiary alcohol or phenol, or C-N of secondary amide) is indicative of an ester, alcohol or amine contamination from solvent or other sources. The C=O function bands at 1741 and 1689 cm^{-1} had a very low intensity, in all other samples apart from A where the band at 1689 cm^{-1} was moderate. This indicates that the samples contain very few or no fatty acids which usually characterize vegetable and waxes and oils as well as beeswax. This suggests that the major organic constituents of these waxes are of petrochemical origin such as paraffin and mineral oils (RESCOLL, 2011). These moderate and weak bands may be from the solvent (conjugated ketone or alkyl carbonates), or adulteration with vegetable wax (esters).

Apart from sample “A” spectrum that showed moderate intensity band at 1689 cm^{-1}, all the major peaks for all the samples were identical to the spectrum of paraffin wax reported by Al-Zubaidi et al. (2016). Thus, the FTIR spectra for the samples was characteristic of a long-chain saturated wax of petrochemical origin (Figure 2) mixed or adulterated with very little wax from vegetable source.

The issue of adulteration of beeswax and other vegetable waxes used for food purposes with paraffin wax is not new and in fact remains a huge challenge for beeswax authentication and control (Svečnjak, 2015; Svečnjak et al., 2015). Virgin beeswax (Figures 3 to 5) contained less than 5% of paraffin wax while a highly adulterated one can contain over 70% of paraffin wax.

The result of the minerals (Cu, K, Mg, Mn and Zn) and the toxic heavy metal element (pb) of the samples indicated variable concentrations (Table 4). The concentration ranges of Cu, K, Mg, Mn, Zn and Pb were 1.73 to 15.66, 10.80 to 1357, 26.46 to 173.12, 0.11 to 0.22, 2.05 to 41.80 and 3.78 to 11.66 mg/g, respectively for wax from normal size apple. All the samples contained copper and zinc above WHO/FAO (2001) permissible limit (0.02 mg/g) and (0.05mg/g) for edible

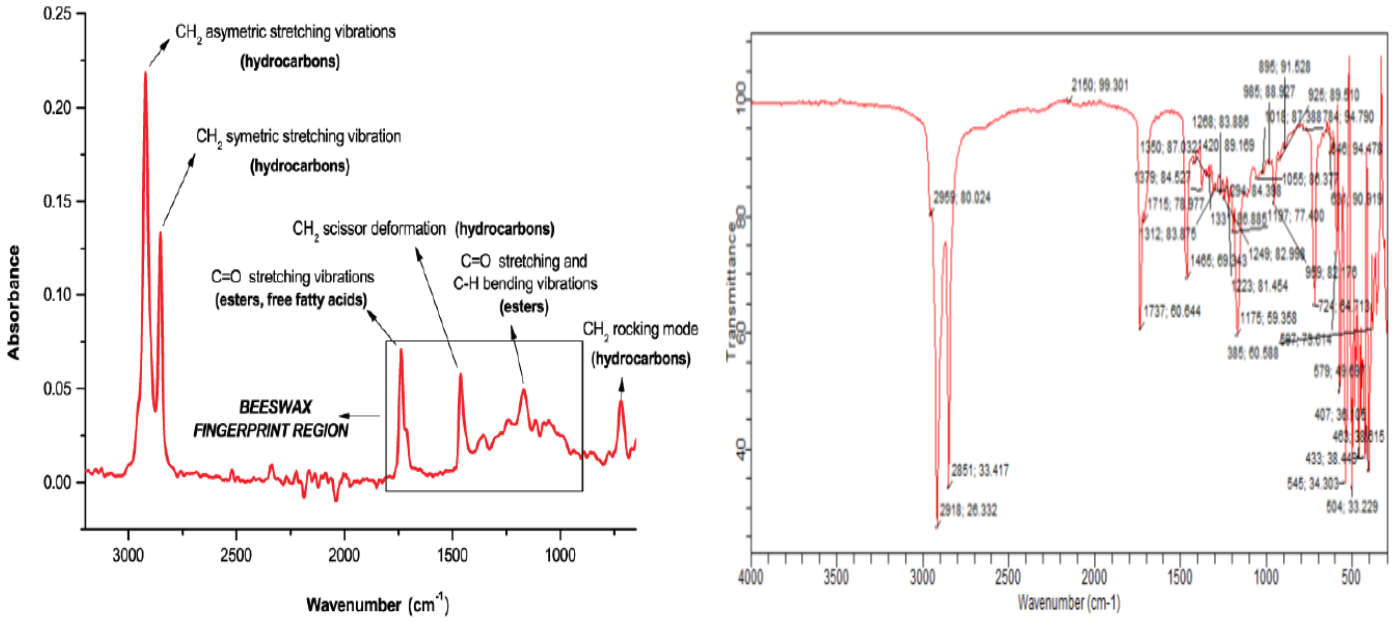


Figure 3. Characteristic FTIR-ATR spectrum of virgin beeswax with assignment of underlying absorption bands.
Source: Svečnjak et al. (2015).

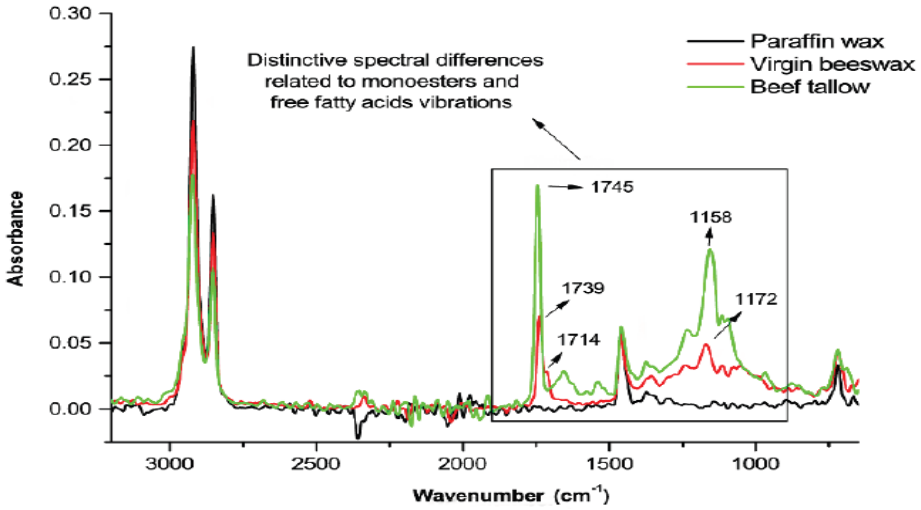


Figure 4. Distinctive spectral differences between virgin beeswax, paraffin, and tallow occurring in the fingerprint region.
Source: Svečnjak et al. (2015).

plants, while sixty percent (60%) of the samples contained lead above WHO permissible limit (0.010 mg/g). In terms of the Recommended Daily Allowance (RDA) of Cu, K, Mg, Mn and Zn established for both sexes at all age groups, all the samples (100%) contained Cu above and K below RDA values, while 80% contained Mg, 100% contained Mn and 20% of the samples contained Zn below RDA values (Table 4). High level of sample's contamination with Cu, Zn and Pb could be from copper compounds or lead arsenate-based pesticides which are usually used to control agricultural pests in fruits orchard, since historically high amount of lead and arsenic are found in former apple orchards (Schooley et al., 2008). Pavan and Gardey (2019) had earlier reported Pb concentrations of 26 ppb (ng/g) and other elemental contamination from petroleum waxes (candle waxes). Therefore, if petroleum waxes which contained complex organometallic compounds (Ali and

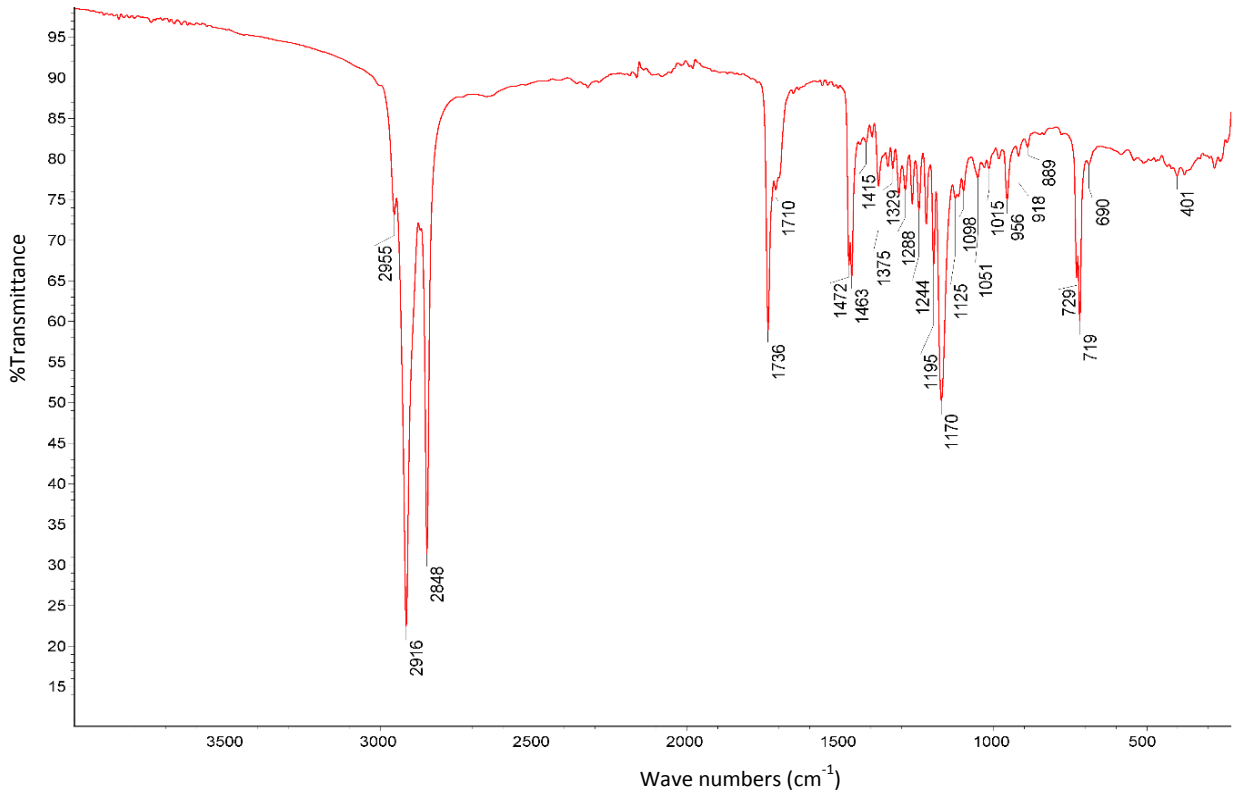


Figure 5. Image of virgin beeswax: Database of ATR-FT-IR spectra of various materials. ATR-FT-IR spectrum of Beeswax (4000 – 225 cm^{-1}). http://lisa.chem.ut.ee/IR_spectra/wp-content/uploads/2015/12/Beeswax.png [Accessed 15 Dec 2018]

Table 4. Mineral elements content.

Sample codes	Mean concentration (mg/g)					
	Cu	K	Mg	Mn	Pb	Zn
C	2.81	ND	34.99	ND	11.66	2.05
E	1.73	ND	26.46	0.216	5.29	18.9
F	1.84	ND	40.61	0.108	3.78	11.88
D+B	15.66	10.8	78.62	0.108	ND	11.61
I+H	7.56	1358.1	173.12	0.108	ND	41.8
RDA	0.34-0.90 mg/day	3000-4700 mg/day	80-420 mg/day	1.2-2.6 mg/day	-	3-12 mg/day
WHO/FAO	0.02 mg/g	-	-	-	0.01 mg/g	0.05 mg/g

RDA = Recommended daily allowance, WHO = World Health Organization, FAO = Food Agriculture Organization.

Abbas, 2006) are used instead of food-grade waxes which are expected to be void of heavy metals, there could be possibility of such contamination.

Conclusion

The results of analysis of waxes used in the preservation of apples imported into Nigeria, suggested that, the waxes are from petrochemical origin with some level of contamination with beeswaxes or vegetable waxes. It also indicated high level of contamination of the waxes with copper, zinc and lead above WHO/FAO permissible limits. The tendency of contamination of these waxes with other waxes and chemical preservatives like insecticides and fungicides used in orchards or farmlands against agricultural pest is possible; therefore quality of waxes used in foods and cosmetics should be monitored especially with regard to origin and mineral element content, as well as quantity applied. The food-grade waxes which are edible and safe allowed by US FDA for coating apples and other fruits are food grade petroleum

jelly, beeswax and vegetable waxes such as shellac, carnauba and others, any waxes other than these may not be easily absorbed by the body and can be harmful for the colon or the small intestine. Apples that have been treated must be labelled: “coated with food-grade vegetable, beeswax, or shellac-based wax to maintain freshness”. It is therefore, pertinent to adhere to food-grade waxes for preservation of apples and other related fruits. In Nigeria, you may not always be sure that the wax used for coating the apple you buy, is food-grade and safe. It is recommended to get rid of the waxes from the apples and other related fruits before consumption.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ali MF, Abbas S (2006). A review of methods for the demetallization of residual fuel oils. Fuel Processing Technology 87:573-584.

Al-Zubaidi AB, Abas OF, Abass RA, Abdulredhi NJ (2016). Thermal behaviour of paraffin wax / polyvinyl alcohol composite material. Al-Khwarizmi Engineering Journal 12(2):18-26.

Bajwa U, Sandhu K (2014). Effect of handling and processing on pesticide residues in food- a review. Journal of Food Science and Technology 51(2):201-220.

Bringe K, Schumacher CFA, Schmitz-Eiberger M (2006). Ontogenic variation in chemical and physical characteristics of adaxial apple leaf surfaces. Phytochemistry 67:161-170.

Buschhaus C, Jetter R (2011). Composition differences between epicuticular and intracuticular wax substructures: how do plants seal their epidermal surfaces? Journal of Experimental Botany 62:841-853.

Coates J (2000) Interpretation of Infrared Spectra: A Practical Approach. In: Meyers, R.A., Ed., Encyclopaedia of Analytical Chemistry, John Wiley & Sons Ltd., Chichester pp. 10881-10882.

Dietary Reference Intakes (DRI) (2011). Recommended Dietary Allowances and Adequate Intakes, Element; Food and Nutrition Board, Institute of Medicine, National Academies pp. 1-8.

El-Anany AM, Hassan GFA, Rehab AFM (2009). Effects of edible coatings on the shelf-life and quality of Anna apple (*Malus domestica* Borkh) during cold storage. Journal of Food Technology 7:5-11.

European Pharmacopoeia (2005). European Pharmacopoeia Monographs. EDQM Publication (European Directorate for the Quality of Medicines and Health Care).

Foraminifera Market Research (2018). Apple importation, storage and distribution in Nigeria, the feasibility report. Available at: https://foramfera.com/marketresearchreports/importexport-wholesale-and-retail-trade/apple-importation-storage-and-distribution-in-nigeria-the-feasibility-report/.

Hassan ZH, Lesmayati S, Qomariah R, Hasbianto A (2014). Effects of wax coating applications and storage temperatures on the quality of tangerine citrus (*Citrus reticulata*) var. Siam Banjar. International Food Research Journal 21(2):641-648.

Jetter R, Schäffer S (2001). Chemical composition of the *Prunus laurocerasus* leaf surface. Dynamic changes of the epicuticular wax film during leaf development. Plant Physiology 126:1725-1737.

Müller C, Riederer M (2005). Plant surface properties in chemical ecology. Journal of Chemical Ecology 31:2621-2651.

Pavan B, Gardey L (2019). Determination of nickel and lead in wax samples by ICP-MS. Impactanalytical.com, 1940 N. Stark Rd., Midland, MI 48642, 855.IA Available from: http://resources.impactanalytical.com/Impact_Analytical_ICP MS_ White paper. pdf [Accessed 12 July 2019].

RESCOLL (2011). Chemical characterization of waxes. Available at: http://www.surfgz.com/descargas/Chemical%20Characterization%20of%20Waxes.pdf [Accessed 15 Dec 2018].

Samali A, Mohammed MI, Ibrahim MB, Gamaniel KS (2017). Metal content determination of some sexual dysfunction medicine samples in Northern Nigeria. Bayero Journal of Pure and Applied Sciences 10(1):234-238.

Schooley T, Weaver MJ, Mullins D, Eick M (2008). The history of lead arsenate use in apple production: Comparison of its impact in Virginia with other States. Journal of Pesticide Safety Education 10:22-52.

Shaffer C (2019). Macro-minerals and Trace minerals in the diet. News -medical. Available at: http.net/health/macrominerals-and-Traceminerals-in-the-diet.aspx. [Accessed 27 May 2019].

Svečnjak L (2015). Beeswax adulteration issue: aspects of contamination and outcome. Available at: http://www.izslt.it/wp-content/uploads/sites/4/2017/04/Svecnjak.pdf [Accessed 15 Dec 2018]

Svečnjak L, Baranović G, Vinceković M, Prđun S, Bubalo D, Gajger IT (2015). An approach for routine analytical detection of beeswax adulteration using FTIR-ATR Spectroscopy. Journal of Apicultural Science 59(2).

Syed MS, Syed ZA, Abida A (2004). Effect of chemical treatment, wax coating, oil dipping and different wrapping materials on physico-chemical characteristics and storage behaviour of apple. Pakistan Journal of Nutrition 3(2):122-127.

Thompson K (2003). Fruit and Vegetables: Harvesting, Handling and Storage, 2nd ed. of Postharvest Technology of Fruits and Vegetables, Oxford: Blackwell P 287.

WHO/FAO (2001). WHO/FAO Food Addictive Data System. Evaluation by the Joint WHO/FAO Expert Committee on Food Additives 1956 – 1984. FAO Food and Nutrition, Paper 30/Review, Rome pp. 2-51.

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Effects of diet substitution with defatted kernels of mango (*Mangifera indica*) and wild mango varieties (*Irvingia gabonensis* and *Irvingia wombolu*) on weight and plasma lipid profile of Wistar rats

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Effects of defatted mango (*Mangifera indica*) and wild mango (*Irvingia gabonensis*, *Irvingia wombolu*) kernels in substituted diets of commercial feed, on weight and plasma lipid profile were analysed using Wistar rats after 21-day feeding. Lipid profiling was conducted using enzymatic/colorimetric techniques. The substitution with each kernel type in the commercial feed was 0, 25, 50, 75 and 100%. Diet substitution of up to 75% inversely correlated with weight gained (P<0.05). Up to 50% diet substitution was over 93% acceptable for consumption by the animals. Lipid profile analysis indicated that total cholesterol (TC) and low density lipoprotein (LDL) decreased with increasing diet substitution. The reverse was observed with HDL. TG increased up to 50% defatted *M. indica* kernel (DMIK) substitution and 25% defatted *I. gabonensis* kernel (DIGK)/defatted *I. wombolu* kernel (DIWK) but decreased thereafter. LDL/Cholesterol degradation could have increased TG level and possibly inhibited at higher diet substitutions due to increased residual polyphenolic substances present in the kernel samples. These variables also significantly improved the lipid profile status of the experimental animals (P<0.05).

Key words: Lipid profile, *Mangifera indica*, *Irvingia gabonensis*, *Irvingia wombolu*, cholesterol.

INTRODUCTION

Mango (*Mangifera indica*) is one of the most notable fruits in the sub-tropical and tropical regions of the world (Legesse and Emire, 2012). Mango kernel is a non-conventional source of food which has drawn attention due to its suitability to combat nutritional need of human beings when incorporate into composite flour (Menon et al., 2014). The kernel was reported to be a useful source of protein, carbohydrate and fat except for the presence of anti-nutritional factors such as tannin (Diarra, 2014). Previous studies successfully showed that mango kernel could be converted to edible state through processing; these studies include the composition, functionality, and toxicology of the kernel before and after processing into flour (Arogba, 1997). In India cultural foodstuff, about 20 to 30% of the kernel flour could be used without adversely affecting acceptability (Legesse and Emire, 2012). The seeds are useful in compounding animal feed (Elgindy, 2017). Furthermore, Arogba (1997, 2002) had

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also shown the potential use of the processed kernel in human diet.

In like manner, wild mango (*Irvingia* species) is of the Irvingiacaea family and exists in two varieties (*gabonensis* and *wombolu*). Both varieties are found naturally in the tropical rainforest of West Africa countries (Kuyooro et al., 2017). *Irvingia gabonensis* was reported to constitute an important part of the natural diet in West Africa for controlling dietary lipids and weight gain. The seed extract of *I. gabonensis* is an effective weight reducing herbal medication, with no knownside effects (Etta et al., 2014). Studies on some tropical Africa kernels have found *I. gabonensis* beneficial in lowering undesirable low density lipoprotein (LDL) cholesterol level with athero-protective properties. Hypolipidemic effects in rats have been demonstrated (Kuyooro et al., 2017).

High blood cholesterol associated with elevated levels of oxidised LDL is a risk factor for cardiovascular diseases such as atherosclerosis and myocardial infarction (Omodamiro and Nwankwo, 2013). Hence, the utilization of lipid lowering effect of various plants products is considered to be an important therapeutic approach (Manna and Maiti, 2016). Plasma cholesterol levels apparently decrease with lowering cholesterol content in diet (Ajayi and Ajayi, 2009).

Recently, the effects of undefatted powder and extract of these kernels in several studies with Wistar rats were reported (Alhassan and Arogba, 2018; Egbuonu, 2018; Irondi et al., 2018; Kuyooro et al., 2017; Arogba and Matanmisi, 2014), and an *in-vitro* study (Arogba et al., 2016). In contrast, Abel et al. (2018) described digestibility and nutrient intake of undefatted mango (*M. indica*) kernel in substituted feeds of West African rams. However, scanty literature exist on defatted form of these kernels (Arogba, 2015), and their effects on nutritional status, particularly lipid profile of animals. Therefore, the effect of diet substituted with defatted kernels of mango and wild mango at different percent ratios on weight and lipid profile of Wistar rats is hereby reported.

MATERIALS AND METHODS

Sample collection and processing

Ripe mango (*M. indica*) fruits were plucked directly from trees at Anyigba town, Kogi State. The seeds were dissected using stainless steel knife to obtain the testa and kernel, dried at ambient temperature (25 ± 3°C) and pulverized using mortar and pestle into powdery form. Dry wild mango (*I. gabonensis* and *Irvingia wombolu*) kernels were procured separately from Anyigba market, Kogi State. The kernels were sorted manually for wholesomeness. Like the mango kernels, they were pulverized into powdery form. The commercial feed used was “Broiler Top Feed Finisher”, produced by Premier Feed Mills Company Limited Ibadan, Oyo State, Nigeria.

Fat extraction

Each sample type 10% (w/v) was added to petroleum ether (60 to 80° grade) contained in a beaker, kept at ambient temperature of 30 ± 2°C and shaken periodically for 24 h. The process was repeated twice and the defatted kernel was oven-dried to constant weight and stored for further analysis. The percentage oil yield was calculated. To assess efficiency of the extraction, residual oil in the defatted samples were determined using Soxhlet extraction technique as described by Williams (2007).

Procurement and management of experimental animals

Thirty nine (39) healthy, adult male albino Wistar rats (weighed 122 - 136 g) were procured from the animal house of the Department of Veterinary Medicine, Benue State University, Makurdi, Nigeria. They were acclimatized in clean rat cages at the Experimental Animal House of the Department of Biochemistry, Kogi State University, Anyigba for a period of ten (10) days at ambient temperature with 12-h light and dark cycle. Within the period, they were fed with a commercial “Broiler Top Feed Finisher” with water *ad-libitum*.

Experimental design

Weight measurement

The weights of the rats were taken before and after twenty one (21) days feeding period. The percentage weight difference was calculated.

Diet substitution/animal

Thirty-nine (39) rats were fed with commercial “Top Feed Finisher” diet and substituted with defatted *M. indica* kernel (DMIK), defatted *I. gabonensis* kernel (DIGK) and defatted *I. wombolu* kernel (DIWK) each, at 0, 25, 50, 75 and 100% levels. Water was provided *ad-libitum* grouping (Table 1).

Estimation of diet consumed

Weights of the diets provided were taken and those of the remnants after 21 days feeding. Percentage diet consumed, %DC = (Weight of diet consumed/Weight of substituted diet provided) × 100.

Blood sample collection

On the 22nd day, the animals were sacrificed by anaesthesia using chloroform in desiccator. The blood was obtained through cardiac puncture into heparinized (EDTA) bottles using 5 ml syringe.

Assay of plasma lipid profile

The plasma lipid profile [Total Cholesterol, Triglycerides and High Density Lipoprotein Cholesterol] was determined by spectrophotometric technique, using enzymatic/colorimetric assay kits (Randox Laboratories, United Kingdom). However, by calculation LDL=TC-[HDL + TG/5].

Statistical analysis

The statistical analysis was conducted using SEM software available at miniwebtool.com. Results were expressed as mean ± standard error of mean (SEM). Separation of mean was conducted for test of significance at P = 0.05.

Table 1. Experimental design for albino rats on substituted diets.

Sample: Comm. Feed (%)	Number of rats per sample type		
	DMIK	DIGK	DIWK
0:100	(3)*	-	-
25:75	3	3	3
50:50	3	3	3
75:25	3	3	3
100:0	3	3	3
Total rats/sample type	15	12	12

DMIK = Defatted *Mangifera indica* kernel, DIGK = defatted *Irvingia gabonensis* kernel, DIWK = defatted *Irvingia wombolu* kernel and (3)* implies the three animals in (DMIK) group also served as control for (DIGK) and (DIWK) groups.

Table 2. Mean weight of animals before and after feeding for 21 days (n = 3).

Sample type	Sample: Comm. feed (%)	Before (g)	After (g)	Difference (g)	Difference (%)	SEM
DMIK	0:100	124	178	54	44^{e}	8.8
	25:75	132	163	31	24^{d}	
	50:50	127	145	18	14^{c}	
	75:25	129	133	4	3^{b}	
	100:0	136	127	-9	-7^{a}	
DIGK	0:100	124	178	54	44^{d}	8.6
	25:75	125	147	22	18^{c}	
	50:50	131	139	8	6^{b}	
	75:25	128	129	1	1^{ab}	
	100:0	134	128	-6	-4^{a}	
DIWK	0:100	124	178	54	44^{d}	8.3
	25:75	127	156	29	23^{c}	
	50:50	125	137	12	10^{b}	
	75:25	122	127	5	4^{ab}	
	100:0	133	129	-4	-3^{a}	

Values with the same superscripts on the same column are not significantly different at $p > 0.05$. DMIK = Defatted *Mangifera indica* kernel, DIGK = defatted *Irvingia gabonensis* kernel, DIWK = defatted *Irvingia wombolu* kernel, SEM = Standard error of mean, Comm. Feed = commercial feed (Broiler Top feed Finisher).

RESULTS AND DISCUSSION

On defatting, the oil yield of 21.9% from *M. indica* kernel in this study was similar to that reported by Arogba (2015) as the variety was obtained from the same locality. The oil yields from *I. gabonensis* and *I. wombolu* kernels were also similar (67% ± 1.0) and agreed with the report of Bamidele et al. (2015). Estimated by Soxhlet extraction technique, residual oil in these kernels was between 1 and 3%.

Weight difference and diet consumption by experimental animals

The percentage weight difference of the experimental animals in Table 2 showed that the substituted *Irvingia* kernel samples (DIGK and DIWK) had similar effects on weight gain ($P < 0.05$). Similar to *M. indica* kernel (DMIK) sample, there was positive but decrease in weight gained as sample substitution in diet increased up to 75% ($P<0.05$). However, 100% substitution had negative effect on weight gain. The “bitter principle” in these higher substitutions possibly had adverse effect on diet consumption. Arogba (1997, 2000) had identified and analysed the tannin constituents of *M. indica* kernel.

Table 3 further supported the aforementioned observation. 100% test samples were least acceptable for consumption by the animals followed by 75% substituted samples, while diets substituted by 50% or less were favourably consumed by over 93%. It further

Table 3. Estimated diet consumed by the animals within 21 days.

Sample:Comm. Feed (%)	DMIK (%)	DIGK (%)	DIWK (%)
0:100	100^{c}	100^{c}	100^{c}
25:75	98^{c}	99^{bc}	99^{c}
50:50	93^{c}	98^{b}	99^{c}
75:25	75^{b}	98^{b}	97^{b}
100:0	53^{a}	90^{a}	94^{a}
SEM	8.9	1.8	1.1

Values with the same superscripts on the same column are not significantly different at p > 0.05. DMIK = Defatted *Mangifera indica* kernel, DIGK = defatted *Irvingia gabonensis* kernel, DIWK = defatted *Irvingia wombolu* kernel, SEM = standard error of mean, Comm. Feed = commercial feed (Broiler Top feed finisher).

Table 4. Effect of diet substitution with DMIK on plasma lipid profile after 21 days.

Sample:Comm. Feed (%)	TC (mg/dL)	TG (mg/dL)	HDL(mg/dL)	LDL (mg/dL)
0:100	131.60 ± 2.52^{c}	219.49 ± 2.71^{c}	32.14 ± 2.05^{a}	55.21 ± 1.77^{d}
25:75	114.41 ± 2.59^{b}	223.66 ± 6.93^{c}	37.83 ± 1.82^{b}	33.75 ± 1.67^{c}
50:50	109.40 ± 4.35^{b}	228.52 ± 5.65^{c}	39.14 ± 2.34^{b}	24 .53 ± 2.23^{b}
75:25	96.44 ± 2.02^{a}	147.60 ± 3.97^{b}	47.55 ± 1.92^{c}	20.78 ± 0.96^{ab}
100:0	90.40 ± 1.88^{a}	122.42 ± 2.90^{a}	49.08 ± 1.71^{c}	16.81 ± 0.76^{a}
SEM	7.22	22.18	3.16	6.85

Values are expressed as mean ± SEM (n=3). Values with the same superscripts on the same column are not significantly different at p > 0.05. DMIK = Defatted *Mangifera indica* kernel, TC = total cholesterol, TG = triglyceride, HDL = high density lipoprotein, LDL = low density lipoprotein, SEM = standard error of mean, Comm. Feed = commercial feed (Top feed finisher).

inferred that the defatted kernels probably contained significant proportion of water-soluble than fat-soluble polyphenolic components.

Plasma lipid profile assessment

The plasma lipid profile of the animals after 21 days of feeding (Tables 4 to 6) showed that total cholesterol (TC) and LDL decreased with increasing defatted test sample substitution (P< 0.05). For instance, the commercial feed had the highest TC level. Substitution by 25 and 50% kernel samples caused average reduction by 10 and 15%, respectively, indicating the significant physical effect of kernel substitution. The composition of the commercial feed, therefore, appeared to promote cholesterol synthesis. In similar manner, LDL proportionally decreased. However, HDL variation was observed to be inversely correlated with TC and LDL.

The results on TG variation raised some curious attention, while TC and LDL decreased with increasing physical substitution of the kernel samples in the diets, TG levels increased in diets with up 50% DMIK and 25% DIGK or DIWK substitution. The TG levels decreased significantly there-after (P<0.05). Two views were proposed to possibly explain these observations:

Since LDL comprises apolipoprotein B, cholesterol, phospholipids, and triglyceride in varied concentrations (Nelson and Cox, 2005; Prass, 2011), hydrolysis of its components could elevate the HDL and TG levels at those levels of diet substitution mentioned. Furthermore, since cholesterol serves as a starting molecule for steroid hormone synthesis, the side chain when cleaved to give 4-methylpentanal, or 4-methyl-4-hydroxypentanal, could be oxidised for glyceride synthesis. On the contrary, at higher levels of kernel substitution in the diets, the corresponding increased levels of residual (water-soluble) polyphenolic substances (e.g. tannins) could have significantly and adversely inhibited the TC/LDL hydrolysis.

100% test samples gave between 100 and 124 µg/dl TG, classified as “desirable” nutritionally in contrast to those substituted with commercial feed which gave a range of 148 to 256 µg/dl. A range of 150 to 500 was described as “borderline high” (Ma and Shieh, 2006). The results of TC and LDL-cholesterol in this study agreed with the report of Kuyooro et al. (2017) on hypolipidemic effects of undefatted kernel of *I. gabonensis*. The decrease in TC and LDL cholesterol recorded in this study shows that the test samples could have athero-protective potential and their consumption could avert the onset of developing atherosclerosis and cardiovascular disease.

Table 5. Effect of diet substitution with DIGK on plasma lipid profile after 21 days.

Sample: Comm. Feed (%)	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
0:100	131.60 ± 2.52^{d}	219.39 ± 2.71^{c}	32.14 ± 2.05^{a}	55.20 ± 1.77^{c}
25:75	118.82 ± 3.93^{c}	255.61 ± 4.90^{d}	36.51 ± 2.11^{b}	31.16 ± 2.21^{b}
50:50	102.66 ± 3.11^{b}	173.13 ± 3.30^{b}	44.93 ± 1.51^{c}	23.10 ± 2.89^{a}
75:25	104.96 ± 1.11^{b}	187.89 ± 2.71^{b}	46.13 ± 0.67^{c}	21.25 ± 1.04^{a}
100:0	94.48 ± 3.10^{a}	100.54 ± 2.11^{a}	54.77 ± 1.62^{d}	19.35 ± 1.31^{a}
SEM	6.57	25.91	3.95	6.61

Values are expressed as mean ± SEM (n=3). Values with the same superscripts on the same column are not significantly different at p > 0.05. DIGK = Defatted *Irvingia gabonensis* kernel, TC = total cholesterol, TG = triglyceride, HDL = high density lipoprotein, LDL = low density lipoprotein, SEM = standard error of mean, Comm. Feed = commercial feed (Top feed finisher).

Table 6. Effect of diet substitution with DIWK on plasma lipid profile after 21 days.

Sample: Comm. Feed (%)	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
0:100	131.60 ± 2.52^{d}	219.49 ± 2.71^{c}	32.14 ± 2.05^{a}	55.21 ± 1.77^{d}
25:75	121.30 ± 5.08^{c}	249.01 ± 3.84^{d}	35.42 ± 1.87^{ab}	33.45 ± 4.10^{c}
50:50	106.21 ± 2.58^{b}	211.85 ± 3.87^{c}	36.95 ± 2.44^{b}	26.55 ± 2.09^{b}
75:25	102.12 ± 4.63^{ab}	159.24 ± 3.22^{b}	42.85 ± 2.34^{c}	27.57 ± 2.67^{bc}
100:0	95.73 ± 3.10^{a}	123.46 ± 1.38^{a}	51.29 ± 2.63^{d}	19.57 ± 1.90^{a}
SEM	6.58	22.55	3.37	6.10

Values are expressed as mean ± SEM (n=3). Values with the same superscripts on the same column are not significantly different at p > 0.05. D DIWK = Defatted *Irvingia wombolu* kernel, TC = total cholesterol, TG = triglyceride, HDL = high density lipoprotein, LDL = low density lipoprotein, SEM = standard error of mean, Comm. Feed = commercial feed (Top feed finisher).

Conclusion

The study has shown that substitution of defatted kernels of *M. indica* and *Irvingia* species in animal diets were optimally palatable at 50%, as it was also concomitant with weight gain. Furthermore, TC and LDL decreased with increasing defatted test sample substitution. The levels of water-soluble polyphenolic substances in the defatted samples of the diets were implicated in these observations. These observations revealed that the test samples possessed hypolipidemic effects and could be utilized in the prevention of atherosclerosis and cardiovascular disease when incorporated in animal diet.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abel IO, Winifred PM, John, AA (2018). Digestibility and nutrient intake of undefatted mango (*Mangifera indica*) diets by growing West African dwarf sheep straw. Journal of Research Report on Genetics 2(1):1-3.

Ajayi OB, Ajayi DD (2009). Effect of oilseed Diet on Plasma Profile in Albino Rats. Pakistan Journal of Nutrition 8(2):116-118.

Alhassan A, Arogba, SS (2018). Wild Mango (*Irvingia wombo*lu) Kernel: Proximate Composition, Mineral Content and Haematological Profile of Wistar Albino Rat. International Journal of Innovative Research and Development 3(7):222-226.

Arogba SS (1997). Physical, chemical and functional properties of Nigeria mango *(Mangifera indica)* kernel and its processed flour. Journal of Science, Food and Agriculture 73(3):321-328.

Arogba SS (2000). Mango (*Mangifera indica*) kernel: Chromatographic analysis of tannin and stability study of the associated polyphenol oxidase activity. Journal of Food Composition and Analysis13:149-156.

Arogba SS (2002). Quality characteristics of a model biscuit containing processed mango *(M. indica)* kernel flour. International Journal of Food Properties 5(2):249–260.

Arogba SS (2015). Effect of Processing on Antioxidant Activity of Conventional Mango (*Mangifera indica*) Seed. Journal of Environmental Science, Toxicology and Food Technology 9(4):50-55.

Arogba SS, Matanmisi JA (2014). Proximate Biological Value and Protein Efficiency Ratio of processed Bush mango (*Irvingia gabonensis*) kernel. Journal of Pharmacy and Biological Sciences 2(9):18-20.

Arogba SS, Itemuagbor JS, Medayedupin OA, Ekeyi Y, Alikali EO (2016). *In-vitro* digestibility profile of wild mango (*Irvingia* spp.) kernel and wheat (*Triticum* spp.) grain. Nigerian Journal of Biochemistry and Molecular Biology 31(1 and 2):15-23.

Bamidele OP, Ojedokun OS, Fasogbon BM (2015). Physico-Chemical Properties of Instant Ogbono (*Irvingia gabonensis*) mix Powder. Journal of Food Science and Nutrition 3(4):313-318.

Diarra SS (2014). Potential of mango (*Mangifera indica L*.) seed kernel as a feed ingredient for poultry: a review. World's Poultry Science Journal 70:279-288.

Egbuonu ACC (2018). Effect of Ethanolic Extract of Pulverized *Mangifera indica* (Mango) Seed Kernel on Some Haematological Parameters in Normal and Monosodium Glutamate-intoxicated Rats. International Journal of Research in Environmental Science 4(1):47-55.

Elgindy AAE (2017). Chemical and Technological Studies of Mango

Seed Kernel. European Journal of Food Science and Technology 5(2):32-40.
Etta HE, Olisaeke CC, Iboh CI (2014). Effect of *Irvingia gabonensis* (Aubry Lecomte ex O'Rorke) Seeds on the Liver and Gonads of Male Albino Rats. Journal of Biology, Agriculture and Healthcare 1(4):10-15.
Irondi EA, Mohammed RO, Balogun AF, Tajudeen HI Aleshinloye OM (2018). Antioxidant and modulatory properties of kernel flour extracts of three Nigerian local mango varieties on enzymes relevant to metabolic syndrome. *Oxidants and* Antioxidants in Medical Science 8(1):1-10.
Kuyooro SE, Abam EO Agbede EB (2017). Hypolipidemic Effects of Irvingia gabonensis - Supplemented Diets in Male Albino Rats. Biochemistry and Analytical Biochemistry 6(2):1-5.
Legesse MB Emire SA (2012). Functional and Physicochemical Properties of Mango Seed Kernels and Wheat Flour and their Blends for Biscuit Production. African Journal of Food Science and Technology 3(9):193-203.
Ma H, Shieh K (2006). Cholesterol and Human Health. Journal of American Science 2(1):46-50.
Manna E, Maiti S (2016). Cardio-protective Effect of Natural Bioactive Compound (polyphenol) by Inhibiting LDL Oxidation with the Scavenging of Reactive Oxygen Species (ROS). Journal of Clinical and Experimental Cardiology 7(6):2-4.
Menon L, Majumdar SS, Ravi U (2014). Mango (*Mangifera indica* L.) kernel flour as a potential ingredient in the development of composite flour bread. Indian Journal of Natural Products and Resources 5(1):75-82.
Nelson DL Cox MM (2005). Principle of Biochemistry. (4^{th} ed.). NewYork: W. H. Freeman and Company.
Omodamiro OD, Nwankwo CI (2013). The Effect of *Voacanga africana* Leaves Extract on Serum Lipid Profile and Haematological Parameters of Albino Wistar Rats. European Journal of Experimental Biology 3(3):140-148.
Prass R (2011). Human low density lipoprotein: the mystery of core lipid packing. Journal of Lipid Research 52(2):187-188.

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Influence of different traditional production processes on the antioxidant capacity and vitamin C content of baobab (*Adansonia digitata*) juice

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This study aims to assess the effect of traditional production processes of baobab juice on its antioxidant and vitamin C content. A survey was conducted in the North region of Cameroon and the 4 main processes identified were subsequently used to prepare juice samples at two different baobab pulp/sugar ratios per litre (42 g/62 g and 87.5 g/231 g) that were analysed. The main difference between the 4 processes reported by the 92 respondents was at the steeping step. Steeping was mostly done for 6 h in fresh water (84.3% of them); 3 h in warm or boiling water; or by boiling the pulp-water mixture for ± 5 min. A high pulp/water ratio and temperature led to a significant increase in the antioxidant properties of the juice. The juice with the highest pulp content produced using the last steeping approach had the highest quality (Total Antioxidant Capacity, 5.76 ± 0.26 g AAE/100 ml; Total Phenolic Content, 46.72 ± 0.61 mg GAE/100 ml; Radical Scavenging Activity: 33.08 ± 2.48% of DPPH· Inhibition/100 ml; Vitamin C, 38.51 ± 5.34 mg/100 ml). High temperature of water during the production of baobab juice is recommended to optimize health value of baobab juice.

Key words*: Adansonia digitata*, juice, production process, antioxidants, vitamin C.

INTRODUCTION

Baobab (*Adansonia digitata* L.) is an indigenous fruit tree belonging to the Malvaceae family and associated with Savannah dry lands of sub-Saharan Africa of which the North and Far North regions of Cameroon are part (Bremer et al., 2009; Muthai et al., 2017). This tree produces relatively large silvery green or brownish

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indehiscent fruits, with an outer hard shell, an inner soft whitish powdery pulp containing kidney-shaped dark brown seeds, all bound together by thin and light brown fibres (Sidibe and Williams, 2002). The baobab fruit is well known in Africa both for its medicinal properties and social uses (Cissé et al., 2013). Biochemical studies have shown that the pulp content is particularly rich in dietary fibres, carbohydrates, essential amino acids, minerals (Potassium, Calcium, Magnesium, Iron, Sodium, Zinc and Manganese) and extremely low in fat (Magdi, 2004; Soloviev et al., 2004; Chadare et al., 2009 and De Caluwé et al., 2010).This pulp has also been described as an excellent source of Vitamin C, since its ascorbic acid content (2.8-3 g/kg) was estimated to be six times higher than in orange (Sidibé et al., 1996; Donatien et al., 2011). Furthermore, the baobab pulp has a higher antioxidant capacity than commonly consumed fruits such as orange, strawberry, apple and kiwi fruits (Silvia et al., 2002; Latifou et al., 2012). Indeed, the Integral Antioxidant Capacity value of baobab fruit pulp is 10 times higher than that of orange pulp, with values of 11.1 mmol/g (Equivalents Trolox) and 0.3 mmol/g (Equivalents Trolox), respectively (Silvia et al., 2002). Epidemiological studies have shown a link between the intake of ascorbic acid and other antioxidant micronutrients to better health. This is associated to their capability in trapping the reactive oxygen species responsible for a broad-spectrum of damages to biological systems (degenerative diseases, cancer) (Elsayed, 2001).

The baobab fruit is exploited traditionally for the treatment of microbial diseases (dysentery, diarrhoea) in many African countries including Cameroon; it also has hepatoprotective effect (Hanafy et al., 2016), cardioprotective (Ghoneim et al., 2016), antidiabetic (Irondi et al., 2017) and is used by many communities as an alternative to imported Western drugs (FAO, 1993; Kamatou et al., 2011). This indigenous fruit contributes to nutrition and food security, health and income generation of local communities in Sub-Saharan Africa (Muthai et al., 2017), especially during times of seasonal food shortages or emergencies such as drought and floods (Saka et al., 2004). Baobab fruits may be processed into different products including juice, yoghurt, gruel, sour dough, oil and coffee-like drinks. It can also be dried as food reserve (Saka et al*.,* 2002). Despite its nutritional and health properties, it is not extensively studied (Russo et al., 2019) and remains underutilized in Cameroon. The small section of the population who knows about this indigenous fruit usually consumes it in the form of juice, a locally made beverage for home consumption or sold on the streets. This leads to a variety of production processes involved, each with a different impact on health value of the final juice. Eulalia and Agnieszka (2009) showed that the antioxidant capacity of a product is influenced by the technological processes involved. This study is therefore aimed at identifying the traditional processes of baobab juice production in Cameroon and their impact on its antioxidant and vitamin C contents.

MATERIALS AND METHODS

Survey on the consumption and production of baobab juice

A survey was carried out in June 2016 in the Benoue Subdivision (North Region of Cameroon) where baobab trees are naturally found and its juice is highly consumed by the population. A total of 92 persons (either sellers or consumers) randomly selected took part in the study. The only inclusive criterion was the consumption or the production of baobab juice. A questionnaire was used to collect information on the level of consumption and motivations, ingredients and production process used for preparation, factors determining its quality, and preservation methods.

The influence of traditional production processes on baobab juice antioxidants and vitamin C

Baobab fruits were harvested in the Benoue Subdivision and immediately transported to the laboratory. Sugar (from sugarcane) was purchased from a local market. The baobab fruit pods were broken and lightly pounded in a traditional mortar to separate the pulp from the seeds. Samples for juice were then prepared following the main traditional production processes identified from the survey and their antioxidant content directly assessed by determining their Total Antioxidant Capacity (TAC), Total Phenolic Content (TPC), Radical Scavenging Activity (RSA) and Vitamin C (ascorbic acid) content. All the analyses were performed in triplicate.

Determination of total antioxidant capacity

One millilitre of juice was diluted in 3 ml of distilled water; then 0.3 ml of the diluted juice was mixed with 3 ml of phosphomolybdenum reagent solution and put in a boiling Water Bath at 95°C for 90 min. The mixture was cooled to room temperature (25°C) and the absorbance was measured at 695 nm against the reagent blank. Ascorbic acid was used as standard at a concentration of 5 mg/mL. The TAC of the samples was calculated using Equation 1 and results expressed in mg Ascorbic Acid Equivalent (AAE)/100 mL of juice (Prieto et al., 1999).

$$TAC = (DO\ Sample/DO\ Standard) \times Concentration\ of\ Standard \quad (1)$$

Determination of total phenolic content

The Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu method (Medina, 2011). 0.5 ml of diluted juice (1 ml of juice in 3 ml of distilled water) was mixed with 4.3 ml of distilled water and 0.2 ml of Folin-Ciocalteu reagent. The mixture was homogenized and incubated for 5 min at room temperature. Into each tube, 0.5 ml of 20% sodium carbonate was added, followed by 4.5 ml of distilled water. The tubes were homogenized and incubated in the dark for 1 h at room temperature and the absorbance was read at 725 nm. A standard curve was plotted using different concentrations of gallic acid which was used as standard (0, 25, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mg/L). The TPC was expressed as mg Gallic Acid Equivalent

Table 1. Consumption of baobab juice.

Parameter	Incidence (%)	Parameter	Incidence (%)
Reasons for consumption		**Frequency of consumption (No of times/month)**	
Taste	37.8	[1-4]	**50.8**
Health	**38.9**	[5-10]	12.8
Pleasure	36.7	[11-20]	16.4
No alcohol	20.0	[21-30]	20
Other	1.1		

(GAE)/100 ml of juice.

Determination of radical scavenging activity

The Radical Scavenging Activity (RSA) was evaluated using the 1.1-diphenyl-2-picryl hydrazyl (DPPH) radical. The analysis was carried out as described by Plaza et al. (2006). 7.8 ml of methanolic solution of DPPH (0.03 g/L) was added to 0.2 ml of diluted juice (1 ml of juice in 3 ml of HCl 1 %). The tubes were homogenized and incubated in the dark for 30 min at room temperature, followed by the reading of the absorbance at 515 nm. The DPPH solution was also read at the same wavelength using the reagent blank (HCl 1% + 0.03 g/L of DPPH). The RSA was calculated using the formula of Yen and Duh (1994) (Eq 2):

$$RSA\ \% = AA\ \% = [(A_{DPPH} - A_{Sam}) / A_{DPPH}] \times 100 \quad (2)$$

Where, A_{DPPH} = the absorbance of the DPPH control and A_{Sam} = the absorbance of the sample.

The RSA was expressed as antioxidant activity (AA %), which is the percentage of inhibition with respect to the control.

Determination of vitamin C content

The vitamin C content was determined by the titrimetric method (Peter et al., 2010). 50 µl of diluted fruit juice (10 times with distilled water) was put in a tube and 450 µl of glacial acetic acid 90% was added. The mixture was homogenized and a dye solution of dichloro-2.6-phenolindophenol (2.6-DCPIP) was added until a permanent light pink colour was obtained. The titre (*T*) was recorded. The titration was repeated and the sample replaced with 50 µl of water (for the control) (*B*1) and 50 µl of ascorbic acid, 0.2 mg Ascorbic acid/20 ml glacial acetic acid 90% (for the standard solution) (*st*).

The vitamin C content of the sample was calculated using the following Equation 3:

$$\text{Vitamin C (mg/100 ml)} = [(T - B1)/ (st- B1)] \times \text{Dilution factor} \quad (3)$$

Statistical analysis

The data collected during the survey were edited, coded and registered into a Microsoft Excel spreadsheet where they were treated, and the mean standard deviation of the values obtained was calculated. The software SPSS 20.0 for Windows was used to perform Analysis of Variance (ANOVA) and the post-hoc Turkey test to assess the significant differences observed (p<0.05) between these values.

RESULTS AND DISCUSSION

Survey

Socio-demographic variables

Among the 92 participants who took part in the survey, 66.3% were females. Most of these respondents were from the Far North (40.7%) and North (37.4%) regions of Cameroon, which are the only regions where baobab trees are naturally found due to the climate (Savannah drylands of sub-Saharan Africa) (Muthai et al., 2017). The other few respondents originated from the West (12.1%), Centre (6.6%), Adamawa (2.2%) and East (1.1%) regions of Cameroon. Baobab is rooted in the culture of the North and Far North regions of Cameroon, where the juice is often used as the main entertainment beverage in traditional ceremonies. This juice (the main product from baobab pulp) is recognised for its nutritional and health value and it is a source of income for these communities (Muthai et al., 2017).

Consumption of baobab juice

The data collected on baobab juice consumption (Table 1) shows that the surveyed population consume it for its healthy value (38.9%), its taste (37.8%), for pleasure (36.7%) and also because it is a non-alcoholic beverage (20%). Half of them consume the juice at a frequency of 4 times maximum/month (50.8%). Up to 20 % of respondents consume this juice, 21 to 30 times/month which means almost every day.

Production of baobab juice

Frequency of production: 52.4% of the respondents prepare baobab juice, maximum 4 times /month. The number of people was almost the same in the 3 other

Table 2. Parameters associated to baobab juice production.

Parameter	Incidence (%)	Parameter	Incidence (%)	Parameter	Incidence (%)
Frequency of production (times/month)		**Reasons for production**		**Ingredients for juice preparation**	
[1-4]	**52.4**	Consumption	**45.8**	Baobab's pulp	100.0
[5-10]	17.5	Commercial	12.0	Sugar	100.0
[11-20]	15.8	Both	42.2	Water	100.0
[21-30]	14.3			Extra ingredients	81.9
Source of baobab fruits		**Extra ingredients used**		**Parameters that affect the quality of juice**	
Market	**78.3**	Chemicals aroma	**84.74**	Quantity of water	**89.5**
Harvest	17.4	Chemicals instant drink (powder)	42.37	Quantity of pulp	25.6
Directly from Producers	4.3	Colouring	30.50	Temperature	18.6
		Other fruit juice	5.08.	Duration	9.3
		Natrum	3.38		
		Milk	1.69		
Volume of water (L) for 175 g of pulp (One plate)		**Quantity of sugar (g) used for the preparation of 1L of Juice**		**Steeping of pulp**	
[0.5-1]	2.8	<50	4.2	In fresh water (25°C)	**84.3**
[1-2]	14	[50-100]	28.0	In lukewarm water (40°C)	11.4
[2-3]	**42.3**	[100-150]	**42.2**	In hot water (100°C)	2.9
[3-4]	18.3	[150-200]	8.4	Boiling the mixture of pulp and fresh water	1.4
[4-5]	8.5	[200-250]	4.2		
[5-6]	9.9	[250-300]	8.4		
[6-8]	4.2	[300-500]	4.2		

classes: [5-10]; [11-20] and [21-30] times of juice production/month (Table 2).

Reasons for production: From the people interviewed, 45.8% prepare baobab juice only for home consumption, 12% for sale and 42.2% for both reasons.

Source of Baobab fruits: Most of respondents (78.3%) buy baobab fruits from the markets, 17.4% harvest it by themselves and 4.3% buy directly from producers. This tree is mostly found in bushes in mountainous areas, while some people have it in their land. It is difficult to climb because of the large size of the tree, which reaches, 18-25 m tall (Chadare et al., 2009), the reason why most of them buy it.

Main ingredients for Baobab juice*:* Baobab pulp, water and sugar were the main ingredients used by all respondents (100 %) in the formulation of baobab juice.

Extra ingredients for Baobab juice: 81.9% of the people add extra ingredients (chemical aroma, 84.74%; chemical instant drink, 42.37%; colouring agents, 30.50%; other fruit juices 5.08%; natron, 3.38% and milk, 1.69%) for commercial and/or sensory reasons. They add other sweeteners to reduce the quantity of sugar used, colouring agents to diversify the colour of the juice and other ingredients to modify the taste. These food additives were said to reduce the cost of production, attract customers and increase profits.

Volume of Water for one plate of pulp: The volume of water generally used for 175 g (one plate) of the baobab fruit pulp varied from 0.5 to 8 L. For this quantity of pulp, most people (42.3%) used between 2-3 L of water.

Quantity of sugar used/litre of juice: The quantity of sugar used for the preparation of 1 L of baobab juice varied from 31.25 g to 500 g, but the most widely used quantity ranged from 100-150 g (42.2%) followed by the range, 50-100 g (28%). The pulp/water ratio and the quantity of sugar used for 1 L of juice tended to change depending on the aim of production. Those who prepare it for their own consumption focused on the health value and the taste. They therefore apply a high pulp/water ratio to have a thick juice, and little or much sugar according to their preference. Sellers rather apply a low pulp/water ratio, less sugar and additional sweeteners, in

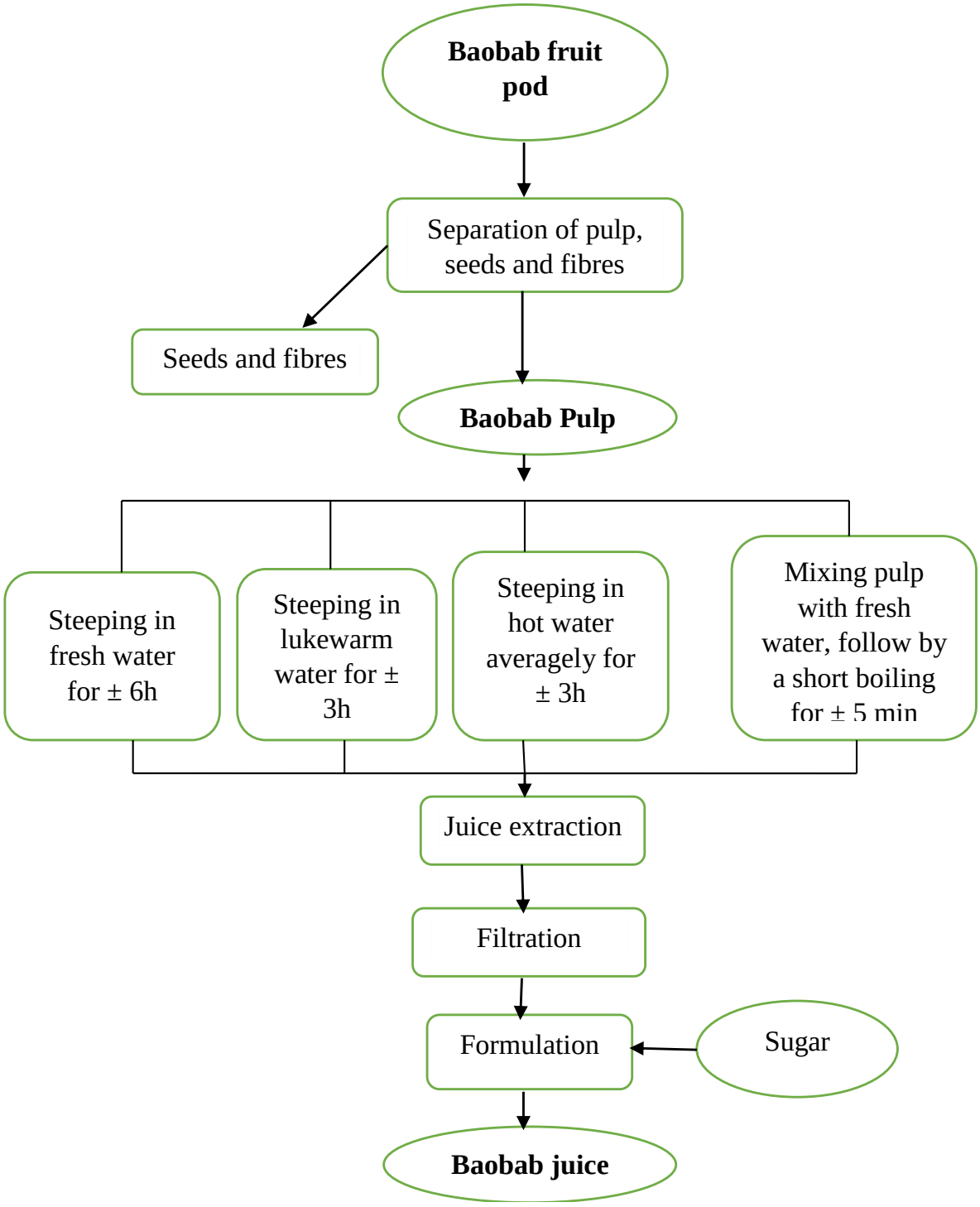

Figure 1. Diagram of the traditional production processes of baobab juice.

order to have more benefits.

Production processes: Figure 1 gives an overview of the 4 traditional production processes identified. They all include the following steps: The separation of baobab pulp from seeds and fibres, steeping with water, extraction and filtration of the juice and addition of sugar. It is at this last step that the other extra ingredients previously cited are added by the producers who use

Table 3. Preservation of baobab juice.

Parameter	Incidence (%)	Parameter	Incidence (%)
Storage methods		Duration of preservation	
Freezing	51.1	Less than one week	75.6
Refrigeration	35.9	Between one-four weeks	13.3
Traditional methods	13.0	Between one-three months	5.6
		More than three months	5.6

them. The way steeping is conducted was the key step differentiating the processes.

Steeping of pulp: Most of the respondents (84.3%) used the method which consisted of steeping the pulp in fresh water for an average of 6 h, while 11.4% steeped the pulp in warm water and 2.9% in hot water for about 3 h; and 1.4 % put the pulp in fresh water followed by boiling for a short time, ± 5 min (Table 2).

In order of importance, respondents estimated that the volume of water (89.5%), quantity of pulp (25.6%), steeping temperature (18.6%) and duration of steeping (9.3%) determine the quality of the baobab juice (Table 2). As reported by Charles et al. (2007), the pulp/water ratio influences the texture of juice. It determines whether the juice will be dense or light. The temperature affects the colour, odour, flavour and taste of the juice.

Preservation of baobab juice

Methods of preservation: Freezing was the most commonly used preservation method (51.1%), followed by refrigeration (35.9%) (Table 3). 13% of the surveyed population use other traditional preservation methods like steeping the bottle of juice in clay pots containing water (limited efficiency). This was due to poverty or electricity problems.

Duration of preservation: These different methods of preservation were usually needed to preserve juice for less than one week (75.6% of respondents). Generally, the consumers prepare juice only when they need to consume it, they do not have to preserve it for long. For traders, they sell it every day, so they do not also keep it for long.

Preparation of Baobab juice samples using different traditional processes

Eight different juice samples were prepared following the 4 traditional processes identified in the survey. In fact, for each process, baobab juice with two different pulp / sugar ratios (42 g/62 g, Process 1-4 and 87.5 g/231 g, Process 1'-4') per litre was prepared. These ratios were chosen to simulate those used when preparing this juice for sale and home consumption, respectively. As a pilot study, the first three processes were those where the pulp was steeped in fresh water at 25°C (Process 1), warm water at 40°C (Process 2) and hot water at 100°C (Process 3) all for 15 min. For process 4, the pulp was mixed with fresh water and directly boiled for 2 min. These durations were the shortest reported from the survey. The temperature profile for this steeping step, under the 8 conditions tested is presented in Figure 2. It was not affected by the change of pulp/water ratio. All the extracted juice samples were filtered using a sieve of 0.5 mm diameter before addition of sugar.

Antioxidants and vitamin C contents of baobab juice

The results obtained from the biochemical analysis of the juice samples are presented in Table 4.

Baobab pulp is known to be a rich source of antioxidants and vitamin C (Donatien et al., 2011). As expected, an increase in the pulp/water ratio of baobab juice led to an increase in the values obtained for the different parameters analysed. However, this rise was not statistically significant in the case of vitamin C.

The Total Antioxidant Capacity (TAC) of the 8 samples varied between 0.65 g AAE/100 ml and 5.76 g AAE/100 ml. For the ratio of pulp/sugar/water of 42 g/62 g/1 L, there was a significant difference ($p < 0.05$) between Process 1 and the 3 others which had the same TAC. For a high ratio of pulp/sugar/water (87.5 g/231 g/1 L), there was no statistical difference ($p > 0.05$) among processes (1'&2'; 2'&3' and 3'&4'). The use of boiling water for the steeping step (Process 3) or boiling of water and pulp mixture for a short time (Process 4) significantly ($p < 0.05$) enhanced the TAC of the juice. In each Process, the TAC significantly ($p < 0.05$) increased with the amount of pulp.

The Total Phenolic Content (TPC) of the juice ranged from 22.97-46.72 mg GAE/100 ml. Irrespective to the ratio, the temperature did not show any effect on TPC ($p > 0.05$). In each process, increase of pulp amount was associated with a significant increase in TPC ($p < 0.05$). Process 4' had the highest content of total phenolic

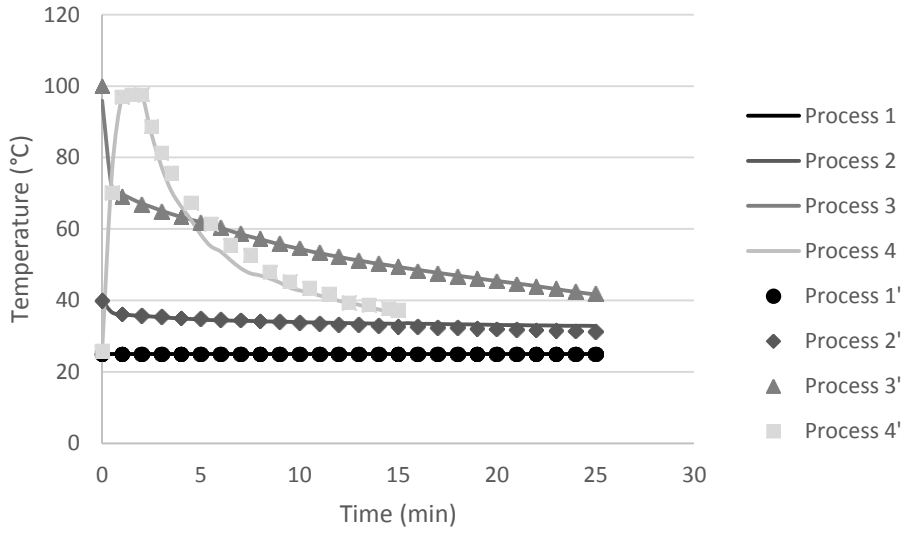

Figure 2. Temperature profiles during the steeping step. Processes 1, 2, 3 & 4= Pulp / sugar ratio of 42 g / 62 g per litre of juice; Processes 1', 2', 3' & 4'= pulp / sugar ratio of 87.5 g /231 g per litre of juice.

Table 4. Biochemical characteristics of baobab juice samples produced.

Samples*		TAC (g AAE /100 ml)	TPC (mg GAE/100 ml)	DPPH (% of Inhibition)/100 ml	Vitamin C (mg/100 ml)
Process 1 (Fresh water : 25°C, 15 min)	1	0.65±0.05^{a}	24.05±0.70^{a}	10.85±0.70^{a}	19.25±1.48^{a}
	1'	4.06±0.17^{c}	39.67±1.61^{bc}	28.83±2.33^{b}	22.22±0.00^{ab}
Process 2 (Lukewarm water : 40°C, 15 min)	2	1.85±0.02^{b}	22.97±0.44^{a}	8.69±1.66^{a}	22.96±0.74^{ab}
	2'	4.45±0.19^{cd}	37.14±0.45^{bc}	25.94±3.29^{b}	25.92±0.74^{ab}
Process 3 (Boiling water : 100°C, 15 min)	3	2.06±0.16^{b}	26.16±1.13^{a}	14.57±1.00^{a}	24.44±1.28^{ab}
	3'	5.24±0.43^{de}	41.95±0.42^{cd}	28.40±1.74^{b}	31.11±0.00^{bc}
Process 4 (Boil the mixture of pulp and water for 2 min)	4	2.51±0.12^{b}	34.12±3.13^{b}	25.79±1.58^{b}	30.37±0.74^{bc}
	4'	**5.76±0.26^{e}**	**46.72±0.61^{d}**	**33.08±2.48^{b}**	**38.51±5.34^{c}**

*1, 2, 3, 4= Pulp / sugar ratio of 42 g / 62 g per litre of juice; **1', 2', 3', 4'=** pulp / sugar ratio of 87.5 g /231 g per litre of juice

Means in the same row with different superscript letters are significantly different (p < 0.05), **AAE**= Equivalent Ascorbic Acid; **GAE**= Equivalent Gallic Acid

compounds (46.72 ± 0.61 GAE/100 ml). The TPC of our samples was much lower than the values (260.80 ± 0.27 mg GAE/100 ml), reported by Tembo (2016) for baobab juice samples from Malawi made with fresh water (pulp/water ratio of 100 g/1 L), and by Konan et al. (2015) with commercialised baobab juice (CBJ) in Ivory Coast (50.1 ± 15.5 mg GAE/ml). However, the use of Process 4 (boiling pulp-water mixture for 2 min) led to a significantly (p < 0.05) high TPC of the final products. Balunkeswar et al. (2015) showed that thermal processing increased the total antioxidant activity of some vegetables. It may cause complex physical and chemical reactions affecting the phenolic composition, such as release of phenolic compounds from their bound forms, degradation of polyphenols and the breakdown and transformation of phenolic compounds (Lo Scalzo et al., 2004; Chen et al., 2013).

The radical scavenging activity (RSA) of the juice ranged from 8.69-33.08% of Inhibition/100mL. The DPPH scavenging activity from the different processes and the two ratios was not temperature-dependent (p > 0.05). An increasing amount of baobab pulp had a positive effect on DPPH inhibition (p < 0.05), except in Process 4. The highest percentage of inhibition obtained was 33.08 ± 2.48% (Process 4') which was lower than the DPPH inhibition value (80.94 ± 0.72%) of commercial baobab juice (CBJ) in Malawi. The higher DPPH values observed in CBJ could be attributed to high TPC and sugar

metabolites (Tembo, 2016).

Vitamin C content

The Vitamin C content was from 19.25 mg/100 ml (with fresh water) to 38.51 mg/100 ml (boiled mixture of pulp and water for 2 min). The vitamin C was also highest in the sample that was prepared using Process 4, as for TAC, TPC and DPPH. Since, vitamin C is known to be a thermosensitive compound (Ranu and Uma, 2012), its highest content in samples produced by boiling (using Process 3 and 4) suggests that the short duration at high temperature during the steeping step (Figure 2) helps to enhance its extraction and also that of the bioactive compounds studied. The vitamin C content of these samples (19.25-38.51 mg/100 ml) was higher than the one obtained with traditional baobab juices in Senegal (12-14 mg/100 ml) made by steeping pulp in fresh water for 5 to 480 min at the ratio of 1/3 (w/v) and to the values obtained with commercialised baobab juice in Malawi (5.09 ± 0.39 mg/100 mL) (Cissé et al., 2009; Tembo, 2016). Besides the differences in the production processes, the difference in values may be also be associated to the variation of baobab pulp composition from one region to another, as noticed by Tembo (2016) when comparing the TPC of pulp samples from Malawi (1866.81 ± 1.61 mg /100 g FW) to those from Burkina Faso (3518-4058 mg GAE/100 g by Lamien-Meda et al., 2008) and Madagascar (1085 mg GAE /100 g, by Cissé et al., 2013),

Conclusion

This study reveals that, both men and women produce and consume baobab juice in the North and Far North regions of Cameroon. The main ingredients used are baobab pulp, water and sugar, and many people add extra ingredients for commercial and/or sensory reasons. The pulp/water ratio and the quantity of sugar used for 1 L of juice varies depending on the aim of production (consumption or for sale). Four main traditional processes of baobab juice production (steeping the pulp in fresh water for 6 h, in warm water or in hot water for about 3 h; and boiling the pulp-water mixture for a short time, ± 5 min) in Cameroon were identified, the main difference being the temperature of water during the steeping step. In order of importance, the volume of water, quantity of pulp, steeping temperature and duration of steeping determine the quality of the baobab juice prepared. These production methods differently affect the final antioxidants and vitamin C contents of the juice. The highest TAC, TPC, DPPH and vitamin C was in the sample that was prepared using a short boiling step.
The process involving a short boiling step or steeping with hot water shall be recommended to optimize their extraction from the pulp.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Balunkeswar N, Rui HL, Juming T (2015). Effect of processing on phenolic antioxidants of fruits, vegetables, and grains -A review. Critical Reviews in Food Science and Nutrition 55:887-918.

Bremer B, Bremer K, Chase MW, Fay MF, Reveal JL, Bailey LH, Soltis DE, Soltis PS, Stevens PF (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society 161:105-121.

Chadare F, Linnemann A, Houhouigan J, Nout MJ, Van Boekel MAJ (2009). Baobab Food Products: A Review on their Composition and Nutritional Value. Critical Reviews in Food Science and Nutrition 49:254-274.

Charles-Rodríguez AV, Nevárez-Moorillón GV, Zhang QH, Ortega-Rivas E (2007). Comparison of Thermal Processing and Pulsed Electric Fields Treatment in Pasteurization of Apple Juice. Food Bioproducts and Processing 85:93-97.

Chen Y, Yu L, Rupasinghe H (2013). Effect of thermal and non-thermal pasteurisation on the microbial inactivation and phenolic degradation in fruit juice: a mini-review. Journal of the Science of Food and Agriculture 93:981-986.

Cissé M, Sakho M, Dornier M, Diop C M, Reynes M, Sock O (2009). Caractérisation du fruit du baobab et étude de sa transformation en nectar. Fruits 64:19-34.

Cissé I, Montet D, Reynes M, Danthu P, Yao B, Boulanger R (2013). Biochemical and nutritional properties of baobab pulp from endemic species of Madagascar and the African mainland. African Journal of Agricultural Research 8:6046-6054.

De Caluwé E, Damme P, Van, Halamova K (2010). *Adansonia digitata L*. A review of traditional uses, phytochemistry and pharmacology. Afrika Focus 23:11-51.

Donatien K, Hagrétou S-L, Bréhima D, Clarisse SC, Mamoudou HD, Mogens J (2011). A review of baobab (*Adansonia digitata*) products: Effect of processing techniques, medicinal properties and uses. African Journal of Food Science 5:833-844.

Elsayed NM (2001). Antioxidant mobilization in response to oxidative stress: a dynamic environmental-nutritional interaction. Nutrition 17:828-834.

Eulalia JB, Agnieszka N (2009). Antioxidant properties of Berry fruit juices as dependent on raw material quality and technological processing: A Review. Functional Plant Science and Biotechnology 3:39-45.

Food and Agriculture Organization (FAO) (1993). Indigenous Multipurpose Trees of Tanzania: Uses and Economic Benefits for People. Available at: http://www.fao.org/docrep/x5327e/x5327e0g.htm.

Hanafy A, Aldawsari HM, Badr JM, Ibrahim AK, Abdel-Hady SS (2016).

Evaluation of hepatoprotective activity of *Adansonia digitata* extract on acetaminophen-induced hepatotoxicity in rats. Evidence-Based Complementary and Alternative Medicine, 7p.
Ghoneim MA, Hassan AI, Mahmoud MG, Asker MS (2016). Protective effect of *Adansonia digitata* against isoproterenol-induced myocardial injury in rats. Animal Biotechnology 27:84-95.
Irondi EA, Akintunde JK, Agboola SO, Boligon AA, Athayde ML (2017). Blanching influences the phenolics composition, antioxidant activity, and inhibitory effect of *Adansonia digitata* leaves extract on α-amylase, α-glucosidase, and aldose reductase. Food Science and Nutrition 5:233-242.
Kamatou GPP, Vermaak I, Viljoen AM (2011). An updated review of *Adansonia digitata*: A commercially important African tree. South African Journal of Botany 77:908-919.
Konan A G, Konan Y, Kone MW (2015). Polyphenols content and antioxidant capacity of traditional juices consumed in Côte d'Ivoire. Journal of Applied Biosciences 87:8015-8021.
Lamien-Meda A, Lamien CE, Compaoré MMYY, Meda RNTT, Kiendrebeogo M, Zeba B, Millogo JF, Nacoulma OG (2008). Polyphenol Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso. Molecules 13:581-594.
Latifou L, Madjid A, Yann A, Ambaliou S (2012).Antifungal, antibacterial and antioxidant properties of *Adansonia digitata* and *Vitex doniana* from Bénin pharmacopeia. Journal of Pharmacognosy and Phytotherapy 4:44-52.
Lo Scalzo R, Iannoccari T, Summa C, Morelli R, Rapisarda P (2004). Effect of thermal treatments on antioxidant and antiradical activity of blood orange juice. Food Chemistry 85:41-47.
Magdi AO (2004). Chemical and nutrient analysis of baobab (*Adansonia digitata*) fruit and seed Protein solubility. Plant Foods for Human Nutrition 59:29-33.
Medina MB (2011). Determination of the total phenolics in juices and superfruits by a novel chemical method. Journal of Functional Foods 3:79-87.
Muthai KU, Karori MS, Muchugi A, Indieka AS, Dembele C, Mng'omba S, Jamnadass R (2017). Nutritional variation in baobab (*Adansonia digitate* L.) fruit pulp and seeds based on Africa geographical regions. Food Science and Nutrition 5:1116-1129.
Ranu P, Uma G (2012). Effect of thermal treatment on ascorbic acid content of pomegranate juice. Indian Journal of Biotechnology 11:309-313.
Peter AI, John JM, Mohammed A (2010). Effects of Storage Period on Some Nutritional Properties of Orange and Tomato. Technical Report, AU Journal of Technology 13:181-185.
Plaza L, Sánchez-Moreno C, Elez-Martínez P, De Ancos B, Martín-Belloso O, Cano MP (2006). Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization. European Food Research and Technology 223:487-493.
Prieto P, Pineda M, Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry 269:337-341.
Russo M, Ronci MB, Vilmercati A, Gionfriddo M, Fanali C, Dugo L, Locato V, Mondello L, De Gara L (2019). African baobab (*Adansonia digitata*) fruit as promising source of procyanidins. European Food Research and Technology. DOI: 10.1007/s00217-019-03342-9.
Saka JDK, Mwendo-Phiri E, Akinnifesi FK (2002). Community processing and nutritive value of some miombo indigenous fruits in central and southern Malawi. In: Akinnifesi FK, Gumya AA, eds. Proceedings of 14th Southern African Regional Review and Planning Workshop, 3-7 September 2001, Harare, Zimbabwe, International Centre for Research in Agroforestry. Harare: ICRAF, pp. 164-169.
Saka JDK, Mkonda A, Schomburg A, Kwesiga F, Akinnifesi FK, Swai R (2004). Processing and utilisation of indigenous fruits of the miombo in southern Africa (F. Rao, M.R and Kwesiga, ed.). Nairobi, Kenya: World Agroforestry Centre.
Sidibé M, Scheuring JF, Tembely D, Sidibé MM, Hofman P, Frigg M (1996). Baobab – Homegrown vitamin C for Africa. Agroforesty Today 8:13-15.
Sidibé M, Williams JT (2002). Baobab, *Adansonia digitata* L. International centre for underutilized crops, University of Southampton, United Kingdom 102 p. ISBN 0854327649.
Silvia V, Elena B, Valentina B, Stefano M (2002). Antioxidant capacity of *Adansonia digitata* fruit pulp and leaves. Acta Phytotherapeutica 2(5):2-7.
Soloviev P, Niang TD, Gaye A, Totte A (2004). Variabilité des caractères physico-chimiques des fruits de trois espèces ligneuses de cueillette, récoltés au Sénégal: *Adansonia digitata*, *Balanites aegytiaca* et *Tamarindus indica*. Fruits 59:109-119.
Tembo DT (2016). Optimisation of Baobab *(Adansonia digitata)* fruit processing and handling techniques for increased human nutrition and commercialisation in Malawi. 286p.
Yen GC, Duh PD (1994). Scavenging Effect of Methanolic Extracts of Peanut Hulls on Free-Radical and Active-Oxygen Species. Journal of Agricultural and Food Chemistry 42:629-632.

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Micronutrients, antinutrients composition and sensory properties of extruded snacks made from sorghum and charamenya flour blends

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The effect of process variables (feed composition, feed moisture, and exit barrel temperature) on the vitamins, antinutrients, and sensory profile of extruded snacks produced from sorghum and charamenya flour blends using response surface methodology were investigated in this study. Feed composition (FC, 10-30%), feed moisture content (FMC, 25-30%) and exit barrel temperature (EBT, 110-130°C) were the independent process variables considered while vitamins, anti-nutrients and sensory evaluation were the response variables. Flour produced from sorghum (SF) and charamenya (CF) seeds were blended in the ratio of 3.2:96.8, 10:90, 20:80, 30:70 and 36.8:63.2%. The blends were conditioned to desired moisture content and allowed to equilibrate overnight in a refrigerator (4°C). Feeding temperature, cooking temperature, screw speed, and pressure of the extruder were set at 90 and 100°C, 250 rpm, and >300 psi, respectively. Extruded snacks from each run were collected when steady state extrusion conditions were attained and dried overnight at 40°C in a cabinet dryer. The vitamins (A, B_1, B_2, B_6, B_{12}, C, and D) and antinutrients (phytate, oxalate and tannin) content reduced post extrusion when compared with the flour blends prior to extrusion. They were significantly ($p<0.05$) affected by FC, FMC, and EBT. There were also observed significant ($p<0.05$) differences in the sensory scores of all the attributes tested except flavor ($p>0.05$), indicating the extrusion process's ability to reduce the beany flavor associated with legumes.

Key words: Extrusion, snacks, micronutrient, antinutrients, sensory, cereal-legume.

INTRODUCTION

Currently, snack consumption has increased in its popularity in Nigeria, shown by their conspicuous presence in the market. Cereal based snacks are the most widely consumed snack food items, many of which are low in nutrient density but high in calories and/or fat content (Hess et al., 2016). There has been huge concern from nutritionists over the years on snack consumption pattern of children, especially pre-schoolers; they frequently consume in-between meal snacks; thus, their diets are often nutritionally poor (Hess et al., 2016).

Processing methods reduced the level of anti-nutritional factors and minimize micronutrients losses are of great interest, both to manufacturers and consumers. Mechanical, thermal, or biological processes had the

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potential to improve the nutrient availability in foods (Hotz and Gibson, 2007). Extrusion cooking technology, a high-temperature-short-time process has been advocated for the production of half- or completely- cooked safe and acceptable foods (Guy, 2001). Raw materials of interest included flour and starch granules from cereal, tubers, and legumes. Extruded foods such as breakfast cereals, snacks, flakes, quick cooking pasta and texturised vegetable protein and breakfast gruel are important products from this process (Iwe, 2001; Nwabueze and and Iwe 2010; Leszek, 2011). Like other processes for heat treatment of food, extrusion cooking may have both beneficial and undesirable effects on nutritional value. Beneficial effects included the destruction of anti-nutritional factors and gelatinization of starch. However, heat-labile vitamins may be lost to varying extents. Thus, retention of vitamins and anti-nutrients post extrusion cooking is of great importance to food technologists and consumers, to assess the effects of food processing on these chemical components (Baskar and Aiswarya, 2016).

More still, consumers have higher interest in snacks that taste good, smell good, feel good, look good, and in addition, are nutritionally superior and healthy. These desirable product qualities are mostly influenced by conditions employed during processing; conditions of processing such as the type of extruder, quantity of moisture in the feed material, temperature of different sections of the barrel, screw speed and feed rate into the extruder (Thymi et al., 2005). The importance of bioactive compounds in human health and nutrition cannot be overemphasized. The bioactive compounds in extruded foods are mostly influenced by extrusion process variables such as shear, temperature, resonance time, and moisture content of the feed material (Brennan et al., 2011). The nutrient composition of sorghum indicated that it is a good source of energy, proteins, carbohydrates, vitamins, and minerals, including the trace elements, particularly iron and zinc, except calcium (Dicko et al., 2006).

Sorghum seeds are considered to be one of the most important sources of carbohydrates, vitamins, mineral elements, and dietary fiber (Stefoska-Needham et al., 2015). Legumes contained high levels of protein and important amino acids – lysine, and tryptophan; as such, their combination with cereals will make well-balanced food. Legumes such as cowpea, soybean, and groundnuts with diverse varieties are nutritionally important, containing about 17 to 25% protein, and good sources of phosphorus and iron; they are considered one of the underutilized crops that have great potentials for becoming industrial raw materials (FAO, 2004).

The cowpea variety (*Vigna unguiculata* Fabaceae*)* used for this study is a local species, small-sized, white in color with a black ringed eye called c*haramenya* in Nsukka, Nigeria. The seeds are traditionally consumed as *otipi* maize or yam based meals; they contained about 24 to 26% crude protein (Akinwale and Obiesan, 2012) and have great potential for industrial use to generate assorted snack products when suitable processing method is used.

The aim of this study was to validate extrusion cooking and conditions' ability to retaining more of the heat labile micronutrients (vitamins), reducing antinutrients and beany flavour which restricted legumes' use in snack making and establish or predict how much consumers are willing to accept products from a novel base ingredient.

MATERIALS AND METHODS

Sorghum grains and cowpea seeds used for this study were purchased from the Orba market in Nsukka L.G.A, Enugu State of Nigeria. Sorghum seeds (5 kg) were cleaned and dehulled after mild wetting, using a grain dehuller. The dehulled grains were washed, and the remaining hulls floated off, then dried for 6 h to a moisture content of 10%. The dried seeds were milled to flour in a locally fabricated attrition mill thrice in order to obtain fine flour. The flour was passed through a 150 µm pore sized sieve and stored in an airtight plastic container at room temperature until further use. Cowpea (*Charamenya*) seeds, on the other hand, were thoroughly cleaned and soaked in tap water at ambient temperature (30 ± 2°C) for 3 h to loosen the seed coats. The seeds were dried at a temperature of 30 ± 2°C for 8 h to approximately 12% moisture content. The seeds were dehulled with an attrition mill set to the right tolerances. The dehulled mass was manually winnowed and then milled to flour in a locally fabricated attrition mill. The flour was passed through a 150 µm pore sized sieve and stored in an airtight plastic container at room temperature until further use.

Experimental design

Independent variables considered were Feed composition, X_1 (10-30%), Feed moisture content, X_2 (25-35%) and Barrel exit temperature, X_3 (110-130°C). A three-factor (variable) central composite rotatable design (CCRD) (Box and Hunter, 1957) at five levels, shown in Table 1, was adopted for the study.

Composite flour formulation and moisture adjustment

Sorghum and cowpea flours were mixed at defined weight ratios (3.2:96.8, 10:90, 20:80, 30:70, and 36.8:63.2%) to obtain five composite flour blends. The initial moisture content of the blends was measured, M_1, prior to conditioning to the desired moisture content (M_2). This was done by spraying with a calculated amount of water and mixing continuously at medium speed in a small scale food mixer. The samples were kept in an airtight container and left in the refrigerator overnight for moisture equilibration. The amount of water to be added was calculated using the equation below:

$$W_w = W_d \times \frac{(M_2 - M_1)}{(1 - M_1)(1 - M_2)}$$

Where: W_w= weight of water to be added, W_d= weight of the raw flour, M_1 = initial moisture content, and M_2 = desired moisture content.

The outlines of the 15 experimental runs in their coded and natural values are presented in Table 2.

Table 1. Independent variable levels used for central composite design.

Variable	Coded variable				
	-α	Low	Medium	High	+α
	-1.68	-1	0	1	1.68
Feed composition (X_1)	3.2	10	20	30	36.8
Feed moisture content (X_2)	21.6	25	30	35	38.4
Barrel exit temperature (X_3)	103.2	110	120	130	136.8

Table 2. Experimental design for extrusion cooking exercise in their coded forms and natural units.

Run	Coded form			Natural form		
	X_1	X_2	X_3	X_1(%)	X_2(%)	X_3(^oC)
1	-1	-1	-1	10	25	110
2	+1	-1	-1	30	25	110
3	-1	+1	-1	10	35	110
4	+1	+1	-1	30	35	110
5	-1	-1	+1	10	25	130
6	+1	-1	+1	30	25	130
7	-1	+1	+1	10	35	130
8	+1	+1	+1	30	35	130
9	-1.68	0	0	3.2	30	120
10	+1.68	0	0	36.8	30	120
11	0	-1.68	0	20	21.6	120
12	0	+1.68	0	20	38.4	120
13	0	0	-1.68	20	30	103.2
14	0	0	+1.68	20	30	136.8
15	0	0	0	20	30	120

-1 = Lowest value, 1=Highest value, 0=Medium value, -1= -α, 1 = +α. feed composition (X_1), feed moisture content (X_2) and barrel exit temperature (X_3) Each design point will be run in triplicates and the average recorded. The experimental runs were randomized.

Production of extruded snacks using a single screw extruder

A small scale laboratory single screw extruder (Cosmic Controls, India), equipped with three heating zones (entry, mid and exit), 20 flighted screw feeder, 4 mm diameter die nozzle, and 30 cm barrel length was used to extrude the different runs. The extruder was fed gradually but continuously through a conical-shaped hopper mounted vertically above the end of the extruder. Experimental samples were collected when a steady state has been achieved. The extrudates were further dried in a cabinet dryer (GENLAB DC 500, Germany) overnight at 60°C. The resulting dried extrudates were packaged in zip lock polyethylene bags and coded according to the corresponding runs. Extrudates were subjected to sensory and laboratory analysis.

Analytical methods

Determination of Beta carotene, Vitamins B_1, B_2, B_6, B_{12}, C, and D

The pro-vitamin A and Vitamins B_1, B_2, B_6, B_{12}, C, and D contents of the extruded snacks were determined according to AOAC (2010).

Determination of phytate, tannin and oxalate contents

Phytate content was determined by the method described by Latta and Eskin (1980), tannin content was determined by the Folin-Denis colorimetric method described by Kirk and Sawyer (1998) while oxalate content of the extruded snacks was determined by the method described by Onwuka (2005).

Sensory evaluation of the extruded snacks samples

Extruded snacks from sorghum-cowpea blends were coded, placed in identical containers, and presented randomly. Twenty semi-trained panel members were used to assess the sensory attributes. The attributes tested were appearance, color, flavor, taste, mouthfeel, and overall acceptability using a 9-point hedonic scale (with 9 = extremely like and 1 = extremely dislike) as described by Ihekoroonye and Ngoddy (1985).

Data analysis

All data generated were subjected to statistical analysis of variance

Table 3. Vitamin contents of the extruded snacks produced from sorghum and charamnya flour blends.

Run	Pro-vitamin A (µg/100 g)	Vitamin B_1 (mg/100 g)	Vitamin B_2 (mg/100 g)	Vitamin B_6 (mg/100 g)	Vitamin B_{12} (mg/100 g)	Vitamin C (mg/100 g)	Vitamin. D (IU)
1 (10:25:110)	$24.67^{d} \pm 0.11$	$0.94^{bcd} \pm 0.04$	$0.56^{abc} \pm 0.01$	$0.74^{cd} \pm 0.01$	$1.94^{d} \pm 0.03$	$0.28^{b} \pm 0.03$	$0.65^{g} \pm 0.03$
2 (30:25:110)	$13.98^{k} \pm 0.07$	$0.62^{f} \pm 0.04$	$0.44^{d} \pm 0.05$	$0.52^{f} \pm 0.03$	$1.02^{h} \pm 0.01$	$0.34^{ab} \pm 0.01$	$1.08^{c} \pm 0.02$
3 (10:35:110)	$19.18^{h} \pm 0.08$	$0.93^{cd} \pm 0.05$	$0.56^{abc} \pm 0.05$	$0.73^{cd} \pm 0.03$	$1.49^{g} \pm 0.03$	$0.31^{ab} \pm 0.04$	$0.87^{ef} \pm 0.01$
4 (30:35:110)	$24.42^{de} \pm 0.06$	$0.78^{e} \pm 0.03$	$0.50^{cd} \pm 0.06$	$0.63^{e} \pm 0.01$	$1.92^{d} \pm 0.03$	$0.34^{ab} \pm 0.02$	$1.07^{c} \pm 0.03$
5 (10:25:130)	$13.48^{l} \pm 0.36$	$0.64^{f} \pm 0.02$	$0.44^{d} \pm 0.01$	$0.62^{e} \pm 0.06$	$1.06^{h} \pm 0.04$	$0.28^{b} \pm 0.02$	$0.66^{g} \pm 0.04$
6 (30:25:130)	$18.57^{j} \pm 0.01$	$0.76^{e} \pm 0.04$	$0.50^{cd} \pm 0.03$	$0.53^{f} \pm 0.03$	$1.44^{g} \pm 0.01$	$0.31^{ab} \pm 0.02$	$0.84^{f} \pm 0.01$
7 (10:35:130)	$18.94^{hi} \pm 0.11$	$0.93^{cd} \pm 0.04$	$0.50^{cd} \pm 0.06$	$0.63^{e} \pm 0.04$	$1.47^{g} \pm 0.03$	$0.31^{ab} \pm 0.04$	$0.86^{ef} \pm 0.05$
8 (30:35:130)	$24.35^{e} \pm 0.04$	$0.78^{e} \pm 0.05$	$0.56^{abc} \pm 0.02$	$0.73^{cd} \pm 0.01$	$1.92^{d} \pm 0.04$	$0.31^{ab} \pm 0.04$	$1.07^{c} \pm 0.01$
9 (3.2:30:120)	$20.45^{g} \pm 0.18$	$1.10^{a} \pm 0.09$	$0.62^{a} \pm 0.04$	$0.85^{a} \pm 0.01$	$2.42^{a} \pm 0.02$	$0.32^{ab} \pm 0.04$	$0.68^{g} \pm 0.01$
10 (36.8:30:120)	$30.41^{a} \pm 0.21$	$0.82^{e} \pm 0.02$	$0.51^{bcd} \pm 0.03$	$0.66^{e} \pm 0.01$	$1.60^{f} \pm 0.01$	$0.37^{a} \pm 0.04$	$1.31^{a} \pm 0.02$
11 (20:21.6:120)	$28.65^{b} \pm 0.03$	$1.05^{ab} \pm 0.02$	$0.60^{ab} \pm 0.07$	$0.81^{ab} \pm 0.03$	$2.27^{b} \pm 0.04$	$0.36^{a} \pm 0.04$	$1.24^{b} \pm 0.02$
12 (20:38.4:120)	$18.72^{ij} \pm 0.04$	$0.77^{e} \pm 0.04$	$0.49^{cd} \pm 0.04$	$0.62^{e} \pm 0.04$	$1.45^{g} \pm 0.03$	$0.31^{ab} \pm 0.01$	$0.85^{f} \pm 0.01$
13 (20:30:103.2)	$28.65^{b} \pm 0.03$	$1.05^{ab} \pm 0.05$	$0.60^{ab} \pm 0.03$	$0.81^{ab} \pm 0.01$	$2.27^{b} \pm 0.03$	$0.36^{a} \pm 0.03$	$1.24^{b} \pm 0.04$
14 (20:30:136.8)	$21.49^{f} \pm 0.04$	$0.85^{de} \pm 0.06$	$0.52^{abcd} \pm 0.04$	$0.68^{de} \pm 0.02$	$1.68^{e} \pm 0.01$	$0.34^{ab} \pm 0.01$	$0.96^{d} \pm 0.01$
15 (20:30:120)	$25.98^{c} \pm 0.15$	$0.97^{bc} \pm 0.07$	$0.57^{abc} \pm 0.01$	$0.76^{bc} \pm 0.03$	$2.05^{c} \pm 0.04$	$0.35^{a} \pm 0.01$	$1.13^{c} \pm 0.04$

Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly ($p<0.05$) different. Sample /Run ratio = Feed composition (%): Feed moisture content (%): Exit barrel temperature (Celsius).

(ANOVA) using the Statistical Package for Service Solution (SPSS) version 20.0 (SPSS Inc., USA). Means were separated using Duncan's new multiple range test. Significance was accepted at $p<0.05$, according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Vitamins contents of the extruded snacks

The vitamin contents of the extruded snack samples are presented in Table 3. Extrusion cooking conditions significantly ($p<0.05$) affected all the vitamins. This is expected as vitamins differed in composition and stability during thermal processing.

Provitamin A content of the extruded samples ranged from 13.48 to 30.41 µg/100 g. Sample No. 10 had the highest pro-vitamin A retention, while sample No.5 had the least vitamin A retention. The reason could be due to the unstable nature of vitamins during heat processing (FAO/WHO, 2001). However, pro-vitamin A content significantly ($p<0.05$) increased as levels of cowpea flour and feed moisture content increased, and as exit barrel temperature decreased. Samples Nos. 4 and 8 appeared similar; this could be attributed to similar feed composition effects. Vitamin A has been indicated for the development of good eyesight in children and also to improve color blindness.

Vitamin B_1 content of the extruded snacks ranged from 0.62 to 1.10 mg/100 g. Significant ($p<0.05$) differences exist in the Vitamin B_1 retention of extruded snacks post extrusion cooking. Samples with lower feed composition (10%) had higher vitamin B_1, which reduced as feed composition levels increased. This is not surprising as cereals have been implicated as rich sources of B vitamins. Lower exit barrel temperature (110°C) also favored more retention of thiamine when compared at an exit barrel temperature of 130°C. However, Thiamine content appeared to be highest and stable at 120°C EBT. Thiamine help the body maximize the use of carbohydrates, its major source of energy. It is also important for the proper functioning of the heart, nervous system, and muscle coordination (ICMR, 2002). Thiamine values obtained for this study were higher than the RDI value (0.5 mg/100 g) for feeding infants below three years (Nestle, 2000).

Vitamin B_2 content of the extruded snack samples ranged from 0.44 to 0.62 mg/100 g, as shown in Table 3. Samples Nos. 2 and 5 had the least, while sample No. 9 had the highest riboflavin (B_2) content. Significant ($p<0.05$) differences exist in the vit.B_2 contents of the products, although some samples appeared similar. Samples Nos. 1, 3, and 8 were similar despite higher variations in feed moisture content. This showed that feed moisture content had no significance ($p>0.05$) on the extruded products. Extruded snack samples with lower feed composition levels had higher riboflavin content; this is shown in sample No.9, which had the least feed composition value (3.2%). At higher exit barrel temperature (130°C), riboflavin content reduced. However, it appeared to stabilize at 120°C. Vit. B_2 is important for human nutrition and health (ICMR, 2002).

Extruded samples met the RDA (0.5 mg/day) for 1 to 3 years, as recommended by the report of a joint FAO/WHO (2001).

Table 4. Antinutrient composition of the extruded snacks produced from sorghum and charamnya flour blends.

Run	Phytate (%)	Oxalate (%)	Tannin (%)
1 (10:25:110)	0.039^{cd} ± 0.002	0.013^{gh} ± 0.001	0.087^{a} ± 0.007
2 (30:25:110)	0.037^{de} ± 0.004	0.040^{ef} ± 0.028	0.031^{de} ± 0.030
3 (10:35:110)	0.047^{b} ± 0.002	0.036^{efg} ± 0.003	0.083^{a} ± 0.003
4 (30:35:110)	0.046^{b} ± 0.004	0.033^{efgh} ± 0.004	0.087^{a} ± 0.003
5 (10:25:130)	0.023^{gh} ± 0.005	0.028^{efgh} ± 0.003	0.042^{d} ± 0.008
6 (30:25:130)	0.022^{h} ± 0.004	0.068^{cd} ± 0.003	0.074^{b}± 0.002
7 (10:35:130)	0.028^{fg} ± 0.003	0.008^{h} ± 0.002	0.057^{c}± 0.067
8 (30:35:130)	0.022^{h} ± 0.003	0.016^{fgh} ± 0.001	0.001^{g} ± 0.008
9 (3.2:30:120)	0.033^{ef} ± 0.004	0.155^{a} ± 0.002	0.087^{a} ± 0.002
10 (36.8:30:120)	0.071^{a} ± 0.003	0.046^{de} ± 0.003	0.001^{g} ± 0.002
11 (20:21.6:120)	0.043^{bc} ± 0.006	0.021^{efgh} ± 0.002	0.067^{b} ± 0.002
12 (20:38.4:120)	0.043^{bc} ± 0.002	0.021^{efgh} ± 0.006	0.021^{ef} ± 0.030
13 (20:30:103.2)	0.067^{a} ± 0.004	0.088^{bc} ± 0.002	0.069^{b} ± 0.007
14 (20:30:136.8)	0.029^{fg} ± 0.003	0.038^{efg} ± 0.003	0.007^{g} ± 0.003
15 (20:30:120)	0.031^{ef} ± 0.003	0.100^{b} ± 0.002	0.003^{g} ± 0.001

Values are means ± standard deviation of duplicate determinations. Means with different superscripts in the same column are significantly different ($p<0.05$). Sample /Run ratio = Feed composition (%): Feed moisture content (%): Exit barrel temperature (Celsius)

Pyridoxine (B_6) content of the extruded snack samples ranged from 0.52 to 0.85 mg/100 g. Sample No.2 had the least, while sample No.9 had the highest pyridoxine content. There were significant ($p<0.05$) differences in the vit. B_6 contents of the extruded snack products, although some samples appeared similar. Vit. B_6 contents followed a similar trend as vitamins $B_{1,}$ and $B_{2,}$ that is, samples containing more sorghum flour had higher pyridoxine values. Feed moisture content and exit barrel temperature had no significant ($p>0.05$) effect on the products as samples extruded at various conditions were similar.

Vitamin B_{12} content of the extruded snack samples ranged from 1.02 to 2.42 mg/100 g. Cobalamin (B_{12}) values were higher than other B vitamins in all extruded snack samples. At higher feed moisture content, a significant ($p < 0.05$) increase was observed in the vitamin, which could be attributed to cobalamin is a water-soluble vitamin.

Vitamin C contents of the extruded snack samples ranged from 0.28 to 0.37 mg/100 g. Sample No.1, as shown in Table 3, had the least vitamin C content, while sample No.10 had the highest. There were no significant ($p>0.05$) differences in the vitamin C contents of the extrudates, as most of the extrudates appeared similar. The vitamin D contents of the extrudates ranged from 0.65 to 1.31%. The result followed the same trend as vitamin C. However, samples that had a higher level of the legume flour and extruded at lower exit barrel temperatures appeared to have better retention of vitamins C and D.

Antinutrients composition of the extruded products

Phytate content of the extruded snack samples ranged from 0.022 to 0.067%, as shown in Table 4. Sample No. 13 had the highest phytate retention, while samples Nos. 5, 6, and 8 had the least retention for phytate. These values were very much lower than 0.26% reported by Anuonye et al. (2012), for extruded pigeon pea and unripe plantain. It was observed that increasing feed composition caused significant ($p<0.05$) reduction in phytate values. Also, higher exit barrel temperature was found to have reduced phytate significantly ($p<0.05$), while higher feed moisture content caused slight increases. It would be expected that lowering this compound should be enhanced the bioavailability minerals like zinc and iron in the extrudates. This is expected as phytic acid has been implicated in making certain minerals unavailable, as reported by Anuonye et al. (2012). Oxalate content of the extrudates which ranged from 0.008 to 0.155% was higher when compared with 0.04% reported by Anuonye et al. (2012), for extruded pigeon pea and unripe plantain. Although, there were significant ($p<0.05$) differences in the retention of oxalates among the extruded snack samples, most of the extruded samples were similar. From the result, a significant ($p<0.05$) increase was observed as cowpea flour increased in the blend.

The tannin composition of the extruded snack samples ranged from 0.001 to 0.087%. Low levels of tannin observed could be attributed to sorghum variety (white) used and proper dehulling given to the seeds during

Table 5. Sensory scores of the extruded snack produced from sorghum and charamnya flour blends.

Run/sample	Appearance	Color	Flavor	Taste	Mouth feel	Overall acceptability
1 (10:25:110)	$5.73^{bcd} \pm 1.62$	$5.93^{bcd} \pm 1.62$	$5.07^{a} \pm 1.43$	$4.33^{a} \pm 2.06$	$5.27^{ab} \pm 2.06$	$5.47^{abc} \pm 1.88$
2 (30:25:110)	$6.07^{bcde} \pm 1.49$	$5.93^{bcd} \pm 1.44$	$5.47^{a} \pm 2.17$	$4.93^{ab} \pm 2.40$	$5.27^{ab} \pm 2.58$	$5.47^{abc} \pm 4.46$
3 (10:35:110)	$6.53^{cde} \pm 1.60$	$6.93^{cd} \pm 1.49$	$6.20^{a} \pm 1.42$	$6.13^{ab} \pm 2.23$	$5.80^{ab} \pm 2.88$	$6.73^{bc} \pm 1.71$
4 (30:35:110)	$7.40^{c} \pm 1.55$	$7.20^{d} \pm 1.70$	$5.80^{a} \pm 2.08$	$6.47^{b} \pm 2.39$	$6.33^{ab} \pm 2.38$	$7.07^{c} \pm 1.71$
5 (10:25:130)	$5.80^{bcde} \pm 2.43$	$5.80^{bc\backslash d} \pm 2.51$	$5.40^{a} \pm 1.96$	$5.07^{ab} \pm 2.63$	$5.73^{ab} \pm 2.74$	$5.67^{abc} \pm 2.26$
6 (30:25:130)	$5.27^{abc} \pm 2.31$	$5.20^{ab} \pm 2.31$	$6.13^{a} \pm 1.85$	$5.80^{ab} \pm 2.39$	$5.20^{ab} \pm 2.45$	$5.73^{abc} \pm 2.28$
7 (10:35:130)	$4.73^{ab} \pm 1.98$	$5.40^{ab} \pm 2.10$	$5.53^{a} \pm 1.77$	$5.20^{ab} \pm 2.78$	$6.13^{ab} \pm 2.42$	$5.53^{abc} \pm 2.10$
8 (30:35:130)	$6.07^{bcde} \pm 2.15$	$6.00^{bcd} \pm 2.07$	$5.67^{a} \pm 1.95$	$5.67^{ab} \pm 2.66$	$6.60^{b} \pm 2.20$	$5.93^{abc} \pm 1.75$
9 (3.2:30:120)	$5.00^{abc} \pm 1.65$	$5.13^{ab} \pm 1.88$	$4.87^{a} \pm 1.60$	$5.33^{ab} \pm 2.06$	$5.00^{ab} \pm 1.96$	$5.00^{ab} \pm 1.89$
10 (36.8:30:120)	$5.07^{abc} \pm 1.83$	$5.27^{ab} \pm 1.62$	$5.67^{a} \pm 1.84$	$5.80^{ab} \pm 1.66$	$5.47^{ab} \pm 2.42$	$4.80^{a} \pm 2.04$
11 (20:21.6:120)	$3.93^{a} \pm 2.37$	$4.00^{a} \pm 2.42$	$5.93^{a} \pm 1.79$	$5.20^{ab} \pm 2.31$	$4.40^{a} \pm 2.67$	$4.33^{a} \pm 2.29$
12 (20:38.4:120)	$5.87^{bcde} \pm 2.03$	$6.00^{bcd} \pm 2.10$	$5.53^{a} \pm 1.88$	$5.27^{ab} \pm 1.75$	$6.00^{ab} \pm 1.89$	$5.20^{ab} \pm 2.14$
13 (20:30:103.2)	$5.40^{abc} \pm 1.80$	$5.13^{ab} \pm 1.77$	$4.87^{a} \pm 1.81$	$5.33^{ab} \pm 2.41$	$5.60^{ab} \pm 1.72$	$5.47^{abc} \pm 2.03$
14 (20:30:136.8)	$7.33^{de} \pm 1.40$	$7.07^{d} \pm 1.67$	$5.47^{a} \pm 2.64$	$5.93^{ab} \pm 2.71$	$5.80^{ab} \pm 2.40$	$5.80^{abc} \pm 2.78$
15 (20:30:120)	$3.87^{a} \pm 2.36$	$4.00^{a} \pm 2.07$	$5.07^{a} \pm 2.63$	$4.73^{ab} \pm 2.31$	$5.87^{ab} \pm 2.47$	$4.93^{ab} \pm 2.89$

Values are means ± standard deviation of 15 determinations. Means with different superscripts in the same column are significantly different ($p<0.05$). Sample /Run ratio = Feed composition (%): Feed moisture content (%): Exit barrel temperature (Celsius).

processing into flour. Higher feed moisture content caused a slight significant ($p<0.05$) increase in tannin contents of the products. According to Obadoni and Ochuko (2001), tannins are polyphenols, and all polyphenolic compounds are water-soluble in nature. Tannins form insoluble complexes with proteins, thereby decreasing the digestibility of proteins (Uzeochina, 2007). They have also been found to decrease palatability, caused damage to the intestinal tract, and enhanced carcinogenesis (Kumari and Jain, 2012).

Sensory evaluation of the extruded snack samples

The mean sensory scores of the extruded snack samples for appearance, crust color, flavor, taste, mouth feel, and overall acceptability are presented in Table 5. The mean score for appearance ranged from 3.87 to 7.40. There were significant ($p<0.05$) differences in the appearance of the extruded snack samples. Sample No. 4 had the highest value, while sample 15 had the least appearance mean score. The appearance however, improved with increased inclusion of the cowpea flour. There was observed reduction in appearance score as exit barrel temperature increased. This could be attributed to the darkening / browning effect (Leonel et al., 2010). The color score of extruded snacks ranged from 4.00 to 7.20. There were significant ($p<0.05$) differences in the color rating of extruded snack samples by the panelist. Mean scores for color were within the range of values (6.13 to 7.40) reported by Sawant et al. (2013) for ready-to-eat finger millet based composites. Samples Nos. 11 and 15 had the least, while sample No.14 had the highest mean score for color. Color is an important quality factor directly related to the acceptability of food products and is an important physical property to report for extruded products (Mesquita et al., 2013). The mean score for flavor ranged from 4.87 to 6.20. Sample No.3 had the highest flavor rating, while samples Nos.9 and 13 had the least score. There was no significant ($p>0.05$) difference in the flavor score of the extrudates. This could justify that beany flavor associated with legumes, which limit its use in industrial application, was significantly ($p<0.05$) reduced by extrusion cooking. Taste mean scores ranged from 4.37 to 6.47, with sample 1 having the least, while sample 4 had the highest value. Sample No. 1 differed significantly ($p<0.05$) than sample No.4, while the rest of the samples were similar in their taste rating. Mean scores for taste were, however, lower than the range of values (7.13 to 8.47) reported by Sawant et al. (2013), for ready-to-eat finger millet based composites. The low taste mean scores observed could be attributed to the non-use of basic snack ingredients such as sugar, butter, etc. From the results, taste perception by the panelist increased as the level of cowpea flour increased, while there were decreases in the taste scores as temperature decreased. This could be attributed to the fact that cowpea is enjoyed by many especially, in combination with cereal, for example, rice; at lower temperatures, the beany flavor maybe more intense, which may leave sour taste in the mouth, respectively. Mouthfeel means score ranged from 4.40 to 6.60 with sample No.11 having the least while sample No.8 had the highest mean score, while the rest of the samples were similar. The overall acceptability score ranged from 4.33 to 7.07 with sample No.11 having the least, while sample No. 4 had the

highest overall acceptability score. The result implied that cowpea flour could be incorporated in extruded snacks production with up to 30% substitution without fear of product rejection by consumers.

Conclusion

Vitamin retention stabilized at the exit barrel temperature of 120°C and 20% feed composition. High retention in vitamins post extrusion showed that extrusion conditions were adequate. The extrusion process considerably reduced most of the antinutrients. Extrudates are good sources of antioxidants, which will help in scavenging the activities of free radicals in the body asides nutrients supply. In this regard, low level of phytic acid in the extruded snack samples is desirable. The overall acceptability by panelists showed that extrudates have good potentials as a cheap source of ready-to-eat diet, safe in antinutrients, and could be utilized to improve food security against malnutrition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Akinwale RO, Obisesan IO (2012). Collection and characterization of some underutilized cowpea *(Vigna unguiculata* L.*)* cultivars of southwestern Nigeria. Journal of Sustainable Development and Environmental Protection 2(2):2-9.

Anuonye JC, Jigam AA, Ndaako GM (2012). Effects of extrusion cooking on the nutrients and antinutrients composition of pigeon pea and unripe plantain blends. Journal of Applied Pharmaceutical Science 2(5):58-162.

Association of Official Analytical Chemists (AOAC) (2010). Official methods of analysis. Association of Official Analytical Chemists.18th edition. (Horwitz, W. and Latimer, G. eds.). Gaithersburg, Maryland USA.

Baskar G, Aiswarya R (2016). Role of extrusion Technology in food processing and its effect on nutritional values. International Journal of Modern Science and Technology 1(1):1-4.

Box GEP, Hunter JS (1957). Multifactor experimental design for exploring response surfaces. The Annals of Mathematical Statistics 28(1):195–242.

Brennan CS, Brennan M, Derbyshire E, Kumar TB (2011). Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. Trend in Food Science and Technolog 22(10):570-575

Dicko MH, Gruppen H, Zouzou OC, Traore AS, Vanberkhel WJH, Voragen AGJ (2006). Effects of germination on the amylases and phenolics related enzymes in fifty sorghum varieties grouped according to food-end use properties. Journal of Science, Food and Agriculture 86(6):953-963.

Food and Agriculture Organisation (FAO) (2004). Cowpea: Post-harvest Operation. INPho – Post-harvest Operations. Food and Agriculture Organisation of the United Nations, Rome.

Food and Agriculture Organisation (FAO) (2001). Human Vitamin and Mineral Requirements. Report of a joint FAO/WHO expert consultation Bangkok, Thailand. Food and Nutrition Division, Food and Agriculture Organisation of the United Nations, Rome.

Guy R (2001). Raw materials for extrusion cooking. In: Extrusion Cooking Technology and Applications. CRS Press, Woodhead Publishing Limited, Cambridge England P 217.

Hess JM, Jonnalagadda SS, Slavin JL (2016). What Is a Snack, Why Do We Snack, and How Can We Choose Better Snacks? A Review of the Definitions of Snacking, Motivations to Snack, Contributions to Dietary Intake, and Recommendations for Improvement. Advances in Nutrition 7(3):466-475.

Hotz C, Gibson RS (2007). Traditional food-processing and preparation practices to enhance bioavailability of micronutrient in plant-based diets. Journal of Nutrition 137(4):1097-1100.

Ihekoroonye AI, Ngoddy PO (Eds.) (1985). Integrated Food Science and Technology of the Tropics. Macmillan publishers, London. pp. 265.

Indian Council of Medical Research (ICMR) (2002). Nutrient requirements and recommended dietary allowances for Indians P 83.

Iwe MO (2001). Organoleptic assessment of extruded blends of soy and sweet potato flour by response surface analysis. Plants Foods for Human Nutrition 60:1-14.

Kirk RS, Sawyer R (1998). Pearsons' composition and analysis of foods.9th Edition. Addison Wesley Longman Ltd, England pp. 609-655.

Kumari M, Jain S (2012). Tannins: an antinutrient with positive effect to manage diabetes. Journal of Recent Sciences 1(12):1–8.

Latta M, Eskin M (1980). A simple and rapid colorimetric method for phytate determination. Journal of Agricultural and Food Chemistry 28:1213-1215.

Leonel M, Martins JC, Mischan MM (2010). Production of functional snacks made from sour flour and soy flour. Rura Science 1(40):1418-1423.

Leszek M (2011). Extrusion Cooking Techniques Applications, Theory and Sustainability. Wiley - VCH Verlag & Co. Weinheim, Germany.

Mesquita CD, Leonel M, Mischan MM (2013). Effects of processing on physical properties of extruded snacks with blends of sour cassava starch and flaxseed flour. Food Science and Technology 33(3):1678-1687.

Nestle (2000). Food politics. University of California Press, Berkeley. pp. 45-46.

Nwabueze TU, Iwe MO (2010). Residence time distribution (RTD) in a single screw extrusion of African breadfruit mixtures. Food Bioprocess Technology 3:135-145.

Obadoni BO, Ochuko PO (2001). Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. Global Journal of Pure and Applied Science 8:203-208.

Onwuka GI (2005). Food analysis and instrumentation theory and practice. Naphthdi prints Lagos Nigeria P 160.

Sawant AA, Thakor NJ, Swami SB, Divate AD, Balasaheb SK, Krishi V (2013). Physical and sensory characteristics of ready-to-eat food prepared from finger millet based composite mixer by extrusion. Journal of Agricultural Engineering International 15:11-13.

Steel RCD, Torrie JH (1980). Principles and procedures of statistics. McGraw Hill Book Co., New York pp. 336-337.

Stefoska-Needham A, Beck EJ, Johnson SK, Tapsell LC (2015). Sorghum: an underutilized cereal whole grain with the potential to assist in the prevention of chronic disease. Food Reviews International 31(4):401-437.

Thymi S, Krokida MK, Pappa A, Marourlis ZB (2005). Structural properties of extruded corn starch. Journal of Food Engineering 68(4):519-526.

Uzeochina OB (2007). Evaluation of the effect of processing techniques on the nutrient and antinutrients contents of pigeon pea (*Cajanus cajan*) seed flours. Journal of Food Science 28:76-77.

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