African Journal of Food Science

Volume 11 Number 5, May 2017
ISSN 1996-0794

Academic Journals

ABOUT AJFS

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

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

Contact Us

Editorial Office: ajfs@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://academicjournals.org/AJFS

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

Editors

Editorial Board

African Journal of Food Science

Table of Contents: Volume 11 Number 5 May, 2017

ARTICLES

academicJournals

Vol. 11(5) pp. 124-129, May 2017
DOI: 10.5897/AJFS2016.1574
Article Number: 9F03FE563925
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Qualitative evaluation and biocompounds present in different parts of camu-camu (*Myrciaria dubia*) fruit

Maria Luiza Grigio[1*], Edvan Alves Chagas[2], Bala Rathinasabapathi[3], Pollyana Cardoso Chagas[1], Antonia Raniely Vieira da Silva[2], Sara Thiely Moreira Sobral[1] and Railin Rodrigues de Oliveira[1]

[1]Federal University of Roraima (UFRR), Boa Vista, Roraima, Brazil.
[2]Brazilian Agricultural Research Corporation, Boa Vista, Roraima, Brazil.
[3]Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, U.S.A.

Received 7 February, 2017; Accepted 29 March, 2017

Camu-camu fruits have high functional importance and great potential as food. Knowing and determining the nutrients present in its fruitsis crucial, in order to understand the relationship between consumption and human health. For this reason, the objective of this work was to evaluate qualitative attributes, such as organic acids content, antioxidant capacity and functional potential of different parts of camu-camu fruit. The fruit used in the present study were collected in the state of Amazonas, Brazil, and analyzed at the University of Florida, Gainesville, U.S.A. Seed, peel, pulp, pulp+peel, and whole fruit were evaluated regarding pH, soluble solids, titratable acidity, and levels of ascorbic acid, phenolic compounds, anthocyanins, and flavonoids. Antioxidant activity was evaluated by the Ferric Reducing Antioxidant Power (FRAP) and Diphenyl-1-picrylhydrazyl (DPPH) methods. Based on the results, camu-camu fruit proved to be a good source of acids, and the pulp is the part that is most concentrated in organic acids. Also, the pulp presents the highest amount of phenolic compounds and highest antioxidant activity. The peel had relatively higher amount of pigments, anthocyanins and flavonoids, and higher concentration of ascorbic acid, proving that it can also be used as a source of bioactive compounds.

Key words: Functional food, antioxidant activity, Ferric Reducing Antioxidant Power (FRAP), Diphenyl-1-picrylhydrazyl (DPPH), pigments, Caçari.

INTRODUCTION

Camu-camu (*Myrciaria dubia* (H.B.K.) McVaugh), also known as 'caçari', 'araçád'água', or 'sarão', belongs to the family Myrtaceae, and is native to the Amazonian floodplains and lake (Maeda et al., 2007). For being a fruit species with great nutraceutical and technological potential, the interest of the scientific community on this species has increased in recent years.

Fruits are important nutritional sources, since they are

*Corresponding author. E-mail: luizagrigio@hotmail.com.

rich in vitamins, minerals and carbohydrates. Some species have higher content of a specific nutrient when compared with others. The increasing interest in camu-camu fruit is due to its remarkable ascorbic acid (vitamin C) content, and it is known as "King of Vitamin C" or "Super Fruit". In the state of Roraima, Brazil, the fruit is known as ‘caçari’, presenting mean values of 3,571 to 7,355 mg ascorbic acid 100 g^{-1} pulp (Aguiar and Souza, 2016; Chagas et al., 2015; Grigio et al., 2015; Grigio et al., 2016).

In addition to vitamin C, these fruits contain other anti-oxidant compounds, such as carotenoids, anthocyanins and other phenolic compounds which are valuable in human nutrition (Silva, 2012). These compounds help in the fight against and prevention of free radicals, increasing immune resistance and delaying the early or natural aging of cells (Santos et al., 2009).

However, the *in natura* consumption of camu-camu fruits is low due to the high content of acids (Azevedo et al., 2014), limiting its current consumption to the producing regions. The rest of the production is exported in the form of pulp, mainly to the United States, Europe and Japan (Akter et al., 2011), for the development of products that are considered natural sources of biocompounds or antioxidants.

Several studies have been carried out with the by-products obtained from fruit pulping, and they denote that the peel has great potential as promising sources of bioactive compounds, even though it is underutilized. These compounds can be used as functional food, not only in the Amazon region, but also in other parts of the world (Fracassetti et al., 2013; Azevedo et al., 2014; Azevedo et al., 2015). Moreover, other by-products that are considered waste could have great economic, nutritional, and functional potential. The seed, for instance, has also proved to be excellent source of minerals, with potential to be used in biotechnological development (Sousa et al., 2015).

Vitamin C has vital effect on the brain, since neurodegenerative diseases usually involve high levels of oxidative stress. Moreover, ascorbic acid has positive therapeutic implications against Alzheimer's disease, Parkinson's disease, Huntington's disease and stroke (Harrison and May, 2009). Thus, the potential of camu-camu to minimize the risks of these diseases is extremely significant.

Camu-camu has great functional and bioactive potential. Besides being rich in vitamin C, it has high levels of phenolic compounds, such as flavonoids. Some of these phenolic compounds also have antioxidant and anti-inflammatory properties, with potential to combat chronic diseases induced by the stress, when the fruits are consumed as part of the diet (Fujita et al., 2015). Antioxidants are chemical compounds that can prevent or reduce the oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen. Antioxidants have the ability to react with free radicals and thus restrict their effects on the organism (Couto and Canniatti-Brazaca, 2010).

Several studies have reported camu-camu as a food that helps spermatogenesis. The species also present antimutagenic potential, and helps treat diseases such as Alzheimer's and Parkinson's, diabetes, hypertension and obesity (Schwertz et al., 2012; Gonzales et al., 2013; Borges et al., 2014; Carvalho-Silva et al., 2014; Gonçalves et al., 2014; Azevedo et al., 2015; Fujita et al., 2015; Langrey et al., 2015).For this reason camu-camu has attracted great interest in the scientific environment, in order to exploit all its functional potential, nutraceutical and nutritional, which are still unknown and under-exploited.

Due to the high functional importance and to the great potential of camu-camu fruit, the knowledge and determination of its nutrients is imperative in order to understand the relationship between consumption and human health. And mainly phytochemical concentration differences between different parts of the camu-camu fruit are not known. For this reason, the objective of this work was to evaluate qualitative attributes, antioxidant capacity and functional potential, of different parts of camu-camu fruit.

MATERIAL AND METHODS

The camu-camu fruit used in the experiment were collected froma private property,in planted area, in Amazonas, located on Highway AM 010, km 98, Rio Preto da Eva-Brazil.

The fruit were collected at a mature stage, which was established in previous studies (Grigio et al., 2015). After harvest, fruit were taken to the Post-Harvest Laboratory of Embrapa Roraima, where they were cleaned and selected for the absence of damages, washed in running water, and sanitized with 0.02% sodium hypochlorite (NaClO) for 30 min, following Brazilian National Health Surveillance Agency (ANVISA) recommendations. After the hygienization, fruits were processed according to the treatments. Some fruits were properly pulped in an industrial pulper, with no water, by separating the pulp from the peel and the seeds; other fruit were kept intact; and some other fruit had only the seeds removed, keeping the pulp+peel for the treatments.

The treatments consisted of: peel+pulp, pulp, peel, whole fruit and seed. Totaling five treatments composed of four replicates each, where each replicate was from a sample of 30 fruits.

After the separation of the material according to the treatments, samples were lyophilized, stored in aluminized bags, and transported to the University of Florida, Gainesville, FL, U.S.A. for analysis.

The material was rehydrated according to the previously calculated moisture content equivalent to each treatment, until obtaining 10 g of fresh sample. The material was centrifuged in a refrigerated centrifuge at 4°C, 12,000 rpm, for 20 min, and the supernatant was removed for the following analyses:

pH

This was determined by reading 5 mL aliquot of the sample, using

an 814 USB sample processor.

Soluble solids (SS)

Soluble solids were estimated by the reading, using a portable refractometer (SOLOESTE, model RT-30ATC), with automatic temperature compensation (10 to 30°C). Results were expressed in °Brix (Institute Adolf Lutz**,** 2008).

Titratable acidity (TA)

This was determined by titration, following the methodology described by the Institute Adolf Lutz (2008). Results were expressed in mg of citric acid 100 g^{-1} sample.

Ratio (SS/TA)

This was calculated by the solid soluble:titratable acidity ratio.

Ascorbic acid

The samples were extracted using 0.5% oxalic acid and titrated with 2,6-dichlorophenolindophenol (Ranganna, 1986). Results were expressed in mg of ascorbic acid 100 g^{-1}sample.

Total flavonoid content

This was determined by the aluminum chloride colorimetric assay (Zhishen et al., 1999), using quercetin as standard. For the extraction, methanol solution and 5% aluminum chloride were added. After 30 min, spectrophotometer readings were performed at 441 nm. For each sample, a blank containing only sample and methanol was made. Results were expressed in µg of quercetin equivalent g^{-1} sample.

Total anthocyanins content

This was determined according to the method described by Lee and Francis (1972), using cyanidin as standard. An acidified solution of methanol (methanol:HCl, 85:15, v:v) was added to the samples. Once being homogenized, samples were stored in the dark. After 24 h, samples were read in a spectrophotometer at 520 nm. Results were expressed in µg of cyanidin equivalent g^{-1} sample.

Phenolic compounds

This was determined according to the Folin-Ciocateau spectrophotometric method described by Singleton et al. (1999). A 20 µl aliquot of the sample was diluted in 1.58 mL water. Afterwards, 100 µL of the Folin-Ciocalteu reagent was added and wellmixed. After 30 s to 8 min, 300 µl of the sodium carbonate solution was added and stirred. Solutions were allowed to stand at 20°C for 2 h, and the absorbance of each solution was determined at 765 nm. Results were expressed in mg of gallic acid g^{-1} sample.

Antioxidant activity (DPPH)

Total antioxidant activity was determined by the oxidation inhibition potential, using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) as reference (Brand-Williams et al., 1995). One gram of the sample was weighed, 10 mL of ethyl alcohol was added and homogenized, and the mixture was taken to centrifuge at 6000 rpm for 50 min. Subsequently, the supernatant was separated using a pipette, and the solution was stored in a dark bottle in an ice-bath, added to 3 mL ethanol. The standard curve was made with ascorbic acid. Absorbance was read using a spectrophotometer at 517 nm, in 500 µL of the sample extract, added to 300 µL of the DPPH stock solution. Results were expressed in mg of ascorbic acid equivalent g^{-1}sample.

Antioxidant activity (FRAP)

Total antioxidant activity was also estimated by the iron reduction method (FRAP), following the procedure adapted by Rufino et al. (2006). One gram of sample was added to 40 mL 50% methanol, which was homogenized and allowed to stand for 60 min at room temperature. Afterwards, samples were centrifuged (15,000 rpm) for 15 min, and the supernatant was transferred to 100 mL volumetric flask. Forty millimeter 70% acetone was added to the residue of the first extraction, which were then homogenized and allowed to stand for 60 min at room temperature.

One hour later, samples were centrifuged again (15,000 rpm) for 15 min, and the supernatant was transferred to the volumetric flask containing the first supernatant, and the volume was completed with distilled water. The extract and the FRAP reagent (Acetate buffer, 0.3 mol/L, 2,4,6-Triz(2-pyridil)-s-triazine (TPTZ) solution, 8.0 mmol/L and ferric Chloride solution, 20 mmol/L) were brought to a water bath at 37°C.The standard curve was made with ascorbic acid. Absorbance reading was performed at 595 nm. Results were expressed in mg of ferrous sulfate g^{-1} fruit.

Data were subjected to analysis of variance, and the means were tested by the Tukey test at 5% probability using the SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

Results show statistically significant difference for all the treatments tested. When comparing the pH, only the seed was different from the others, with mean value higher than that of the other treatments (Table 1). Thus, the seed presented lower acidity when compared with the other parts of the fruit. Values lower than those observed in the present study have been reported in the literature (Akter et al., 2011; Barreto et al., 2013; Rufino et al., 2009).

The seed had the highest content of soluble solids, followed by whole fruit, peel, and peel+pulp, which did not show statistically significant difference between them. Finally, the pulp had the lowest soluble solids, with mean value of 5.53 °Brix, similar to the reports found in the literature regarding the pulp (Barreto et al., 2013; Fujita et al., 2013; Grigio et al., 2016) and the fruit (Rufino et al., 2009). Thus, it can be inferred that camu-camu seeds likely have the highest sugar content. Similar behavior was observed by Daiuto et al. (2014), who detected higher sugar contents when evaluating avocado seed. This greater amount of sugar is possibly the necessary source for seed germination.

Table 1. pH, soluble solids (SS), titratable acidity (TA), ratio (SS/TA) in different parts of camu-camu fruit.

Parameter	pH	Soluble solids (°Brix)	Titratable acidity (mg citric acid 100 g^{-1} sample)	Ratio (SS/TA)
Peel+Pulp	3.12±0^{B}	6.93±0.2^{B}	4.05±0^{B}	10.79±0.2^{BC}
Pulp	3.09±0.1^{B}	5.53±0^{C}	4.86±0^{A}	10.39±0^{BC}
Peel	3.16±0^{B}	7.00±0.2^{B}	2.96±0.1^{C}	9.93±0^{C}
Whole fruit	3.09±0^{B}	7.26±0^{B}	4.69±0.1^{A}	11.79±0.1^{B}
Seed	3.93±0^{A}	13.86±1.3^{A}	4.33±0.1^{B}	19.19±1.3^{A}

*Means±standard deviation followed by the same letter in the column do not differ by the Tukey test at 5% probability.

Table 2. Anthocyanins, flavonoids, phenolic compounds, antioxidant activity (FRAP and DPPH) and ascorbic acid in different parts of camu-camu fruits.

Sample	Anthocyanins (µg g^{-1}sample)	Flavonoids(µ g g^{-1}sample)	Phenolic compunds(mg g^{-1} sample)	Antioxidant activity (FRAP) (mg g^{-1} sample)	Antioxidant activity (DPPH) (mg g^{-1} sample)	Ascorbic acid (mg 100 g^{-1} sample)
Peel + Pulp	50.7±1.5^{B}	440.9±4.9^{C}	130.6±2.9^{B}	11.7±0.2^{A}	8.53±0^{AB}	4,426±90^{A}
Pulp	13.6±1.3^{D}	91.5±6.5^{D}	141.2±3.9^{A}	11.7±0.3^{A}	8.46±0^{B}	2,919±55^{C}
Peel	107.2±2.6^{A}	550.8±12.3^{A}	98.1±0.6^{D}	11.7±0^{A}	8.53±0^{AB}	3,068±135^{BC}
Whole fruit	29.9±0.6^{C}	517.6±21.0^{B}	117.7±5.9^{C}	11.5±0.4^{A}	8.59±0^{A}	3,204±61^{B}
Seed	n.^{d}	49.6±2.8E	n.^{d}	8.4±0.2^{B}	2.45±0^{C}	551±31^{D}

*Means±standard deviation followed by the same letter in the column do not differ by the Tukey test at 5% probability. ** n.d = not detected.

The highest titratable acidity index was observed in the pulp, according to the method of the Instituto Adolf Lutz, and it did not statistically differ from the whole fruit, followed by the seed and the peel + pulp, which also did not have statistically significant difference between them. The peel had the lowest acidity index. These results are consistent with those found in the literature (Rufino et al., 2009; Akter et al., 2011; Barreto et al., 2013; Fujita et al., 2013).

The pulp and the whole fruit had higher values of acid. Results indicated that a large number of acids are present in the pulp of camu-camu fruits, corroborating the literature that reports camu-camu fruit to be an extremely acidic one (Rufino et al.,2009; Grigio et al., 2015; Maeda et al., 2006). On the other hand, the lowest values were observed in the peel. This fact is consistent with the fact that camu-camu pulp is the part of the fruit that contains the highest contents of acids, since in the treatments with pulp these values were always higher than in the treatments with peel or seed.

Regarding the acceptability index, or ratio, higher values were observed for the seed, since it contained a greater quantity of Brix, a proxy for soluble sugars, consequently denoting more expressive values to this ratio, followed by the whole fruit, peel+pulp, and pulp, which did not differ statistically between treatments. The lowest soluble solids/titratable acidity ratio was observed peel+pulp and pulp.

When analyzing the total anthocyanins content, statistically significant difference was observed among all the treatments. As expected, the content was higher in the peel of camu-camu fruits, due to the greater purplish pigment, with mean value of 107.19 µg g^{-1}sample (Table 2), followed by peel + pulp, and whole fruit, with mean values of 50.70 and 29.90 µg g^{-1}sample, respectively. Lower incidence of this pigment was observed in the pulp, and no traces of anthocyanins were detected in the seed. Similar behavior was observed by Solis et al. (2009), when evaluating the pulp, peel and seed of camu-camu fruits. The more mature the fruits, the higher was the anthocyanin content (Pinto et al., 2013).

In relation to the total flavonoids content, statistically significant difference was observed among all treatments. The highest values were: Peel (550.80), whole fruit (517.65), peel+pulp (440.92), pulp (91.54), and seed (49.56 µg g^{-1}sample). The peel presented the highest concentration of these pigments, such as anthocyanins and flavonoids, which have antioxidant potential. Anthocyanins were responsible for the color of the peel, corroborating with other studies that evaluated the pulp, the peel and seed of camu-camu fruits (Solis et al., 2009).

Currently, camu-camu has been considered one of the richest foods, mainly due to its great amount of phenolic compounds, and consequently to its great antioxidant potential. The highest amount of phenolic compounds

was observed in the pulp, followed by pulp+peel, whole fruit, and peel, with mean values of 141.18, 130.57, 117.66 and 98.15 mg of gallic acid g^{-1} sample. Phenolic compounds were not observed in the seed. Similar results have reported the pulp with the greatest amount of phenolic compounds (Solis et al., 2009). In particular this fruit is known for its content of ellagic acid derivatives (Francassetti et al., 2013), which we assume are preserved in our dried powdered samples.

Several studies have been carried out in order to evaluate the amount of phenolic compounds present in the fruits, and a great difference on the mean values had been observed between different studies. This difference may be due to genetic diversity or even to conservation methods and maturation stage of the fruit, resulting in the divergent data found in the literature (Bataglion et al., 2015; Fracassetti et al., 2013; Fujita et al., 2013; Neves et al., 2015; Solis et al., 2009; Rufino et al., 2010; Villanueva et al., 2010).

The FRAP method detected greater antioxidant activity than the DPPH method, and thus, the former is considered one of the most indicated to evaluate camu-camu fruits (Rufino et al., 2010). Higher antioxidant activity was observed in both methods when both pulp and peel were evaluated. However, antioxidant activity is very low in the seed. Much information is found regarding the antioxidant activity of camu-camu fruits, and several methods are being tested. However, all of them lead to the same result, proving that camu-camu is one of the fruits with the highest antioxidant activity, and with great potential for application and development as a functional food (Baldeon et al., 2015; Barreto et al., 2013; Bataglion et al., 2015; Fracassetti et al., 2013; Fujita et al., 2013; Neves et al., 2015; Rufino et al., 2010; Solis et al., 2009; Villanueva et al., 2010).

The highest concentration of ascorbic acid was observed for the peel+pulp, with mean value of 4.426 mg acid 100 g^{-1} sample, followed by the whole fruit and the peel, which did not present statistical difference between them. On the other hand, the lowest values were also observed for the seed. The highest values were always observed in the treatments that contained the peel, which is in agreement with other results found in the literature (Zamudio, 2007; Imán-Correa et al., 2011). According to Fujita et al. (2013), the lyophilization process can generate losses of ascorbic acid and of other polyphenols. However, our study shows that the products can still retain considerable amounts of vitamin C, phenols, anthocyanins and antioxidants, even after being dried.

Conclusion

Results show that camu-camu fruit has good qualitative attributes. The pulp had the greatest amount of phenolic compounds and antioxidant activity. Higher amounts of pigments (such as anthocyanins and flavonoids), and higher concentration of ascorbic acid were found in the peel, making it useful as a source of bioactive compounds as a food colorant with antioxidant qualities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors thank the Research Foundation FAPEAM (Fundação de Amparo à Pesquisa do Estado do Amazonas), Capes (Coordenação de aperfeiçoamento de Pessoal de Nível Superior) for financial support and CNPq (Conselho Nacional de desenvolvimento científico e tecnológico) for financial support and the productivity scholarship of Dr Edvan Alves Chagas.

REFERENCES

Aguiar JPL, Souza FCA (2016). Camu-Camu super fruit *Myrciaria dubia* (H.B.K) Mc Vaugh) at different maturity stages. Afr. J. Agric. Res.11:2519-2523.

Akter MOh S, Eun J, Ahmed M (2011). Nutritional compositions and health promoting phytochemicals of camu-camu (*Myrciaria dubia*) fruit: A review. Food Res. Int. 44:1728-1732.

Azevêdo JCS, Fujita A, Oliveira EL, Genovese MI, Correia RTP (2014). Dried camu-camu (*Myrciaria dubia* H.B.K. McVaugh) industrial residue: A bioactive-rich Amazonian powder with functional attributes. Food Res. Int. 62:934-940.

Azevêdo JCS, Fujita A, Oliveira EL, Genovese MI, Correia RTP (2015). Neuroprotective effects of dried camu-camu (*Myrciaria dubia* HBK McVaugh) residue in *C. elegans*. Food Res. Int. 73:135-141.

Baldeon EO, Alcaniz M, Masot R, Fuentes EM, Barat JM, Grau R (2015). Voltammetry pulse array developed to determine the antioxidant activity of camu-camu (*Myrciaria dubia* (H.B.K.) McVaug) and tumbo (*Passiflora mollisima* (Kunth) L.H. Bailey) juices employing voltammetric electronic tongues. Food Control 54:181-187.

Barreto AG, Cabral LMC, Matta VM, Freitas SP (2013). Clarificação de polpa de camu-camu por microfiltração. Brazilian J. Food Technol. 16:207-215.

Bataglion GA, Silva FMA, Eberlin MN, Koolen HHF (2015). Determination of the phenolic composition from Brazilian tropical fruits by UHPLC–MS/MS. Food Chem. 180:280-287.

Borges LL, Conceição EC, Silveira D (2014). Active compounds and medicinal properties of *Myrciaria* genus. Food Chem. 153:224-233.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28:25-30.

Carvalho-Silva LB, Dionísio AP, Pereira ACS, Wurlitzer NJ, Brito ES, Bataglion GA, Brasil IM, Eberlin MN, Liu RH (2014). Antiproliferative, antimutagenic and antioxidant activities of a Brazilian tropical fruit juice. Food Sci. Technol. 59:1319 -1324.

Chagas EA, Lozano RMB, Chagas PC, Bacelar-Lima CG, Garcia MIR, Oliveira JV, Souza OM, Morais BS, Araújo MCR (2015). Intraspecific variability of camu-camu fruit in native populations of northern Amazonia. Crop Breed. Appl. Biotechnol.15:265-271.

Couto MAL, Canniatti-Brazaca SG (2010). Quantificação de vitamina C

e capacidade antioxidante de variedades cítricas. Ciência e Tecnologia de Alimentos 30:15-19.
Daiuto ER, Tremocoldi MA, Alencar SM, Vieites RL, Minarelli PH (2014). Composição química e atividade antioxidante da polpa e resíduos de abacate 'Hass'. Revista Brasileira de Fruticultura 36:417-424.
Fracassetti D, Costa C, Moulay L, Tomás-Barberán FA (2013). Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (*Myrciaria dubia*). Food Chem. 139:578-588.
Fujita A, Borges K, Correia R, Franco B, Genovese M (2013). Impact of spouted beddrying on bioactive compounds, antimicrobial and antioxidant activities of commercial frozen pulp of camu-camu (*Myrciaria dubia* Mc. Vaugh). Food Res. Int.54: 495-500.
Ferreira DF (2011). Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia 35:1039-1042.
Gonçalves AESS, Lellis-Santos C, Curi R, Lajolo FM, Genovese MI (2014). Frozen pulp extracts of camu-camu (*Myrciaria dubia* McVaugh) attenuate the hyperlipidemia and lipid peroxidation of Type 1 diabetic rats. Food Res. Int. 64:1-8.
Gonzales GF, Vasquez VB, Gasco M (2013). The transillumination technique as a method for the assessment of spermatogenesis using medicinal plants: the effect of extracts of black maca (*Lepidium meyenii*) and camucamu (*Myrciaria dubia*) on stages of the spermatogenic cycle in male rats. Toxicol. Mechanisms Methods 23:559-565.
Grigio ML, Chagas EC, Durigan MFB, Sousa AA, Mota-Filho AB, Chagas PC (2016). Determination of harvest time and quality of native camu-camu fruits (*Myrciaria dubia* (Kunth) Mc Vaugh) during storage. Fruits 71:373-378.
Grigio ML, Durigan MFB, Chagas EA, Chagas PC, Nascimento CR, Almeida MS (2015). Post-harvest conservation of camu–camu fruits (*Myrciaria dubia* (Kunth) Mc Vaugh) using different temperatures and packages. Food Sci. Technol. 35: 652-658.
Harrison FH, May JM (2009). Vitamin C function in the brain: Vital role of the ascorbate transporter (SVCT2). Free Radical Biol. Med. 46:719-730.
Imán-Correa S, Zamudio LB, Sotero-Solís V, Cruz CO (2011). Contenido de vitamina C en frutos de camu camu Myrciaria dubia (H.B.K) Mc Vaugh, en cuatro estados de maduración, procedentes de la Colección de Germoplasma del INIA Loreto, Perú, Scientia Agropecuaria 2:123-130.
Instituto Adolfo Lutz (2008). Métodos físico-químicos para análise de alimentos. São Paulo: Instituto Adolfo Lutz, 2008. 1020 p
Langley P, Pergolizzi J, Taylor R, Ridgway C (2015). Antioxidant and associated capacities of camu-camu (*Myrciaria dubia*): A systematic review. Journal of Alternative and Complementary Medicine 21: 8-14.
Lee DH, Francis FJ (1972). Standardization of pigment analyses in Cranberries. Hort. Sci. 7:83-84.
Liu TZ, Chin N, Kiser MD, Bigler WN (1982). Specific spectrophotometry of ascorbic acid in serum or plasma by use of ascorbate oxidase. Clin. Chem. 28:2225-2228.
Maeda RN, Pantoja L, Yuyama LKO, Chaar JM (2006). Determinação da formulação e caracterização do néctar de camu-camu (Myrciaria dúbia McVaugh). Ciência e Tecnologia de Alimentos 26:70-74.
Maeda RN, Pantoja L, Yuyama LKO, Chaar JM (2007). Estabilidade de ácido ascórbico e antocianinas em néctar de camu-camu (*Myrciaria dubia* (H. B. K.) McVaugh). Ciência e Tecnologia de Alimentos 27:313-316.
Neves LC, Silva VX, Pontis JA, Flach A, Roberto SR (2015). Bioactive compounds and antioxidant activity in pre-harvest camu-camu [*Myrciaria dubia* (H.B.K.) Mc Vaugh] fruits. Sci. Horticulturae 186:223-229.
Pinto PM, Jacomino AP, Silva SR, Andrade CAW (2013). Ponto de colheita e maturação de frutos de camu-camu colhidos em diferentes estádios. Pesquisa Agropecuária Brasileira, 48:605-612.
Ranganna S (1986). Analysis and quality control for fruit and vegetable products. Tata McGraw-Hill Publishing, 1112 p.
Rufino MSM, Alves RE, Brito ES, Jiménez JP, Calixto FS, Filho JM (2010). Bioactive compounds and oxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chem. 121:996-1002.
Rufino MSM, Alves RE, Brito ES, Silveira MRS, Moura CFH (2009). Quality for fresh consumption and processing of some non-traditional tropical fruits from Brazil. Fruits 64:361-370.
Rufino MSM, Alves RE, Brito ES, Morais SM, Sampaio CG, Jiménez JP, Saura-Calixto FD (2006). Metodologia Científica: Determinação da atividade antioxidante total em frutas pelo método de redução do ferro (FRAP). Comunicado Técnico, Embrapa Agroindústria Tropical. 4 p.
Santos JC, Santos AP, Rocha CIL (2009).Estrutura da cadeia produtiva de camu-camu no Brasil. Relatório Final de projeto. Belém: CPATU:. 35 p.
Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. Methods Enzymol. 299:152-178.
Solis VS, Doza LS, Sotero DG, Correa SI (2009). Evaluacion de la actividad antioxidante de la pulpa, cascara y semilla del fruto del camu camu (*Myrciaria dubia* H.B.K.). Revista Sociedad Química Perú. 75:293-299.
Sousa RCP, Chagas EA, Guimaraes PVP, Nascimento Filho WB, Melo Filho AA (2015). Sais minerais em extrato aquoso de coprodutos de *Myrciaria dubia* (Kunth.) McVaugh, Myrtaceae. Revista Virtual de Quimica. 7:1299-1305.
Villanueva-Tiburcio JE, Condezo-Hoyos LA, Asquieri ER (2010). Antocianinas, ácido ascórbico, polifenolestotales y actividad antioxidante, em lacáscara de camu-camu (*Myrciaria dubia* (H.B.K) McVaugh). Ciência e Tecnologia de Alimentos 30:151-160.
Zamudio LHB (2007). Caracterização de vitamina C em frutos de camu-camu (*Myrciaria dúbia* (H.B.K.)) em diferentes estágios de maturação do banco ativo de germoplasma de Embrapa. 121f. Monografia (Especialização em Nutrição Humana). Brasília: Faculdade de Ciências da Saúde, Universidade de Brasília. 2007.
Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on Superoxide radicals, Food Chem. 64:555-559.

academicJournals

Vol. 11(5) pp. 130-133, May 2017
DOI: 10.5897/AJFS2016.1543
Article Number: 1D7F40F63927
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Quality assessment of oil from *Parkia filicoidea* (Mkundi) seeds

Davies Emmanuel Mweta[1]* and Peter Unique Magombo[2]

[1]Malawi University of Science and Technology, P.O. Box 5196, Limbe, Malawi.
[2]The Polytechnic, University of Malawi, Private Bag 303, Chichiri, Blantyre, Malawi.

Received 13 January 2017 ; Accepted 6 February 2017

Oil was extracted from *Parkia filicoidea* seed, an African locust bean seed locally known as Mkundi and its quality was determined in order to unravel its potential as source of edible oils in Malawi. The quality of the oil was determined by analysis of the iodine value, peroxide, saponification value, acid value, impurity percentage, colour and cloud point. The result showed that Mkundi seeds contain about 12.06 ± 0.26% crude oil with an impurity of 4.2%. The colour of the oil was deep red. The iodine (mg I_2/g), saponification (mg KOH/g), peroxide (mEq/g) and acid (mg KOH/g) values ranged from 20.13 to 21.23, 176.72 to 185.13, 1.92 to 2.20 and 3.36 to 4.60, respectively. These values fall within the ranges of edible oils found in the market and, therefore, Mkundi seeds are potentially a source of edible oil in Malawi.

Key words: *Parkia filicoidea*, edible oils, quality, Malawi, African locust bean seed.

INTRODUCTION

Natural vegetable oils and fats are increasingly becoming important in nutrition as well as the manufacturing industry worldwide. Nutritionally, vegetable oils are important sources of dietary energy and antioxidants. In the manufacturing industry, they are used as raw materials for the manufacture of various food, cosmetic, pharmaceutical and chemical products. Vegetable oils account for 80% of the world's natural oils and fat supply (Okullo et al., 2010).

Vegetable oils are sourced from diverse varieties of leguminous plants. With an ever increasing demand for vegetable oils for food and industrial applications, there is need for considerable expansion of oilseed crop production (Çamaş et al., 2007). This expansion can be achieved by exploring other sources of vegetable oils, especially underutilized oilseeds (Popoola et al., 2016).

Africa is one of the continents endowed with richest biodiversity in the world with an avalanche of many food plants used as herbs, health foods and for therapeutic purposes (Farombi, 2003), one of which is the African locust bean. The African locust bean tree is a perennial tree which belongs to sub-family, Mimosodee and family Leguminosae (now Fabaceae) (Akande et al., 2010). Locust bean tree is a leguminous crop peculiar to the tropics. The tree is not normally cultivated but can be seen in population of two or more in the savannah region of West Africa (Hopkins, 1983). The various types of African locust bean tree are *Parkia clappertoniana,*

*Corresponding author. E-mail: dmweta@must.ac.mw. Tel: +265 1 478 278 or +265 888 364444. Fax: +265 1 478 220.

Table 1. Physicochemical properties of *P. filicoidea* seed oil extracted.

Property	Value
Colour (Lovibond)	9.3R, 4.0B, 35.0Y: Deep red
Iodine value, Wij's (g of I/100 g oil)	20.72 ± 0.55
Saponification Value, mg KOH/g	148.46 ± 4.28
Acid value, mg KOH/g	4.04 ± 0.63
Cloud point	4°C
Peroxide value, mg O_2/g	
Fresh	1.95 ± 0.30
After 2 weeks	2.17 ± 0.31

Parkia bicolor, Parkia filicoidea and *Parkia biglobosa.* In Malawi, locust bean (*P. filicoidea*) is found in abundance as the wild uncultivated type. It grows well in Zomba, Nkhatabay and Ntchisi districts where the climate is temperate (Forestry Research Institute of Malawi, 1998).

The locust bean seed is widely used for its remarkable nutritional and dietary value. The seeds are rich in protein, lipids and vitamin B2 and when fermented are rich in lysine. The fat in the beans is nutritionally useful (approximately 60% unsaturated). The fermented locust bean seeds are commonly used in soups and stews (Owolarafe et al., 2011). The locust bean seed is also rich in carbohydrate, soluble sugars and ascorbic acid and the cotyledon is very nutritious, has less fibre and ash contents (Alabi et al., 2005). Its oil is suitable for consumption since it contains very low acid and iodine contents; it has essential acids and vitamins and serves as a protein supplement in the diet of poor families and has been reported to be non-toxic. The oil also has very high saponification value and hence would be useful in the soap industry (Aiyelaagbe et al., 1996).

Currently in Malawi, the focus is on sunflower, groundnuts, soybeans and cotton as the main four oil seeds (Ministry of Trade and Industry, 2014). These conventional sources of vegetable oil have little impact on meeting the increasing demand of vegetable oil for both human and industrial use. Hence, there is need to supplement the supplies with other sources, especially underutilized oilseeds (Popoola et al., 2016). With the current research trend towards the use of unconventional oil seeds for commercial vegetable oil production, it is worthwhile to examine the physico-chemical characteristics of oils from some of these less common seeds that, at present, exist as uncultivated types in order to explore their wider exploitation. Hence, this study was carried out to extract oil from *P. filicoidea* (Mkundi) seeds and evaluate its quality as a natural source of edible oils in Malawi

MATERIALS AND METHODS

Mature seeds were collected from Zomba and Nkhatabay districts of Malawi in the months of January and February. The seeds were sun-dried to facilitate handling during removal of husks and for easy crushing. The husks were carefully removed using a scalpel blade to avoid damaging the seed inside. The seeds were then ground using a mortar and a pestle.

Oil extraction

Oil was extracted from the samples using Soxhlet apparatus with diethyl ether as the extracting solvent. After extraction, the solvent was removed from the extract through evaporation on rotatory evaporator followed by in an oven set at 40 to 60°C. The extracted oil was subsequently used for analysis (Caltest Standard Operating Procedure, 2009). The oil obtained was stored under refrigeration (4°C), until used for further analysis.

Analysis of the oil

Physical and chemical parameters of Mkundi oil

The extracted Mkundi oil was analyzed for some important physical and chemical properties. Oil yield, color, cloud point, acid, iodine, saponification and peroxide values of the oil were determined using standard American Oil Chemists' Society methods (AOCS 1997). The percentage impurity of the oil was determined using standard Official Methods of Analysis of the Association of Analytical Chemists (AOAC, 1990).

Statistical analysis

The statistical analysis was performed using Statistix 8 for Windows (Analytical Software, Tallahassee, USA). All analyses were performed in triplicate. Data were expressed as mean ± standard deviation (SD) and statistical significance was assigned at $P \leq 0.05$ level.

RESULTS AND DISCUSSION

Physicochemical properties

The knowledge of physical and chemical properties of edible oils is important, having a role in processing functionality, storage stability and nutritional behaviour. Physicochemical properties of the extracted Mkundi seed oil are presented in Table 1.

The major characteristics usually included in national

Table 2. Requirements for edible oils from the four main oil seeds in Malawi.

Physicochemical property	Cotton	Groundnut	Sunflower	Soybean
Acid value, mg KOH/g	0.6	4	0.6	4
Colour	Dependent on gossypol content	35Y, 4R	25Y, 3R	Not specified
Free Fatty Acids (% by mass, maximum)	<0.15 as oleic acid	<0.15 as oleic acid	<0.15 as oleic acid	<0.15 as oleic acid
Iodine value, Wij's	100-115	80-106	126-135	120-143
Moisture and volatile matter (% by mass, maximum)	0.1	0.1	0.1	0.1
Peroxide value, mg O_2/kg (maximum)	2	2	2	2
Refractive index	1.4580 - 1.4660 at 40°C	1.4680 - 1.4720 at 2 °C	1.4670 -1.4690 at 4 °C	1.4660 -1.4700 at 4 °C
Saponification Value, mgKOH/g	189-198	188-196	188-194	189-195
Unsaponified matter, (% by mass, maximum)	15	10	15	15

and international standards and trading specifications and used for quality control for crude oils in laboratories include the free fatty acids, iodine value, saponification value, refractive index, specific gravity, unsaponifiable matter, moisture and impurities. These are intended to give a quick impression of the authenticity of the oil and the likely losses in refining (Gunstone, 2002). However, vegetable oils generally exhibit considerable deviations in their composition, thus it is difficult to define single values for chemical and physical properties of edible oils (Shatta et al., 2016). The characteristic requirements for edible oils in Malawi as set by the Malawi Bureau of Standards, a national quality regulatory body (MBS, 2011) are shown in Table 2.

The average oil content of *P. filicoidea* seeds was found to be 12.06 ± 0.26% with an impurity of 4.2%. Its colour was deep red. The results indicate low oil content as compared to oil yield reported by Talabi and Enujiugha (2014) for African locust bean (*P. filicoidea)* as 20.68±0.71%. The cloud point was found to be 4°C, indicating that the oil can be stored at lower temperatures (Roiaini et al., 2015).

Acid value is often used an indicator for edibility of oil and suitability for industrial use (Augustine et al., 2013). Oils with high acid value are not suitable for cooking but can be utilised in the soap making and paint industries. Low acid values indicate stability over long periods of storage and suitability for consumption (Aiyelaagbe et al., 1996). The acid value obtained in this study (4.04 ± 0.63 mg KOH/g) is comparable to those of other vegetable oils most common in Malawi (Table 2). However, the acid value of the oil is lower than the ones reported for *P. biglobosa* seed oil and Shea nut (*Vitellaria paradoxa)* oil of 9.48 and 11.79 mg KOH/g, respectively (Augustine et al., 2013). Thus, the Mkundi seed oil could be utilised as edible oil as well as raw material for soap making and paint industries.

The iodine value is also an important characteristic of seed oils that guides its application in a processing industry and its classification. Oils with iodine value of less than 100 g I2/100 g are classified as non-drying oils (Igunbor et al., 2013). Non-drying oils are not suitable for ink and paint production due to their non-drying characteristics but may be useful in the manufacturing of soaps, leather and dressings, and as candle lubricants and hydraulic break fluids (Aremu et al., 2015; Adelaja, 2006; Kochhar, 1998). The iodine value obtained in this study (20.72 ± 0.55) clearly indicates that the Mkundi seed oil is a non-drying oil and could therefore be utilised as a raw material in the manufacture of leather, dressings, as candle lubricants and hydraulic break fluids (Aremu et al., 2015).

The iodine value also indicates the degree of unsaturation of a fat or oil which reflects the susceptibility of the oil to oxidation (Gunstone, 2002). Aremu et al. (2006) reported that the lower the iodine value, the lesser the number of unsaturated bonds; thus, the lower the susceptibility of such oil to oxidative rancidity. The low iodine values in this study indicate that the oil contains low level of polysaturated fatty acid and hence reduce the susceptibility to oxidative rancidity. This is also supportedby the peroxide value obtained which is also an indicator of deterioration of oil (rancidity); peroxide values (meq O_2/kg) less than 10 signify fresh oils and values between 20 and 40 leads to rancid taste (Adelaja, 2006). The peroxide value (mg O_2/g) for the fresh Mkundi seed oil was 1.95 ± 0.30 which rose to 2.17 ± 0.31 after two weeks of storage at

room temperature. This falls within the required peroxide values for conventional oils and confirms the low susceptibility of the Mkundi oil to rancidity (Augustine et al., 2013; MBS, 2011).

The saponification value gives a measure of the average molecular weight of the fatty acids present in the oil which also govern the utilisation of the oil (Talabi and Enujiugha, 2014; Alabi et al., 2005; Akanni et al., 2005). Talabi and Enujiugha (2014) reported that Locust bean seed *(P. filicoidea Welw)* oil with high saponification value (358.69 mg/g) contained low molecular weight fatty acids and may not be useful in soap making. Aalbi et al. (2005) reported saponification value of 160.60 ± 1.21 mg/g for oil derived from the cotyledon of *P.biglobosa* seed oil and suggested that the oil could be used in soap making industry. Akanni et al. (2005) reported saponification values ranging between 169.98 ± 4.25 and 239.830 ± 1.155 mg/g for some non-conventional seed oils and suggested that the oils contain high molecular weight fatty acids, and could be used in soap making industry. The saponification value obtained in this study (148.46 ± 4.28 mg/g) indicates the presence of lower molecular weight fatty acids in the Mkundi seed oil and thus not suitable for soap making.

Conclusion

The results of this study show that the Mkundi seed is potentially a good source of edible oil with low iodine and acid values; however, the yield of the oil is low. Physicochemical properties of Mkundi seed oil indicate that it is non-drying oil, has low levels of polyunsaturated fatty acids and has low susceptibility to deterioration by oxidation. However, there is need for further research on toxicity levels of the oil before its exploitation as edible oil in Malawi.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adelaja JO (2006). Evaluation of mineral constituents and Physico-chemical properties of some oil seed. M.Sc. Industrial Chemistry, University of Ibadan, Ibadan, Nigeria.

Aiyelaagbe OO, Ajaiyeoba EO, Ekundayo O (1996). Studies on the seed oils of *Parkia biglobosa* and *Parkia bicolor*. Plant Food Hum. Nutr. 49(3):229-33.

Akande FB, Adejumo OA, Adamade CA, Bodunde J (2010). Processing of locust bean fruits: Challenges and prospects. Afr. J. Agric. Res. 5(17):2268-2271.

Akanni M S., Adekunle S A. and Oluyemi E A. (2005). Physico-chemical properties of some nonconventional oil seed. J. Food Technol. 3:177-181.

Alabi DA, Akinsulire OR, Sanyaolu MA (2005). Qualitative determination of chemical and nutritional composition of *Parkia biglobosa* (Jacq.) Benth. Afr. J. Biotechnol. 4(8):812-815.

AOAC (1990). Official Methods of Analysis of the Association of Analytical Chemists, 15th Edition. Arlington, Virginia 22201, USA.

AOCS (1997). Official methods and recommended practices of the American Oil Chemists Society. American Oil Chemist Society, Champaign.

Aremu MO, Ibrahim H, EBamidele TO (2015). Physicochemical characteristics of the oils extracted from some Nigerian plant foods- a review. Chem. Process. Eng. Res. 32:36-52.

Aremu MO, Olaofe O, Akintayo ET (2006). Chemical composition and physicochemical characteristics of two varieties of bambara groundnut (*Vigna subterrenea*) flours. J. Appl. Sci. 6(9):1900-1903.

Augustine A, Okoro IC, Francis EU, Gilbert U, Okuchukwu O (2013). Comparative assessment of lipids and physicochemical properties of African locust beans and shea nut oils. J. Nat. Sci. Res. 3(11):25-31.

Caltest Standard Operating Procedure (2009). Soxhlet extraction method EPA 3540c. Available at: http://www.swrcb.ca.gov/public_notices/petitions/water_quality/docs/a2239/overview/Communications/AR-Comm%20(119).pdf

Çamaş N, Irak C, Esendal E (2007). Seed yield, oil content and fatty acids composition of safflower (*Carthamus tinctorius* L.) grown in Northern Turkey conditions. J. Fac. Agric. Univ. OMU 22:98-104.

Farombi EO (2003). African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. Afr. J. Biotechnol. 2(12):662-671.

Forestry Research Institute of Malawi (1998). Indigenous Plants of Malawi, Zomba, Malawi.

Gunstone F (2002). Vegetable Oils in Food Technology. Blackwell Publishing Ltd. P 225.

Hopkins B (1983). The taxonomy, reproductive biology and economic potentials of *Parkia* in Africa and Madagascar. Bot. J. Linnean Soc. 87:135-167.

Igunbor A, Okoro C, Uduenevwo E (2013). Comparative assessment of lipids and physicochemical properties of African locust beans and shea nut oils. J. Nat. Sci. Res. 3(11):25-31.

Kochhar SL (1998): Economic Botany in the Tropics. 2nd Edition, Macmillan India Ltd. pp. 354-355.

MBS (Malawi Bureau of Standards) (2011). MS 51: 2011 Fortified edible oils – specification (3rd edition).

Ministry of Trade and Industry (2014). Oil Seed Products, Lilongwe, Malawi. Available at: http://www.moit.gov.mw/index.php/export-oil-seed-crops-and-products

Okullo JBL, Omujal F, Agea JG, Vuzi PC, Namutebi A, Okello JBA, Nyanzi SA (2010). Physicochemical characteristics of shea butter (*Vitellaria paradoxa* c.f. Gaertn.) Oil from the shea districts of Uganda. ALFAND 10(1):2070-2080.

Owolarafe OK, Adetan DA, Olatunde GA, Ajayi AO, Okoh IK (2011). Development of a locust bean processing device. J. Food Sci. Technol. 50(2):248-256.

Popoola YY, Akinoso R, Raji AO (2016). Optimization of oil extraction from giant bushel gourd seeds using response surface methodology. Food Sci. Nutr. 4(5):759-765.

Roiaini M, Ardiannie T, Norhayati H (2015). Physicochemical properties of canola oil, olive oil and palm olein blends. Int. Food Res. J. 22(3):1227-1233.

Shatta AA, Rayan AM, El-Shamei ZS, Gab-Alla AA, Moussa EA (2016). Comparative Study of the Physicochemical Characteristics of Oil from Transgenic Corn (Ajeeb YG) with its Non-Transgenic Counterpart. Austin Food Sci. 1(5):1023.

Talabi JY, Enujiugha VN (2014). Physical and chemical evaluation of oils from selected underutilized oilseeds. Chem. Sin. 5(6):9-12.

academicJournals

Vol. 11(5) pp. 134-139, May 2017
DOI: 10.5897/AJFS2017.1584
Article Number: 967FC9D64031
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Effect of aging on the physico-chemical and functional characteristics of maize (*Zea mays* L.) flour produced by a Company at Maroua (Far North of Cameroon), during storage

Kagouanbe Palouma Benjamin[1], Goudoum Augustin[1*], Aboubakar[2], Abdou Bouba Armand[1] and Mbofung Carl Moses[3]

[1]Department of Agriculture, Livestock and Derived Products, The Higher Institute of Sahel, The University of Maroua, P.O. Box 46, Maroua, Cameroon.
[2]Department of Biological Sciences, Faculty of Sciences, The University of Maroua, P.O. Box 814, Maroua, Cameroon.
[3]Department of Food and Bioresource Technology, School of Technology, The University of Bamenda, P.O. Box 39, Bambili, Cameroon.

Received 28 February, 2017; Accepted 30 March, 2017

The effect of aging on the physicochemical and functional properties of maize (*Zea mays L*) flour produced in Maroua (Far North-Cameroon) during storage, were investigated using standard analytical methods. The samples underwent natural (37°C) and accelerated (50°C) aging and packed in aluminum sachets. The results show that dry matter, starch and protein varied significantly ($p<0.05$) with the storage time and temperature. The variation of lipids content was minimal and statistically insignificant for 3 months for the flour storage at 37°C and decreased significantly ($p<0.05$) at 50°C. The functional characteristics shows that water absorption capacity, solubility index, water retention capacity and gel length varied ($p<0.05$) with the storage period and temperature. This study revealed that flours packed in aluminum bags at ambient temperature may be stored for three months without altering their physicochemical and functional characteristics, whereas two months is the maximum storage time at 50°C.

Key words: Maize flour, aging, physicochemical properties, functional properties, Maroua.

INTRODUCTION

Maize (*Zea mays* L.) was the main staple of people worldwide for many centuries (Galinat, 1977). According to FAO (2016a), 885.3 million tons were produced in 2011. The main producer was the United States with

*Corresponding author. E-mail: goudoumaugust@gmail.com.

313.9 million tons, accounting for 35.5% of world production (Hamel and Dorff, 2016). Maize is the main staple of people in developing countries, especially African populations (Grah et al., 2014). This food product is consumed by not less 50% of the sub-Saharan populations and therefore represents the most important cereal crop in this part of world (Grah et al., 2014). However, maize production remains very low in Cameroon, with about 700,000 family, artisanal and modern farms producing about 1.1 tons per farmer and a national consumption of 700,000 tons ; maize is therefore the third foodstuff produced in Cameroon after cassava and plantain. It also contributes more than 150 billion FCFA to the gross domestic product (GDP) (http://www.camerpost.com/cameroun, 2015).

Most of the world's people affected by hunger and extreme poverty live in rural areas. According to FAO (2016b), achieving the Sustainable Development Goals (SDGs) requires moving to more productive and sustainable agriculture where everyone have a place, which strengthens rural livelihoods and ensures security for all while drawing less on natural resources and improving resilience to climate change. According to the New Partnership for Africa's Development (2015), maize is thus a potential source for improving food security and raising incomes for producers. Concerning the consumption and use of maize, the Conference of Ministers of Agriculture of West and Central Africa (2015) states that most of the produced corn is used for animal feed. It also shows that total maize uses are estimated at 583 million tons divided between human consumption (20%), animal consumption (67%) and other uses (13%).

Several products are carried and preserved as flours, because they are susceptible to attack by pests of cereals. Their role in the feed and in food industry is very important (Nip, 1997). Flours generally have low water and in this way, a means for the preservation of cereals. Its consumption became more important in urban areas in most African countries like Cameroon (Bricas et al., 1997).

Several factors influence the stability of food during storage, both among farmers and the food industry. These factors are generally related to the food or its environment (water activity, water content, food composition, pH and temperature) (Aboubakar et al., 2010). Therefore, this is why maintaining the quality of a food is a major industrial concern. Indeed, the acceptance of food by the consumer depends, among other things, on its quality, both organoleptic and nutritional.

The shelf life of food flours in general depends on their lipid content, the moisture content of the grain, the presence of contaminants and storage conditions (packaging material, ambient temperature and humidity). The storage time and conditions have an influence on the technological quality of cereal and result in modifications of the flour parameters (Goudoum et al., 2012). The evolution of maize flour during its storage is the result between chemical, physical and enzymatic phenomena. These results in a depreciation, mainly sensory, of the product. Indeed, the acceptance of a food by the consumer depends, among other things, on its organoleptic quality. However, this quality decreases with time of storage due to complex phenomena of alteration (Mertens, 2009). Loss of some nutritional and organoleptic properties such as flavor and taste of dried maize flour during storage is a crucial problem (Grah et al., 2014). In Far North region of Cameroon, the months of March and April are the hottest, with the monthly average of 40°C in the shade. Thus, a good mastery of these phenomena is an essential element to ensure an optimal conservation of food in this part of Cameroon. The objective of this study was to estimate the changes, the physicochemical and rheological modifications of maize flours produced in Maroua (Far North Region of Cameroon) during storage.

MATERIALS AND METHODS

The flour sample was obtained from milling corn (CMS 8504) in a processing company in Maroua. The products were kept in aluminum bags. The same amount of flour (± 500 g) was introduced into each bag. They were sealed with an adhesive tape. Each bag of flour was provided for a pair (time, temperature) to which corresponds a sample. On each bag is indicated the temperature (37 and 50°C) and the aging period (first day, one, two and three months).

Collection of samples and observation

The samples were allowed to age by natural and accelerated methods. For natural aging, the sachets were just placed on a laboratory shelf (at 37°C). For accelerated aging, one temperature was retained (50°C) and the remaining sachets are therefore introduced in ovens set at the chosen temperature.

Products were analyzed monthly. After opening, bags were sealed with an adhesive tape and stored in a refrigerator at 4°C until all analyzes are carried out (between 2 and 4 days).

Chemical and functional analysis before and after storage

The chemical characteristics and functional properties analysis were conducted before and after 1, 2 and 3 months of storage.

Proportioning of starch

The proportioning of starch was carried out using the method described by Ewers modified (BIPEA, 1978).

Proteins

The protein content was determined according to the Kjeldahl method for the total nitrogen determination (AOAC, 1990).

Table 1. Changes of water activity and dry matter of maize flour during three months storage at 37 and 50°C.

Period	37 °C		50 °C	
	WA	DM (%)	WA	DM (%)
First day	0.155^{ab}	96.670^{c}		
1 month	0.160^{ab}	97.070^{c}	0.150^{ab}	97.400^{b}
2 months	0.165^{ab}	96.895^{c}	0.140^{bc}	97.785^{b}
3 months	0.170^{a}	96.880^{c}	0.115^{c}	98.030^{a}

WA: Water activity ; DM: dry matter. Averages followed by the same letter in the same column are not different significantly with P < 0.05 (Test of Duncan).

Evaluation of oxidation state

The evaluation of oxidation state was made according to the AOAC method (2000).

Sugars

Sugars were extracted and analyzed by Fischer and Stein (1961) method.

Water absorption capacity

The determination of the water absorption capacity was performed by the method of Adebowale et al. (2005).

Solubility index

The solubility index (IS) in water is calculated using the Anderson et al. (1969) method.

Gel length

The gel length was determined by the method developed by Cagampang et al. (1973).

Ash content

Ash content was determined by AOAC method (2000).

Moisture

The hot air oven method, AOAC (2000) was used for moisture determination. The samples were dried in a hot air oven (Memmert) at 130°C for 1 h to constant weights.

Statistical analysis

The results obtained from the evaluation of chemical characteristics and functional properties of maize flour during storage were analyzed with analysis of variance (ANOVA) using the software Xlstat 2014. The average values were classified using Duncan multiple test with the same software.

RESULTS AND DISCUSSION

Evolution of water activity and dry matter of maize flour during storage

The results of the water activity and dry matter of maize flours stored at 37 and 50°C for three months are shown in Table 1. The water activity range from 0.155 to 0.170 when the flours were stored at 37°C and 0.155 to 0.115 at 50°C, respectively at the first day of flours production and 3 months after. The values were significantly different ($p< 0.05$). The result of dry matter varied with the storage period and temperature. The dry matter increased significantly ($p < 0.05$) during the storage of flours from 96.670 (first day) to 96.880 (3 months) and from 96.670 (first day) to 98.030 (3 months), respectively at 37 and 50°C storage temperature. This increase in the dry matter at 50°C would be due to the drying which continued under the experimental conditions. This evolution has been compared with the water activity (Wa). Indeed, during long-term storage, the Wa of a product is in equilibrium with the HR of the storage atmosphere (Aboubakar et al., 2010). Hruskova and Machova (2002) showed that the changes in moisture contents depends on the short time storage conditions.

Evolution of proximate composition of maize flour during accelerated aging

The results of proximate chemical composition of flour storage during 3 months at differents temperatures are shown in Table 2. The result shows that starch and protein varied with the storage period and temperatures ($p < 0.05$). The starch content decreased from 85.940 at the first day to 83.300 after 3 months at 37°C and shows minimal changes at 50°C during the storage. The protein content also decreased significantly ($p < 0.05$) during storage at both temperatures. There was non significant variation in the lipid content during three months storage at 37°C, but at 50°C the variation between average of different months are significant ($p < 0.05$). Changes were minimal and statistically non-significant for the flours storage 3 months at both temperature for ash and carbohydrate.

During storage of cereals, many physicochemical properties were subject to changes (Singh et al., 2006 ; Keawpeng and Venkatachalam, 2015). These changes are mainly dependent on their variety, storage conditions (light, temperature) and amylose content (Keawpeng and Venkatachalam, 2015). In this study, the variation in the protein and starch contents of maize flour was a function of storage time and temperature. These changes have been attributed to proteins, the interaction between proteins, the breakdown products of lipid oxidation and starch-protein interactions (Sodhi et al., 2003). A high

Table 2. Changes of proximate chemical composition of maize flour during the three months storage at 37 and 50°C.

Constituents (%)	37 °C				50 °C			Pr > F
	First day	1 month	2 months	3 months	1 month	2 months	3 months	
Starch	85.03[a]	84.82[ab]	84.15[b]	83.82[b]	85.05[a]	85.52[a]	84.35[ab]	0.032
protein	9.85[a]	9.87[a]	9.48[b]	9.45[b]	10.04[ab]	9.72[ab]	9.66[b]	0.041
Crude Fat	3.51[a]	3.62[a]	3.65[a]	3.77[a]	3.820[a]	3.22[b]	3.35[c]	0.013
Ash	0.86[a]	0.87[a]	0.88[a]	0.81[a]	0.72[a]	0.74[a]	0.89[a]	0.728
Free sugar	0.71[a]	0.75[a]	0.73[a]	0.78[a]	0.71[a]	0.72[a]	0.78[a]	0.486

Averages followed by the same letter in the same line are not different significantly with P < 0.05 (Test of Duncan).

Table 3. Changes of some functional properties of maize flour during three months storage at 37 and 50°C.

	37°C				50°C			Pr > F
	First day	1 month	2 months	3 months	1 month	2 months	3 months	
WAC (%)	131.900 [a]	134.250 [a]	136.250[b]	136.960 [b]	136.730 [b]	136.260 [a]	138.045[c]	0.000
SI (%)	20.975 [a]	20.195 [a]	19.700 [a]	19.645 [a]	18.100 [b]	16.885 [bc]	15.830 [c]	0.000
WRC (%)	197.690 [a]	197.175 [a]	193.700 [ab]	189.915 [bc]	186.885 [cd]	182.760 [de]	179.365 [e]	0.000
GL (mm)	90.110 [a]	88.775 [ab]	86.915 [b]	84.100 [c]	82.810 [c]	80.535 [d]	78.410 [e]	0.0001

WAC: Water absoption capacity, SI: solubility index, WRC: water retention capacity, GL: gel lenght. Averages followed by the same letter in the same line are not different significantly with P < 0.05 (test of Duncan).

temperature and shelf life could contribute to the acceleration of these phenomena. Teo et al. (2000) and Zhou et al. (2003) reported that the storage conditions are important in the aging process and impact on the number of changes in rice physical properties such as textural properties, pasting, color, flavor, composition and eating quality.

Protein content is an important criterion for assessing quality. It represents a technological and nutritional interest in the cereals process (Gate, 2015). These results corroborate with those of Khaly (1998) and Maloumba Kamba et al. (2008), who worked respectively on the techno-functional properties of maize and on the quality control of cereal meal placed on the market in Senegal. However, the small decrease in lipid content during storage time could result from the oxidation, which would be due to the presence of pro-oxidants such as metal ions and enzymes. Another hypothesis is that of cereal grinding, which puts lipolytic enzymes in contact with fat in flour, thus promoting lipid degradation (Khaly, 1998). As for proteins, their small decrease over time is due to proteolysis reactions by proteases. Indeed, during storage, proteases naturally present in certain foods or secreted by the microorganisms that contaminate these foods, can hydrolyze proteins. The decrease in proteins during storage could also be due to the result of Maillard reactions also called non enzymatic browning reactions.

The decrease in ash rate during storage time, however slight, is likely to be related to the intensity of dehulling-degerming and sieving operations (Khaly, 1998). These results are similar to those of Benhania (2013) on the flour processing and control of its quality.

Like other nutrients in corn flour, starch and free sugars degrade over time. After three months storage of maize flour, non significant difference was observed during tree months storage at both temperatures for starch and free sugars decreased. These results are similar to those of Lin (1997), who worked on the physicochemical properties of corn flour and starch.

Evolution of functional composition of maize flour during accelaration aging

The results of functional properties of maize flours stored during 3 months at different temperatures are shown in Table 3. The result shows that water absorption capacity, solubility index (SI), water retention capacity and gel length (GL) varied with the storage period and temperature. The WAC increased from 131.900 to 136.960 at 37°C and from 131.900 to 138.045 at 50°C during 3 months storage. The SI did not show any significant variation at 37°C, but at 50°C the variation was significant ($p < 0.05$) during the storage period. The WRC decreased significantly ($p < 0.05$) from 197.690 to 189.915 and from 193.690 to 179.365 during the storage respectively, at 37 and 50°C. The GL decreased significantly ($p < 0.05$) from 90.110 to 84.100 at 37°C and

90.110 to 78.410 at 50°C during the 3 months storage. The water solubility index of maize flour also decreased significantly ($p < 0.05$) after 2 months at 37°C and after one months at 50°C.

It can be seen from the results that the storage time of the corn flour increases the affinity of the latter to water. According to Anderson (1982), the WAC of food meal increases as the storage temperature increases. Several studies on the distribution of water among wheat flour constituents have shown that starch is the main component involved in the hydration properties of flour due to its presence in large quantities (Feillet, 2000; Goesaert et al., 2005). The increase in the WAC is due to the particle size because the finer the flour, the greater is its capacity of absorption of water (Feillet, 2000). The results show the variation of different rheological properties at different storage times and temperature. The WRC, SI and the GL decrease with the storage time and temperature. Sefa-dedeh and Afoakwa (2001) reported that the WRC increases with increase in the amount of proteins and that hydration of starchy polysaccharides is usually followed by the proteins. Grah et al. (2014) showed that the WRC of maize flour increased with the fermentation time, because of the rearrangement of water holding fiber and hydrophilic polysaccharide components. Goudoum et al. (2012) showed that the change of the GL, WAC and SI found, was due to the modification of the grains parameters such as pH and certain minerals such as calcium, by weevils droppings, and even by the presence of eggs in the middle.

Conclusions

The results obtained showed some differences in the physico-chemical and functional properties of maize flour during storage. The study revealed that during the three months of storage, variations in water content of flours were recorded. Water absorption capacity and water solubility index increased during the first three months for the two temperatures. This result showed that the date line of storing maize flour in Maroua can not exceed three months in allimium bags at ambient temperature.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Aboubakar NYN, Nguimbou RM, Scher J, Mbofung CM (2010). Effect of storage on the physicochemica l, functional and rheological properties of taro (*Colocasia esculenta*) flour and paste. Innov. Rom. Food Biotechnol. 7:37-48.

Adebowale KO, Olu-Owolabi BI, Olawumi E, Lawal OS (2005). Functional properties of native, physically and chemical modified breadfruit (*Artocarpus artillis*) starch. Ind. Crops Prod. 21: 343-351.

Anderson RA (1982). Water absorption and solubility and amylograph characteristics on roll-cooked small grain products. Cereal Chem. 59:265-269.

Anderson RA, Conway FF, Pfeifer VF, Griffin EL (1969). Rolland extrusion-cooking of grain sorghum grits. *Cereal Sci. Today*. 14: 372-380.

AOAC (1990). Official methods of analysis of AOAC intl. 15th ed. Association of Official Analytical Chemists, Washington, USA.

AOAC (2000). Official methods of analysis of the Association of Official Analytical Chemists of AOAC. Intl. 17th ed. Association of Official Analytical Chemists, Arlington, USA.

Benhania Z (2013). *Etude de la fabrication de la farine de maïs et control de sa qualité*. Mémoire de Master, Faculté des Sciences et de la Technologie et des Sciences de la Matière Département de Génie des Procédés, Universite Kasdi Merbah Ouargla.

BIPEA Collection of analysis methods from Europeans Community (1978). International Bureau for Analytical Studies, Paris, France.

Bricas N, Vernier P, Ategbo E, Hounhouigan J, N'kpenu KE, Orkwor GC (1997). The development of yam chip sector in West Africa. *Lebensm Wiss. Technol.* 44: 110-114.

Cagampang GB, Perez CM, Juliano BO (1973). A gel consisting test for eating quality of rice. *J. Sci. Food Agr.* 24: 1589-1594.

Cameroun 6 millions de tonnes de maïs visés par an à l'horizon 2015. Available from http://www.camerpost.com/cameroun. Accessed Nov. 01, 2015.

Conference of Ministers of Agriculture of West and Central Africa (CMA / AOC) (2015). Note technique sur la filière maïs dans la zone CMA/AOC. Available from hubrural.org/IMG/pdf/cmaaoc. Accessed Oct. 18, 2015.

Feillet P (2000). *Le grain de blé: composition et utilisation*. Editions INRA. Paris, France.

Fisher EH, Stein EA (1961). DNS colorimetric determination of available carbohydrates in foods. *Biochem. Prep.* 8: 30-37.

Food and Agriculture Organization of the United Nations (FAO) (2016a). The state of food and agriculture. www.fao.org/docrep/013/i2050e/i2050. Accessed Dec. 15, 2016.

Food and Agriculture Organization of the United Nations (FAO) (2016b). Perspectives agricoles de l'OCDE et de la FAO 2016-2025. www.fao.org/3/a-i5778f.pdf. Accessed Dec. 15, 2016.

Galinat WC (1977). The origin of corn. Sprague Edition Corn and corn improvement. Madison, Wis., Etats-Unis. Am. So. Agron. pp. 1-47.

Gate P (1995). Ecophysiologie du blé, de la plante à la culture. Lavoisier Tec and Doc, Paris, 430 p.

Goesaert H, Brijs K, Veraverbeke WS, Courtin CM, Gebruers K, Delcour JA (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. Trends in Food Sci. Technol. 16 :12-30.

Goudoum A, Ngamo Tinkeu LS, Ngasoum MB, Mbofung CM (2012). Variation in technological and nutritional parameters of maize treated with essential oils during storage. *Afr. J. Food Sci.* 6: 34-40.

Grah AMB, Beda MY, Sika HB, Dago G (2014). Effect of fermentation time on the physicochemical properties of maize flour. *Int. J. Res. Stud. Biosci*, 2 (8), 30-38.

Hamel MA, Dorff E (2016). Corn: Canada's third most valuable crop. Statistics Canada. Available from http://www.statcan.gc.ca. Accessed Dec. 15, 2016.

Hruskova M, Machova D (2002). Changes of wheat flour properties during short term storage. *Czech J. Food Sci.* 20: 125-130.

Keawpeng I, Venkatachalam K (2015). Effect of aging on changes in rice physical qualities. *Int Food Res. J.* 22: 2180-2187.

Khaly S (1998). *Contrôle de qualité des farines céréalières mises sur le marché au Sénégal*. Thèse de Doctorat d'Etat en Pharmacie, Faculté de Médecine, de Pharmacie et d'Odontostomatologie, Université Cheikh Anta Diop de Dakar, Sénégal.

Lin Ying-Pi (1997). Physicochemical properties of corn flour and starch and their relation to gel texture of two dry milled fractions. PhD, Purdue University.

Maloumba Kamba P, Vanderghem C, Deroanne C, Bera F (2008). Influence of drying temperature on the solubility, the purity of isolates and the electrophoretic patterns of corn proteins. Food Chem. 111:564-572.

Mertens C (2009). *Contribution à l'étude du vieillissement physico-chimique de produits pré gélatinisés obtenus par technologie roller et développement d'une méthode d'analyse de leurs volatils par TD-GC-MS*. Mémoire d'Ingénieur, Faculté Universitaire des Sciences Agronomiques de Gembloux, Belgique.

New Partnership for Africa's Development (NEPAD) (2015). Annual Report 2003/2004. Available from https://www.eisa.org.za/.../NEPAD. Accessed Dec. 22, 2015.

Nip WK (1997). Taro root. In Taro: Processing Vegetable and Technology. Smith DS, Cash JN, Nip WK, Hui YH (ed). Technomic Publishing, Pensylvania, USA. pp. 355-387.

Sefa-dedeh KY, Afoakwa EO (2001). Influence of fermentation and cowpea steaming on some quality characteristics of maiez-cowpea blends. Afr. J. Food Sci. 2:65-72.

Singh N, Kaur L, Sandhu KS, Kaur J, Nishinari K (2006). Relationship between physicochemical, morphological, thermal, rheological properties of rice staches. Food Hydrocoll. 20:532-542.

Sodhi NS, Singh N, Arora M, Singh J (2003). Changes in physicochemical, thermal, cooking and textural properties of rice during aging. J. Food Process. Preserv. 27:387-400.

Teo CH, Abd A, Cheah PB, Norziah MH, Seow CC (2000). On the roles of proetein and starch in the aging of non-waxy rice flour. Food Chem. 69: 229-236.

Zhou Z, Robards K, Helliwell S and Blanchard C (2003). Rice aging: effect of changes in protein on starch behviour. *Starch/Stärke*, 55:162-169.

academicJournals

Vol. 11(5) pp. 140-145, May 2017
DOI: 10.5897/AJFS2017.1490
Article Number: AABAC9A64251
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Enrichment of traditional maize snack (Kokoro) with moringa (*Moringa oliefera*) leaf and soybean

Ewulo T. O.[1], Oluwalana I. B.[2], Ewulo B. S.[3] and Awolu O. O.[2*]

[1]Department of Science, Ijapo High School, Akure, Ondo State, Nigeria.
[2]Department of Food Science and Technology, Federal University of Technology, Akure, Ondo State, Nigeria.
[3]Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Ondo State, Nigeria.

Received 21 July, 2016; Accepted 11 January, 2017

Kokoro, a local maize snack was made from white maize (W) flour and supplemented with *Moringa oliefera* leaf (M) and defatted soybean (S). WMS_0 (100:0:0%), WMS_1 (90:10:0%), WMS_2 (90:0:10%), WMS_3 (90:5:5%), WMS_4 (90:7.5:2.5%), and WMS_5 (90:2.5:7.5%) were carried out in triplicates. Kokoro was produced by deep frying in hot refined vegetable oil. The proximate composition, vitamin content and phytochemicals composition were determined. Kokoro formulated with 90% maize flour and 10% deffated soybean (WMS_2) had the highest moisture, crude protein, fat, oxalate, phytic acid and alkaloid, while Kokoro formulated with 90% maize flour and 10% moringa (WMS_1) had the highest crude fibre, vitamin A, B_3 (niacin), C and flavonoid. On the other hand, Kokoro formulated with only 100% maize flour (WMS_0) had the least phytochemical composition and vitamins A, B_3, C contents. Although, the addition of soybean had the highest positive effect on the protein and crude fibre contents of Kokoro, it was the addition of moringa that had the highest positive effect on the vitamins contents. On the other hand, moringa also raised the phytochemical contents significantly ($p \leq 0.05$). Overall, sample WMS_4 (90% Maize + 7.5% Moringa + 2.5% Soybean) had substantial proximate and minerals composition in addition to having the least phytochemical could be considered the best formulation for Kokoro formulation.

Key words: Moringa, phytochemicals, proximate composition, soybean, Kokoro.

INTRODUCTION

The traditional maize (*Zea mays* L. Poaceae) snack (Kokoro) is a popular local snack in Southwestern Nigeria and is made solely from maize flour that contains primarily carbohydrates (Awolu et al., 2016a). Snack containing 100% maize do not meet nutritional requirements of the body, hence the need to fortify with crops rich in protein, fibre, minerals and antioxidants (Awolu et al., 2016a, 2015; Omueti and Morton, 1996). Specifically, toasted had been produced by optimum blends using response surface methodology (Awolu et al., 2016b).

Maize, the primary material for making Kokoro snack is

*Corresponding author. E-mail: ooawolu@futa.edu.ng.

a cereal and is deficient in the essential amino acids such as lysine and tryptophan (Omueti et al., 1992) that are essential for human nutrition (Lasztity, 1984). Research effort has been concentrated on supplementing cereal food with legumes and has successfully enhanced the nutritional value and/or functionality of staple foods (Awolu et al., 2016a).

Moringa oleifera is a highly valuable plant, distributed in many tropical and subtropical countries. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source for protein, vitamins, β-carotene, amino acids and various phenolics. Moringa plant provides a rich and rare combination of zeatin, qurcetin, kaempferol and many other phytochemicals. It is also very important plant for its medicinal value. Various parts of the plant such as leaves, roots, seeds, bark, fruit, flowers and immature pods, etc., as cardiac and circulatory stimulants, possess antitumour, antipyretic, antitiepileptic, anti-inflammatory, antiulcer, antispasmodic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, antibacterial and antifungal (Bukar et al., 2010). *M. oleifera* leaves are highly nutritious. In 100 g dry matter, they contain 29±6 g of protein, 28±6 mg of iron, 1,924±288 mg of calcium, 15,620±6,475 IU of vitamin A and 773±91 mg of vitamin C. This is at least twice the protein in milk and half the protein in egg, and has more iron than in beef, more calcium than in milk, equal vitamin A to carrot and more vitamin C than in orange (Wangcharoen and Gomolmanee, 2013). In addition, the leaves of this plant are reported to have various biological activities such as diuretic, immune boosting and hypotensive, anti-inflammatory, antiulcer, antihepatotoxic, antitumour, thyroid hormone status regulating, hypocholesterolaemic, radioprotective, hypolipidaemic, antiatherosclerotic, antidiabetic, and antioxidant (Jaiswal et al., 2009; Chumark et al., 2008; Singh et al., 2009; Sreelatha and Padma, 2008; Verma et al., 2009).

This study was to evaluate the addition of soybean and moringa into maize for the production of Kokoro with high nutritional and phytochemical properties.

MATERIALS AND METHODS

Sample collection and preparation

Maize (*Zea mays* L., Poaceae) was sourced from the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. It was shelled, winnowed and sorted to remove damaged grains before milling. Soybean (*Glycine max* (L.) Merrill) was sourced from Ondo State Agricultural Development Project (ADP), Akure. The seeds were cracked with hammer mill and seed coat winnowed. It was toasted, cooled and milled into flour. The flour was defatted with n-hexane in a Soxhlet apparatus. *Moringa* leaf was plucked from the branch of the tree raised in Akure, Nigeria. The leaves were cleaned, air dried under shade, dry milled and kept in airtight container. Considerable amount of chlorophyll in the leaves were removed by soaking in ethanol overnight, followed by draining and air-drying.

Composite flour formulation

White maize (W), moringa (M) and soybean (S) were mixed to make 180 g at different ratios and replicated three times in the following percentage: WMS_0 (100:0:0%), WMS_1 (90:10:0%), WMS_2 (90:0:10%), WMS_3 (90:5:5%), WMS_4 (90:7.5:2.5%), and WMS_5 (90:2.5:7.5%). Each of the mixture was replicated three times.

Formulation of Kokoro

Salt was added to 180 g composite flour to taste and was gently stirred into 0.5 L boiling water in a stainless steel pot. The mixture was cooked with continuous stirring until stiff dough was formed. The dough was cooled to a temperature (40°C) at which it could be kneaded by hand for 5 min. The kneaded dough was cut into pieces, rolled into cylindrical shapes and deep fried at 150°C in 1 L of hot refined vegetable oil for 3 min. The fried Kokoro was then cooled and packed in sealed polyethylene bags. The frying was carried out in triplicates.

Proximate analysis of Kokoro

Moisture and protein content were determined using the procedure described by AOAC (1990). The ash content was determined using the procedure described by Pearson (1976).

The fat content determination was carried out by soxhlet extraction method (AOAC, 2005). Oil in 5 g sample was extracted using hexane in Soxhlet extraction equipment for 2.5 h under reflux. The crude fibre content was determined using the procedure described by Kirk and Sawyer (1991). Total carbohydrate was estimated using the formula (Ouzouni et al., 2009):

Total carbohydrates (% f) = [100 - moisture (%) - protein content (%) - crude fat (%) - ash (%)].

Determination of vitamin A

Method of AOAC (2005) was used. Exactly 1 ml of the hydrophilic extracts from the sample was measured to the test-tube I (centrifugal) with a tight stopper and 1 ml of the KOH solution was added, the tube was plugged and shake vigorously for 1 min. The tube was heated in a water bath (60°C, 20 min), and was then cooled down in cold water. About 1 ml of xylene was added, the tube was plugged and shake vigorously again for 1 min. The tube was centrifuged (1500×*g*, 10 min), the whole of the separated extract (upper layer) was collected and transferred into the test tube II made of “soft” (sodium) glass. The absorbance A_1 of the obtained extract was measured at 335 nm against xylene. The extract in the test tube II was irradiated to the UV light for 30 min, then the absorbance A_2 was measured. The concentration (Cx) of vitamin A (μM) in the analyzed liquid was calculated using the equation:

$$Cx = [A_1] – [A_2]. 22.23$$

where 22.23 is the multiplier received on basis of the absorption coefficient of 1% solution of vitamin A (as the retinol form) in xylene at 335 nm in a measuring cuvette, 1 cm thickness.

Determination of vitamin B_3 (Niacin)

Five grams of the sample was treated with 50 ml 1N H_2SO_4 and

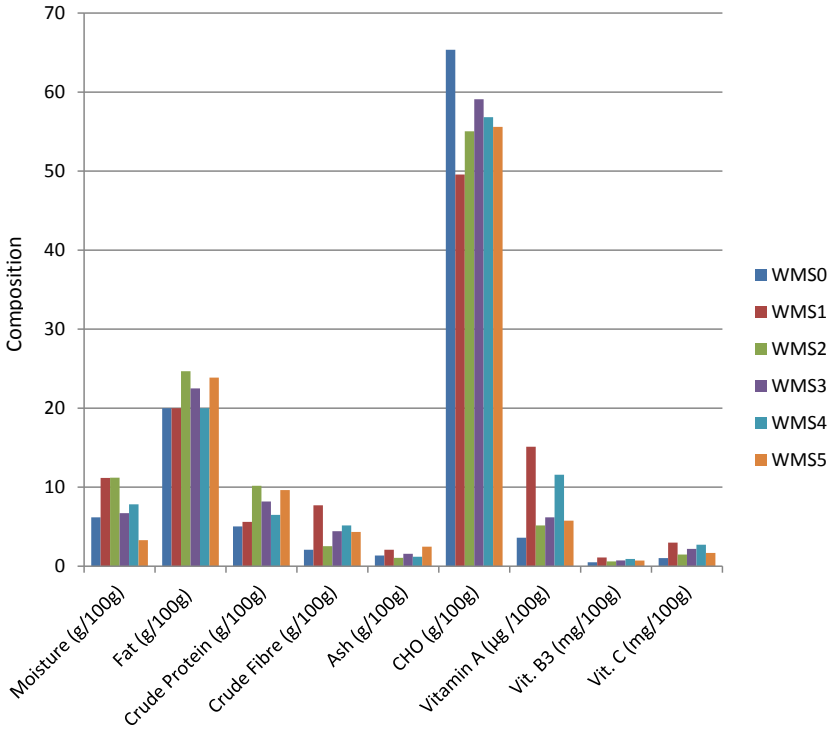

Figure 1. Proximate composition of formulated Kokoro. WMS_0 (100% Maize flour), WMS_1 (90% Maize + 10% *Moringa* + 0% Soybean), WMS_2 (90% Maize + 0% *Moringa* + 10% Soybean), WMS_3 (90% Maize + 5% *Moringa* + 5% Soybean), WMS_4 (90% Maize + 7.5% *Moringa* + 2.5% Soybean) and WMS_5 (90% Maize + 2.5% *Moringa* + 7.5% Soybean).

shaken for 30 min. About 3 drops of ammonia solution was added to the samples and filtered. The filtrate was pipette into a 50 ml volumetric flask and 5 ml potassium cyanide was added. This was acidified with 5 ml of 0.02 N H_2SO_4 and absorbance was measure using spectrophotometer at 470 nm (Okwu and Josiah, 2006)

Determination of vitamin C

The modified method of the method adopted by Awolu et al. (2013) was used. The vitamin C content of the hydrophilic extracts from the sample was determined by the spectrophotometric method using ascorbic acid as a reference compound. Exactly 10 ml of the juice sample was weighed into 10 ml of water and mixed together. 200 µl, that is, 0.2 ml of the extract was pipetted and mixed with 300 µl (0.3 ml) of 13.3% of trichloro-acetic acid (TCA) and 75 µl (0.075 ml) of dinitrophenylhydrazyl (DNPH). The mixture was incubated in water bath at 37°C for 3 h. After 3 h, 500 µl (0.5 ml) of 65% sulphuric acid was added and the absorbance was read with the spectrophotometer at 520 nm. The concentration of vitamin C was calculated as follows:

$$\frac{Absorbance\ of\ standard}{Concentration\ of\ standard} = \frac{Absorption\ of\ sample}{Concentration\ of\ sample}$$

Determination of phytochemicals

Flavonoid was determined using the procedure of Boham and Kocipai (1994). From oxalate through Day and Underwood (1986) procedure, phytin-phosphorus was determined by the method of Wheeler and Ferrell (1971) as modified by Reddy et al. (1978). Phytic acid was calculated by multiplying Phytin-P by the factor of 3.55 (Enujiugha and Olagundoye, 2001). The tannin content was determined by the quantitative method of Makker and Goodchild (1996). Alkaloid was determined using the procedure of Harborne (1973).

Statistical analysis

All analyses were carried out in triplicates. The results obtained were subjected to analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL, USA). Means were separated using the Duncan multiple range test (DMRT) at 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

Proximate composition and vitamin content of formulated Kokoro

The results of proximate composition and vitamin contents of the formulated Kokoro are shown in Figure 1. Kokoro made from WMS_0 formulation had the highest CHO (65.35%) and least crude protein (5.03%) and crude fibre (2.08%) content. The significantly ($p<0.05$) high crude

Table 1. Phytochemical composition of formulated Kokoro.

Formulated Kokoro	Oxalate (mg/g)	Phytic acid (mg/g)	Phytic phosphate (mg/g)	Tannin (mg/g)	Alkaloid (%)	Flavonoid (%)
WMS_0	0.22^{d}	19.86^{d}	5.67^{d}	0.014^{c}	1.57^{c}	1.92^{d}
WMS_1	0.27^{cd}	24.72^{c}	6.96^{c}	0.025^{c}	2.00^{b}	3.23^{a}
WMS_2	0.59^{a}	30.49^{a}	8.57^{a}	0.025^{c}	2.40^{a}	2.63^{b}
WMS_3	0.36^{b}	30.32^{a}	7.31^{b}	0.029^{a}	2.00^{b}	1.95^{cd}
WMS_4	0.32^{bc}	20.60^{d}	5.80^{d}	0.027^{b}	2.00^{b}	1.99^{c}
WMS_5	0.36^{b}	25.98^{b}	7.31^{b}	0.017^{d}	2.40^{a}	1.94^{cd}

Values not followed by the same letter in the same column are significantly different ($P<0.05$). WMS_0 (100% Maize flour), WMS_1 (90% Maize + 10% Moringa + 0% Soybean), WMS_2 (90% Maize + 0% Moringa + 10% Soybean), WMS_3 (90% Maize + 5% Moringa + 5% Soybean), WMS_4 (90% Maize + 7.5% Moringa + 2.5% Soybean), and WMS_5 (90% Maize + 2.5% Moringa + 7.5% Soybean).

carbohydrate and least crude protein found in Kokoro from WMS_0 is consistent with the study of Omueti et al. (1992) and Miracle (1997). This study showed cereal based product to contain primarily carbohydrate and to be low in crude protein. Increased substitution of soybean and moringa flour for maize flour in the formulated Kokoro increased crude protein content and decreased carbohydrate content.

Kokoro (WMS_2) had the highest moisture (11.20%) and crude protein (10.17%). Kokoro (WMS_1) showed no significant ($P>0.05$) difference with WMS_2 with regards to moisture content. The highest moisture and crude protein content observed in Kokoro (WMS_2) was due to defatting effect on soybean flour. Defatting has been reported to increase flour water absorption capacity (Gonzalez-Agramon and Serna-Saldivar, 1988; Ogunsina et al., 2010) and crude protein (Alobo et al., 2009; Uzor-Peters et al., 2008). Serna-Saldivar et al. (1988) reported 35% more crude protein in wheat flour fortified with 11.1% defatted soybean meal (SBM).

The highest crude fibre (7.71%) content was recorded in WMS_1. The highest crude fibre recorded in Kokoro (WMS_1) is consistent with the study of Sanford (2000) and Oduro et al. (2008) that showed dried moringa leaf to contain high crude fibre concentration. Increase in substitution with dried moringa leaf powder from 0 to 15% has been reported to result in increase in dietary fibre (Dachana et al., 2010). Potential health benefits of dietary fibre have been documented in relation to the prevention of cardiovascular disease (Bazzano et al., 2003).

The highest fat content (24.67%) was obtained in WMS_5. The fat content in WMS_1 did not show significant difference ($p<0.05$) from those obtained in WMS_4 and WMS_5. Substituting white maize flour with defatted soybean flour in the formulation of Kokoro at WMS_3, WMS_5 and 10% level of defatted soybean flour substitution (WMS_2) significantly ($p<0.05$) increased Kokoro fat content. This is consistent with the study of Ogunsina et al. (2010) revealing the ability of defatting of soybean in increasing fat absorption capacity of flour.

The highest value of vitamin A, B and C recorded in formulated Kokoro (WMS_1) showed the ability of moringa leaf in improving vitamin composition of Kokoro when substituted for maize in its formulation. This is in agreement with earlier study on moringa leaf that showed it to be substantially rich in vitamin A, B and C (ascorbic acid) (Ramachandran et al., 1980; Sanford, 2000; Anwar et al., 2007). The ascorbic acid acts as antioxidant and hence enhance the shelf life of fat containing food (Dillard and German, 2000; Siddhuraju and Becker, 2003).

Though vitamin B_3 (niacin and niacinamide) has been reported to possess antioxidant properties (Gliszczynska-Swiglo, 2006), and is therefore able to protect the body against free radical induced degenerative diseases due to its antioxidant properties. Dried moringa leaf has been reported to contain seven times more of the vitamin C found in orange and four times more the vitamin A obtained in carrot (Balbir, 2005; Fahey, 2005). Kokoro (WMS_0)–had the least value of vitamin A, B_3 (niacin) and C. The highest value of vitamin A, B_3 (niacin) and C were recorded in Kokoro (WMS_1).

Substitution of soybean or moringa for maize in the formulated flour significantly ($p<0.05$) improved Kokoro vitamin A, B_3 (niacin), and C. Likewise, the increase in the levels of substituting moringa for soybean in the formulated flour.

Phytochemical composition of formulated Kokoro

The results of the phytochemical composition of formulated Kokoro are presented in Table 1. The highest value for oxalate (0.59 mg/g) and phytic acid (30.49 mg/g) were recorded in Kokoro made from 10% level of defatted soybean flour substitution (WMS_2). The presence of phytate has been established in Soybean (Ronald, 2000). Phytates are organic acid, present in the bran or hulls of seeds, which blocks the uptake of essential minerals, calcium, magnesium, iron and especially zinc in the intestinal tract (Ronald, 2000) due to its ability to chelate divalent cations (Nelson et al., 1968).

Phytate content is higher in the raw white variety of the maize than the raw yellow variety (Oboh et al., 2010). It is only a long period of fermentation that can significantly reduce the phytate content. The concentration of high level of oxalate, a salt or ester of oxalic acid in Kokoro formulated with 10% level of defatted soybean flour substitution (WMS_2) was due to its presence in soybean seed. The high concentration of oxalate in Kokoro produced at all levels of moringa flour formulation may be as a result of large amounts oxalate found in the stems and leaves of *M. oleifera* (Dachana et al., 2010; Olson and Carlquist, 2001).

There were no significant differences ($p<0.05$) between 100% white maize flour (WMS_0) formulation and WMS_4 with regards to phytic acid and phytic phosphate. Kokoro (WMS_0) had the least value for all anti-nutritional factors considered. The highest value for phytic phosphate and tannin occurred in WMS_3. The low tannin in 100% white maize flour formulated Kokoro (WMS_0) is consistent with the finding of Oboh et al. (2010) that reported low tannin content in white maize. Tannin has also been found negligible in all fractions of moringa plant (Reyes-Sánchez et al., 2006). Tannin is known to affect nutritive value of food products by forming complex with protein (both substrate and enzyme) thereby inhibiting digestion and absorption (Osuntogun et al., 1987). They also bind Fe making it unavailable (Aletor and Adeogun, 1995).

The highest values for alkaloids were recorded in WMS_5, WMS_2 and WMS_3 formulated Kokoro. There were no significant differences ($p<0.05$) between the three formulations. The highest value for flavonoid (3.23%) was in Kokoro (WMS_1). The least value (1.92%) was revealed in (WMS_0). Flavonoid content in formulated Kokoro increases with increase in moringa flour substitution in the flour mixture. The observed rise in flavonoid content of formulated Kokoro with attendant rise in moringa flour substitution is consistent with earlier study that showed moringa leaf as a good source of natural antioxidants (Dillard and German, 2000; Siddhuraju and Becker, 2003; Anwar et al., 2005). Antioxidants are known for free radical scavenging ability (Scherer and Godoy, 2009) and therefore, neutralize free radicals that have the ability of stimulating reaction that make the cells more vulnerable to cancer causing chemicals, called carcinogen. Reddy et al. (2005) reported that the use of *M. oleifera* as source of natural antioxidant and it was found effective in controlling lipid oxidation during storage of biscuits.

Conclusions

The addition of defatted soybean and *M. oleifera* leaf flours to maize flour in the production enhanced the protein, crude fibre and vitamins contents of the snack (Kokoro). In addition, the flavonoid and alkanoids (antioxidants) contents were improved by the incorporation of deffated soybean and *M. oleifera* leaf flours. Soybean had a negative effect on the anti-nutrients at 5% and above incorporation. Therefore, sample WMS_4 (90% maize + 7.5% moringa + 2.5% soybean) was the best formulation which guaranteed enough protein, crude fibre, vitamins and antioxidants, with considerably low anti-nutrients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Aletor VA, Adeogun OA (1995). Nutrient and anti-nutrient components of some tropical leafy vegetables. Food Chem. 53:375-379.

Alobo AP, Agbo BN, Ilesanmi SA (2009). Physicochemical and functional properties of full fat and defatted cashew kernel flours. Int. J. Food Sci. Technol. 44:581-585.

Anwar F, Ashraf M, Bhanger MI (2005). Inter-provenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan. J. Am. Oil Chem. Soc. 82:45-51.

Anwar F, Latif S, Ashraf M, Gilani AH (2007). *Moringa oleifera*: A Food Plant with Multiple Medicinal Uses. Phytother. Res. 21:17-25.

AOAC (Association of Official Analytical Chemist) (1990). Official method of analysis, 14th Edition, Washington DC.

AOAC (Association of Official Analytical Chemist) (2005). Official method of analysis of the AOAC (W. Horwitz, Ed.) 18th Edition, Washington DC.

Awolu OO, Aderinola TA, Adebayo IA (2013). Physicochemical and rheological behavior of African Star apple (*Chrysophyllum albidium*) juice as affected by concentration and temperature variation. J. Food Process. Technol. 4:229.

Awolu OO, Oluwaferanmi PM, Fafowora OI, Oseyemi GF (2015). Optimization of the extrusion process for the production of ready-to-eat snack from rice, cassava and kersting's groundnut composite flours. LWT Food Sci. Technol. 64:18-24.

Awolu OO, Omoba OS, Olawoye O, Dairo M (2016a). Optimization of production and quality evaluation of maize-based snack supplemented with soybean and tigernut (*Cyperus esculenta*). Food Sci. Nutr. 5:3-13.

Awolu OO, Osemeke RO, Ifesan BOT (2016b). Antioxidant, functional and rheological properties of optimized composite flour, consisting wheat and amaranth seed, brewers' spent grain and apple pomace. J. Food Sci. Technol. 53(2):1151-1163.

Balbir M (2005). Tree for life *Moringa* Book. [Bronchure] Balbir Mathur; Wichita, Kansas.

Bazzano LA, He J, Ogden LG, Loria CM, Whelton PK (2003). Dietary fibre intake and reduced risk of coronary heart disease in US men and women: the National Health and Nutrition Examination Survey I epidemiologic follow-up study. Arch. Intern. Med. 163:1897-1904.

Boham AB, kocipai AC (1994). Flavonoid and condensed tannins from leaves of *Hawaiian vaccinium* vaticulum. Pac. Sci. 48:458-463.

Bukar A, Uba A, Oyeyi TI (2010). Antimicrobial profile of *Moringa oleifera* Lam. extract against some food – borne microorganisms. Bayero J. Pure Appl. Sci. 3(1):43-48.

Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP, Phivthong-ngam L, Ratanachamnong P, Srisawat S, Pongrapeeporn KU (2008). The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. Leaves. J. Ethnopharmacol. 116:439-446.

Dachana KB, Jyotsna RD, Indrani JP (2010). Effect of dried *Moringa* (*Moringa Oleifera* Lam.) leaves on rheological, microstructural, nutritional, textural and organoleptic characteristics of cookies. J. Food Qual. 33(5):225-300.

Day RA Jr, Underwood AL (1986). Quantitative Analysis. 5th edition, Prentice-Hall publication 70 p.

Dillard CJ, German JB (2000). Phytochemicals: nutraceuticals and human health: A review. J. Sci. Food Agric. 80:1744-1756.

Enujiugha VN, Olagundoye TV (2001). Comparative nutritional characteristic of raw, fermented and roasted African oil bean (*Pentaclethra macrophylla* Benth) seed. Rivista Italiana delle Sostanze Grasse 78:247-250.
Fahey J (2005) *Moringa oleifera*: A review of the medical evidence for its nutritional therapeutic, and prophylactic properties. Part 1. Tree Life J. 1(5):1-5.
Gliszczynska-Swiglo A (2006). Antioxidant activity of water soluble vitamins in the TEAC (Trolox equivalent antioxidant capacity) and the FRAP (ferric reducing antioxidant power) assays. Food Chem. 96:131-136.
Gonzalez-Agramon M, Serna-Saldivar S (1988). Effect of defatted soybean and soybean isolate fortification on the nutritional, physical, chemical and sensory properties of wheat flour tortillas. J. Food Sci. 53:793-797.
Harborne JB (1973). Phytochemical Methods. London Chapman and Hall, Ltd. Pp. 49-188.
Jaiswal D, Rai PK, Kumar A, Mehta S, Watal G (2009). Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic rats. J. Ethnopharmacol. 123:392-396.
Kirk RS, Sawyer R (1991). Pearson's Composition and Analysis of Foods. 9th edition. Longman Group. P 646.
Lasztity R (1984). Maize proteins. In: The Chemistry of Cereal Proteins, CRC Press, Inc., Boca Raton, FL. pp. 131-155.
Makker AOS, Goodchild AV (1996). Quantification of tannins. A laboratory manual. International Centre for Agricultural Research in Dry Areas (ICARDA), Aleppo Syria. 25 p.
Miracle MP (1997). Increasing efficiency in maize production in Tropical Africa. Longman, London. 233 p.
Nelson TS, Ferrara LW, Stover NL (1968). Phytate phosphorus content of feed ingredients derived from plants. Poult. Sci. 47: 1372-1378.
Oboh G, Ademiluyi AO, Akindahunsi AA (2010). The effect of roasting on the nutritional and antioxidant properties of yellow and white maize varieties. Int. J. Food Sci. Technol. 45:1236-1242.
Oduro I, Ellis WO, Owusu D (2008). Nutritional potentials of two leafy vegetables: *Moringa oleifera and Ipomea batatas* leaves. Sci. Res. Essays 3(2):57-60.
Ogunsina BS, Radha C, Singh RSG (2010). Physicochemical and functional properties of full-fat and defatted *Moringa oleifera* kernel flour. Int. J. Food Sci. Technol. 45:2433-2439.
Okwu DE, Josiah C (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. Afr. J. Biotechnol. 5:357-361.
Olson ME, Carlquist S (2001). Stem and root anatomical correlation with life form diversity, ecology, and systematics in *Moringa* (*Moringa*ceae). Bot. J. Linn. Soc. 135(4):315-348.
Omueti O, Morton ID (1996). Development by extrusion of soya Bari snack sticks: A nutritionally improved soya-Maize product based on the Nigerian snack (Kokoro). Int. J. Food Sci. Nutr. 47(1):5-13.
Omueti O, Morton ID, Emery PW (1992). Nutritional characteristics of soybean seed flour after processing with sodium bicarbonate or trona. Int. J. Food Sci. Nutr. 43:147-53.
Osuntogun BA, Adewusi SRA, Telek L, Oke OL (1987). Human nutrition. Food Sci. Nutr. 41(F):41-46.
Ouzouni PK, Petridis D, Koller WD, Riganakos KA (2009). Nutritional value and metal content of wild edible mushrooms collected from West Macedonis and Epirus, Greece. Food Chem. 115:1575-1580.
Pearson D (1976). Chemical Analysis of Foods.7th edition, Churchill Livingston, Edinburgh, United Kingdom.
Ramachandran C, Peter KV, Gopalakrishnan PK (1980). Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. Econ. Bot. 34:276-283.
Reddy NR, Balakrishman CV, Salunkhe DK (1978). Phytate phosphorus and mineral changes during germination and cooking of black gram (*Phaseolu mungo* L) seeds. J. Food Sci. 43(2):540-543.
Reddy VP, Garett MR, Perry G, Smith MA (2005). Carnosine: A versatile antioxidant and anti-glycating agent. Sci. Aging Knowl. Environ. 5(2):119-125.
Reyes-Sánchez A, Nchez N, Ledin S and Ledin I (2006). Biomass production and chemical composition of *Moringa oleifera* under different management regimes in Nicaragua. Agroforestry Syst. 66:231-242.
Ronald WH (2000). Use of soybean meals in diets of salmon and trout. Written in cooperation with the United Soybean Board and American Soybean Association. pp. 1-16.
Sanford H (2000). Moringa, Nature's Medicine Cabinet. Sierra Sunrise Publishing, Sherman Oaks, California. 122 p.
Scherer R, Godoy HT (2009). Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chem. 112:654-658.
Serna-Saldivar S, Lopez-Ahumada G, Ortega-Ramirez R, Dominguez RA (1988). Effect of sodium stearoyl-2-lactylate on the rheological and baking properties of wheat bread fortified with defatted soybean and sesame meal. J. Food Sci. 53:211.
Siddhuraju P, Becker K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam.). J. Agric. Food Chem. 15:2144-2155.
Singh BN, Singh BR, Singh RL, Prakash D, Dhakarey R, Upadhyay G, Singh HB (2009). Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. Food Chem. Toxicol. 47:1109-1116.
Sreelatha S, Padma PR (2008). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods Hum. Nutr. 64:303-311.
Uzor-Peters PI, Arisa NU, Lawrence CO, Osondu NS, Adelaja A (2008). Effect of partially defatted soybean or groundnut cake flour on proximate and sensory characteristics of Kokoro. Afr. J. Food Sci. 2:98-101.
Verma AR, Vijayakumar M, Mathela CS, Rao CV (2009). In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. Food Chem. Toxicol. 47:2196-2201.
Wangcharoen W, Gomolmanee S (2013). Antioxidant activity changes during hot-air drying of *Moringa oleifera* leaves. Maejo Int. J. Sci. Technol. *7*(3):353-363.
Wheeler EI, Ferrell RE (1971). A method of phytic acid determination in wheat fractions. Cereals Chem. 48:312-314.

African Journal of Food Science

Related Journals Published by Academic Journals

- *African Journal of Microbiology Research*
- *African Journal of Plant Science*
- *International Journal of Genetics and Molecular Biology*
- *Journal of Cell and Animal Biology*

academicJournals