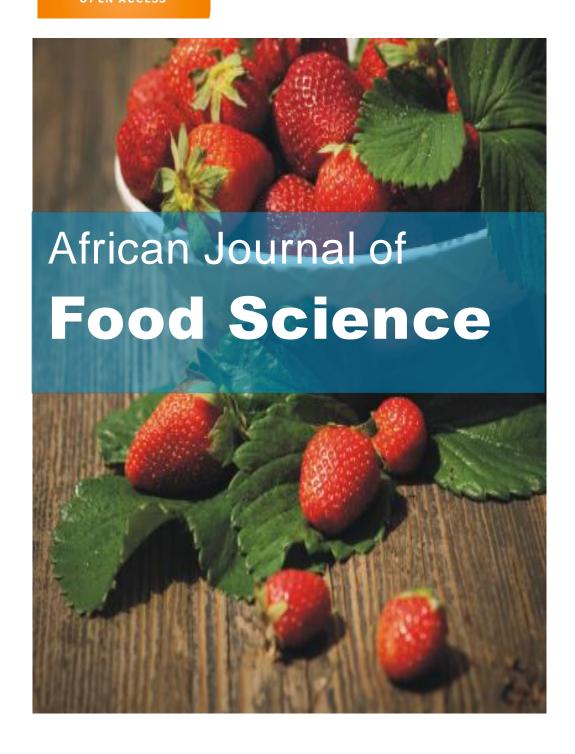
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October 2020 ISSN 1996-0794 DOI: 10.5897/AJFS www.academicjournals.org



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Vol. 14(9) pp. 265-273, October 2020

DOI: 10.5897/AJFS2020.1979 Article Number: 86D731C64930

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Full Length Research Paper

Proximate and phytochemical composition of selected indigenous leafy vegetables consumed in Malawi

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Received 9 June, 2020; Accepted 22 July, 2020

Indigenous vegetables are very important in nutritional wellbeing of low resource rural communities especially in developing countries. Most indigenous vegetables are also believed to contain health promoting compounds such as antioxidants. In this study, nutrient composition of three commonly consumed indigenous leafy vegetables in Malawi namely Amaranth (Amaranthus species), Black jack (Bidens pilosa) and Mwamuna aligone/gallant soldier (Galinsoga parviflora) was determined. Results showed that crude protein expressed on dry weight basis ranged from 15.83±0.19 to 19.04±0.33 with B. pilosa registering the highest value and G. parviflora the lowest. Results on mineral content showed that G. parviflora had the highest (18.84±0.40% DW) p<0.05 mineral/ash content compared to B. pilosa (13.35±0.07% DW) and Amaranthus spp. (15.48±0.14%). Amaranthus spp. had the highest crude fat (13.17±0.20%) content compared to B. pilosa and G. parviflora which had 9.00±0.29 and 8.97±0.25%, respectively. Antioxidant capacity in mg vitamin C Equiv./g DW, ranged from 49.403±0.105 to 59.186±0.0608 with G. parviflora registering the highest value compared to the other two indigenous vegetables. Total phenolic content ranged from 22639±26.0 to 28672±45.1 mg GAE/kg with Amaranthus spp. registering the highest value and G. parviflora the lowest. Results on anti-nutrient content with respect to phytic and oxalic acids showed that all the three indigenous vegetables contained low and safe levels of antinutrients. The study results have demonstrated the significance of these indigenous vegetables in human nutrition and health for rural people in Malawi.

Key words: Indigenous vegetables, proximate composition, total phenolic compounds, antioxidant capacity, phytochemicals.

INTRODUCTION

Indigenous vegetables have a very significant role in the livelihood of rural people in emerging worlds (Zemede and Mesfin, 2001; Uusiku et al., 2010). In developing

worlds, many people in rural areas have less food for their families resulting in deficiency of important nutrients (Tanumihardjo and Yang, 2005; FAO et al., 2012). These

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rural poverty stricken people depend on locally available indigenous vegetables for income and food, as staple food, during lean seasons (Zemede and Mesfin, 2001; Ebert, 2014; Tomori and Obijole, 2000; Flyman and Afolayan, 2006). Therefore, indigenous leafy vegetables are valuable sources of both nutrients, as micronutrients (Nesamvuni et al., 2001) and herbal medicines (Hilou et al., 2006). In Africa, Malawi inclusive, indigenous leafy vegetables are used as relish and are eaten together with starchy staple foods (Schippers, 2000).

Indigenous leafy vegetables play important role in protective foods; used in human health maintenance and disease prevention (Sheela et al., 2004; Nnamani et al., 2007). Plants produce organic compounds that are not directly used in primary growth and development metabolic processes of plants (Buchanan et al., 2000). These compounds are non nutritive plant secondary metabolites that are also called phytochemicals (Krishnaiah et al., 2007). phytochemicals are antioxidant bioactive chemicals that prevent oxidative processes occurrences in animals and plants (Baang et al.. 2015). These phytochemicals include saponins, alkaloids, flavonoids, tannins and phenolic compounds (Baang et al., 2015), fibres, vitamins and water (Adenipenkun and Oyetunji, 2010; Saidu and Jideobi, 2009; Uwah and Ogugbuaja, 2012). They are absorbed by the human body to be utilized as energy sources, body building and protective materials (Saidu and Jideobi, 2009; Uwah Ogugbuaja, 2012). They have high fiber content compared to root vegetables and cereals (Saidu and Jideobi, 2009). The high fiber content has been reported to reduce cholesterol levels in the body resulting in low occurrences of cardiovascular diseases (Chionyedua et 2009). Potassium from leafy vegetables is responsible for preventing body diuretic and hypertensive complications (George, 2003) while oils/fats from vegetables lower blood lipids thereby controlling incidences of coronary diseases (Adenipenkun and Oyetunji, 2010).

However, the presence of some phytochemicals that are called anti-nutritional factors like phytate, oxalate, trypsin inhibitors and lectins threatens the bioavailability of plant micronutrients to human beings (Shi et al., 2003). Other authors have previously reported that oxalate complexes with calcium forming calcium oxalate crystals resulting in both non-absorption and utilization of calcium by the body and renal stones (Ladeji et al., 2004; Akwawo et al., 2000). The non-absorption and utilization of calcium has been reported to cause rickets and osteomalacia (Ladeji et al., 2004). Phytic acid (PA; myoinositol hexaphosphate), a ubiquitous biomolecule is found in plants and PA phosphorus is a major fraction of total phosphorus in seeds and grains (Harland and Overleas, 1987). Phytic acids form insoluble complexes with polycations/micronutrients like Fe, Ca, Zn and P because of reactive phosphorus groups which are

attached to its inositol (Pedersen et al., 2007) resulting in unavailability of the nutrient for human intestinal absorption (Mahesh et al., 2015). Despite being an antinutritional factor, phytate consumption has been associated with some health benefits like prevention against dental and renal caculi, rectal cancer, cardiovascular calcification and as an antioxidant (Shamsuddin, 2002; Grases et al., 2007, 2009).

In Malawi, Amaranths (Amaranthus L.), Black jack (Bidens pilosa) and Mwamuna aligone/gallant soldier (Galinsoga parviflora) are some of the commonly consumed indigenous leafy vegetables. Amaranthus L. belongs to the Amaranthaceae family and there are 60 recognizable species (Anjali et al., 2013). Findings from studies conducted on indigenous vegetables revealed that Amaranthus spp. vegetables have high antioxidant properties, with phenolic values of 275±20 mg GAE/100 g (Baang et al., 2015). This is despite having low proximate composition on fresh basis (Matenge et al., 2017). In addition. other researchers have reported Amaranthus spp. contains crude protein and fat contents of 3.2 and 0.3%, respectively at 7% DM content (Sheela et al., 2004). The leaves are boiled and in some cases groundnut flour is added and is usually consumed as relish.

B. pilosa and G. parviflora belong to Asteraceae family (Essack, 2018). B. pilosa is a small erect weedy plant that grows in tropical countries and is used as a source of food (Grubben and Denton, 2004). It is rich in phytochemicals like phenols, flavonoids, terpenes, phenylpropanoids and lipids (Chang et al., 2001). In Africa, dry powdered leaves of B. pilosa are used to cure syphilis and in East Africa the leaves are used in the treatment of conjunctivitis and constipation in babies (Hutchings et al., 1996). Other authors have previously reported that B. pilosa contains 5% crude protein, 10 mg/100 g copper and 658 mg/100 g magnesium (Odhav et al., 2007). G. parviflora has 13 species and originated from the mountains of Central America (Warwick and Sweet, 1983). It has been reported to contain 5.0 g protein and 0.5 g fat on fresh basis per 100 g of the consumed vegetable (Odhav et al., 2007). Similarly, others have reported that G. parviflora leaves contain high amounts of magnesium (681 mg/100 g) on fresh matter basis (Odhav et al., 2007). They are cooked as spinach and eaten as relish (Tredgold, 1990).

It is widely acknowledged that indigenous leafy vegetables have been underutilized with limited knowledge on their nutritional values (Keatinge, 2012). In Malawi, it has been observed that very few studies have been done on nutritional values of indigenous leafy vegetables (Chitsulo, 2013; Kachiguma et al., 2015) resulting in either limited or scanty information. Against the background of this limited information on nutritional composition of indigenous vegetables, this current study was undertaken to determine the nutritive value of these three selected indigenous leafy vegetables, namely,

Amaranthus spp., B. pilosa and G. parviflora, consumed in Malawi.

MATERIALS AND METHODS

Plant sample collection

Three fresh indigenous leafy vegetables: *Amaranthus* spp., *B. pilosa* and *G. parviflora* were collected from naturally growing plants in the fields in Lilongwe south west, Mitundu area, which is located in Lilongwe district, Malawi. The vegetables were sampled during the rainy season in the month of January 2020. These vegetables represent some of the indigenous leafy vegetables that are mainly consumed by rural people in Malawi.

Sample preparation

Enough samples were thoroughly washed with water to remove dirt and other contaminants and were later oven dried at 40°C for proximate and phytochemicals composition determination. The dried leafy vegetable samples were ground through a 1 mm sieve using a Thomas-WILEY model 4 Laboratory Mill before analyzing the chemical properties.

Determination of nutritional composition

The nutritional/proximate composition of the samples was determined using Association of Official Analytical Chemists (AOAC) 1990 methods.

Dry matter content determination

The dry matter content was determined by using the oven-dry method. 2.5 g of the samples was weighed into a porcelain dish and dried at 105°C for 5 h in the drying oven. After drying, the samples were cooled in a desiccator and weighed to constant weight. The dry matter content was expressed as a fraction of dry weight and presented as a percentage.

Ash content determination

2.5 g of the ground samples was weighed in porcelain dish with a known weight. The samples in the porcelain dishes were ignited in a muffle furnace at 500°C for 2 h to obtain a grey ash. The samples were cooled in a desiccator and weighed to constant weight. The ash content was expressed as a fraction of the sample on dry matter basis and expressed as a percentage.

Protein content determination

Crude protein was determined by using Kjeldahl method. 2.5 g of the sample was digested in 20 ml concentrated sulphuric acid using selenium tablet as a catalyst until the mixture turned colorless/clear. The mixture was then diluted to 250 ml in a volumetric flask and 10 ml of the mixture was mixed with 20 ml of 40% NaOH. The mixture was distilled to liberate ammonia into weak (4%) boric acid and the distillate was titrated against standard HCl using bromocresol green as an indicator. The calculated nitrogen content from the samples in percent was converted to crude protein by multiplying by a factor of 6.25.

Crude fat determination

Crude fat was extracted from the sample by using petroleum ether in a soxhlet extractor/apparatus for 16 h. 2.5 g of finely ground sample was put into a porous thimble in a soxhlet apparatus connected to a weighed 250 ml flat bottomed quick fit flask containing 200 ml petroleum ether. The solvent was continuously boiled at 40 to 60°C extracting the fat from the sample. After 16 h of extraction the petroleum ether was evaporated by using a rotary evaporator. The flask containing the crude oil was then dried to constant weight at 105°C in the laboratory oven for 2 h. The crude oil was calculated as the fraction of original dry weight of the sample expressed in percentage.

Crude fibre determination

2.0~g of the sample was boiled in 150 ml of 0.128 M $H_2 SO_4$ in a beaker for 30 min and the residues were filtered through fluted funnel and was washed three times with hot distilled water. The residues were further boiled in 0.125 M NaOH for another 30 min, filtered and washed with hot distilled water, followed by washing three times with acetone. The residues were oven dried at 105°C to constant weight and then ashed at 500°C for 2 h. The ash was weighed and fibre content was expressed as a fraction of the difference between the weight of the residues and ash of dry weight sample and this was expressed as a percentage.

Determination of phytochemicals

Extraction of phenolic compounds

Phenolic compounds were extracted from 2.5 g of the samples by using 25 ml of methanol (80:20 v/v) and pure distilled water. The mixtures were homogenized using a vortex for 30 s at 30 min intervals for 1 h. The mixture was then filtered and concentrated using a rotary evaporator at 40 to 50°C (SatyaEswari et al., 2018).

Determination of total phenolic compounds

The total phenolic compounds were determined spectrophotometrically by using Folin-Ciocalteau reagent method (Singleton and Rossi, 1965). 1.0 ml of the plant extract was mixed with 0.5 ml of Folin-Ciocalteau (1:10 v/v) in a test tube. The mixture was left to stand for 5 min, 1.5 ml of 20% NaCO2 was added and the volume was made up to10 ml with distilled water. A standard stock solution of 1 mg/ml gallic acid was prepared. A standard curve was plotted as reference gallic acid equivalent (GAE) (0-0.4 mg/ml) after similarly treated as the samples. The absorbance of standards and samples was measured at 765 nm using a spectrophotometer. The phenolic compound was determined by the Folin-Ciocalteau method expressed as gallic acid equivalent per 1000 gram (mg GAE/kg). The TPC was calculated using the standard curve of gallic acid equation (y=1.28x; R²=0.9233) as shown in Figure 1.

Determination of phytic acids

Phytic acid was determined by Davis and Reld method as modified by Abulude (2007). 2.5 g of dried sample was soaked in 100 ml of 2% HCl in 250 ml conical flask for 3 h. The mixture was filtered through Whatman filter paper and 25 ml of the filtrate was mixed with 107 ml of distilled water, 10 ml of 3% ammonium thiocyanate (NHSCH) was added and the solution was titrated against standard FeCl₃ containing 0.00195 g Fe/ml to brownish-yellow color that

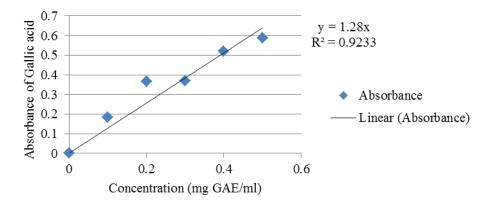


Figure 1. Standard curve of absorption of gallic acid against concentration.

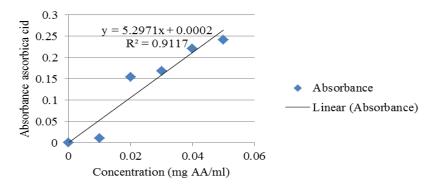


Figure 2. Standard curve of absorption of ascorbic acid against concentration.

persisted for 5 min. The phytate content of the samples was calculated as follows:

Phytate phosphorus = iron equivalent × 1.95 g of titre Phytate = phytate phosphorus × 3.65 g

Oxalate determination

Oxalate composition in the leafy vegetables was determined by Day and Underwood (1986) method with minor modifications. 2.5 g of the samples was mixed with 75 ml of 3 M $\rm H_2SO_4$ and was stirred for 1 h with a magnetic stirrer. The mixture was filtered and 25 ml of the filtrate was titrated while hot against 0.05 M KMnO₄ solution to a faint pink color that persisted for 30 s. The oxalate composition was calculated by assuming that 1 ml of 0.05 M KMnO₄ is equivalent to 2.2 mg oxalate (Chinma and Igyor, 2007; Ihekoronye and Ngoddy, 1985).

Determination of vitamin C

Vitamin C was determined by using methods as prescribed in Food Analysis Laboratory manual (Zvaigzne et al., 2009) and AOC methods (1995) with minor modifications. 5 g of fresh samples was ground using mortar and pestle, 20 ml of oxalic and trichloroacetic acid was added. The mixture was further mixed, filtered through a cotton wool in 100 ml volumetric flask and made up to volume with oxalic acid. 10 ml of the sample extract was pipetted into a 250 ml

conical flask and titrated against 2, 6 phenolindolindol dichlorophenol dye to a persistent rosy pink color. Similarly, 10 ml of 1 mg/ml standard ascorbic acid was titrated against phenolindol indophenol dye.

Determination of total anti-oxidant capacity

The total antioxidant capacity in leafy vegetables was determined by phosphomolybdenum method (Prieto et al., 1999). 0.5 ml of methanol, water and ethanol extract (1 mg/ml) were mixed with 1 ml of 0.6 M $\rm H_2SO_4$, 28 mM $\rm Na_4$ (PO $_4$) $_2$ and 4 mM ammonium molybdate solution in a test tube and incubated in a water bath at 95°C for 90 min. After cooling, the volume was made up to 10 ml with distilled water and absorbance was measured at 695 nm against a blank. Standards (0-0.05 mg/ml) were prepared, treated similarly as the samples and a calibration curve was plotted. Total antioxidant capacity of the leafy vegetables was calculated using the standard curve of ascorbic acid equation (y=5.2971+0.0003; $\rm R^2$ =0.9117) as shown in Figure 2, expressed as ascorbic acid equivalent (AAE) in mg/g of the dry sample.

Determination of reducing power

The reducing power of the leafy vegetables was determined using Chu et al. (2000) method with minor modifications. The reducing power was investigated by observing the formation of Fe³⁺ from F²⁺. The color of the test solution changed to various colors such as

Table 1. Proximate composition of indigenous vegetables.

Indigenous vegetable	DM%	Ash%	Crude fiber%	EE%	CP%
Amaranthus spp.	92.18±0.41 ^a	15.48±0.14 ^a	11.79±0.13	13.17±0.20 ^a	18.09±0.19 ^a
Bidens pilosa	93.77±0.38 ^b	13.35±0.07 ^b	12.83±0.53	9.00±0.29 ^b	19.04±0.33 ^b
Galinsoga parviflora	96.60±0.09 ^c	18.84±0.40 ^c	10.98±0.26	8.97±0.25°	15.83±0.19 ^c

Data represent mean (±SE) of three separate measurements. Different letters in the same column represent significantly different values (P<0.05).

green and blue with reference to the reducing power of test solutions. The sample antioxidants reduces ${\rm Fe^{3+}/ferricyanide}$ complex to ${\rm Fe^{2+}}$ and is evaluated by measuring the formation of Perl's Prussian blue at 700 nm (Yang et al., 2010).

The methanolic extracts were diluted in the range of 0.5 to 2.0 mg/ml using distilled water. 2.5 ml of the diluted extracts was mixed with 2.5 ml of pH 6.6 phosphate buffer, 1% w/v potassium ferricyanide in test tubes and were incubated in a water bath at 50°C for 30 min. After cooling, 2.5 ml of 10% trichloroacetic acid was added and centrifuged for 10 min at 13,000 rpm. 2.5 ml of the supernatant was diluted with 2.5 ml of distilled water and freshly prepared 0.5 ml of 1% ferric chloride solution was added, mixed thoroughly and absorbance of the mixture was measured at 700 nm after 10 min of incubation.

Statistical analysis

Laboratory chemical analyses were done in triplicate and the mean value of each chemical parameter was calculated using Microsoft excel. The data was statistically analyzed by using analysis of variance (ANOVA) in Microsoft Excel ToolPak. Two sample T-test with unequal variances was used to compare mean values and significance was accepted at P≤0.05 level.

RESULTS AND DISCUSSION

Proximate composition

Results on the proximate composition of the three selected indigenous leafy vegetables are presented in Table 1. Crude protein composition ranged from 15.83±0.19 to 19.04±0.33% with the dry matter content ranging from 96.60±0.09 to 93.77±0.38%. The crude protein content in B. pilosa (19.04±0.33%) was higher compared to the other two indigenous vegetables which were 18.09±0.19 and 15.83±0.19%, respectively for Amaranthus spp. and G. parviflora. The crude protein content in Amaranthus subsp. has previously been reported to be 5.60±0.01% at 84.1±0.05% moisture content (Matenge et al., 2017) which translates to 35.22±0.63% crude protein at 92.18±0.41% DM. A study conducted in Zimbabwe found out that crude protein content for Amaranthus spp. and B. pilosa were 4.94±0.46 and 4.40±0.78% on fresh weight basis. respectively (Muchuweti et al., 2011). The mineral composition as ash content of 15.48±0.14% for Amaranthus spp., was comparably similar to the value of 16.43±0.88% reported in a related study in Malawi (Kachiguma et al., 2015). Amaranthus spp. (dubius) has been reported to contain high values of protein (31.13±0.54%), fat (47.20±0.40%) but lower (12.24±0.67%) values than the values obtained in a study conducted by other researchers (Mih et al., 2017). In a study conducted in Nigeria, Amaranth spp. leaves had lower crude fat value of 2.20±0.58% (Funke, 2011) than 13.17±0.20% from this study. Odhav et al. (2007) reported that *G. parviflora* contains 34.91% crude protein on dry matter basis almost twice more than 15.83±0.19 obtained in this study. However, crude fat was 4.36% on dry matter basis almost twice less than 8.97±0.25 from this study (Odhav et al. 2007). On fresh matter basis, B. pilosa has previously been reported by other authors to contain 19.18±0.06% crude protein which is slightly higher compared to the value of 19.04±0.33 obtained in this study (Adedapo et al., 2011). However, comparison was made based on crude fat content for the same authors, it was observed that the crude fat content obtained in this study (9.00±0.29) was higher compared to their results (6.0±1.0%). These differences when compared with our findings might have been attributed to various factors such as geographical locations.

Total antioxidant capacity of the indigenous leafy vegetables

Results on total antioxidant capacity of the leafy vegetables are presented in Table 2. Total antioxidant capacity of the 80% methanolic extracts of the vegetables, in mg AAE/g, ranged from 49.403±0.105 to 59.186±0.0608 for Amaranthus subsp. and G. parviflora, respectively. G. parviflora registered the highest (p<0.05) total antioxidant capacity compared to B. pilosa (55.358±0.0608) and Amaranthus subsp., respectively. Antioxidants are free radical scavengers that either prevent or repair damaged cells by reactive oxygen species (ROS) in human bodies culminating in increased immune defense system and therefore lowering risk of cancer and degenerative diseases (Pham-Huy et al., 2008). The higher values in total antioxidant capacity signify the importance of these indigenous leafy vegetables both for food and medicinal purposes.

Total phenolic compounds content

Results on total phenolic compounds (TPC) of the

Table 2. Antioxidant capacity and Total phenolic compounds of indigenous vegetables.

Indigenous vegetable	Antioxidant capacity (mg Vit. C Equiv./g)	Total phenolics (mg gallic acid Equiv./kg)	Vitamin C (mg/100 g)
Amaranthus spp.	49.403±0.105 ^a	28672±45.1 ^a	45.5026±0.00 ^a
Bidens pilosa	55.358±0.0608 ^b	23464±68.9 ^b	60.7198±0.00 ^b
Galinsoga parviflora	59.186±0.0608 ^c	22639±26.0°	148.8364±0.00 ^c

Data represent mean (±SE) of three separate measurements. Different letters in the same column represent significantly different values (P<0.05).

indigenous vegetables are presented in Table 2. Total phenolic compounds of the 80% methanolic extracts for the three vegetables ranged from 22639±26.0 to 28672±45.1 for G. parviflora and Amaranthus spp., respectively. Amaranthus spp. had the highest (p<0.05) TPC compared to B. pilosa (23464±68.9) and G. parviflora (22639±26.0) leaves. The TPC value for B. pilosa obtained in this study was comparably lower to the values of 27080±2900 (Adedapo et al., 2011) and 51100±5560 mg/kg (Chipurura, 2010) for samples obtained in South Africa and Zimbabwe, respectively. However, G. parviflora TPC value obtained from this study was comparably similar to the value of 20000±5000 mg/kg from a related study conducted in Zimbabwe (Chipurura, 2010). The TPC values obtained in this study for Amaranthus spp. was lower as compared to the value of 40400±0.11 mg/kg DW reported by other authors (Matenge et al., 2017) but comparable to the values (2750±200 mg/kg DW) for Amaranthus triclor for studies conducted in Botswana and Philippines (Baang et al., 2015).

The findings from this study have revealed that the three indigenous vegetables contains high vitamin C content ranging from 45.5026 ± 0.00 to 148.8364 ± 0.00 mg/100 g with *G. parviflora* registering the highest (p≤0.05) value and *Amaranthus* spp. the lowest (p≤0.05) value, respectively. Vitamin C values for *Amaranthus* spp. and *B. pilosa* obtained in this study were lower compared to the values of 64 ± 6 and 70 ± 7 mg/100 g (Muchuweti et al., 2011), respectively reported in a similar study conducted in Zimbabwe.

Total phenolic compounds in plants include phenolic acids, polyphenols and flavonoids which are used as antioxidants in plants protecting them from oxidative damage. Therefore, consumption of phenolic compounds from vegetables, as antioxidants, has medicinal value to humans (Do et al., 2014).

Phytochemical content of the indigenous leafy vegetables

Phytic acid composition

Results on phytic acid composition of the three indigenous vegetables are presented in Table 3. Phytic

acid is a hexaphosphate of inistol that chelate calcium and iron making them biologically unavailable to humans (Gupta et al., 2005). Phytic acid consumption of 4 to 9 mg/100 g results in a decrease in iron absorption of 4-5 fold in humans (Unuofin et al., 2017; Hurrel et al., 1992). It has previously been reported that the general daily phytic acid intake to be 4000 mg (Reddy, 2002) and for rural people in emerging world it is supposed to be 150 to 4000 mg (Reddy et al., 1982). The phytic acid concentration ranged from 0.3264±0.0192 1.3504±0.0450 mg/kg for Amaranthus spp. and B. pilosa, respectively. Amaranthus spp. had the highest (p<0.05) concentration of phytic acid compared to G. parviflora (0.5013±0.0113) and B. pilosa (0.3264±0.0192). The phytic acid content for Amaranthus spp. reported in this study was lower than the value of 6.69 and 13.2 mg/kg reported in Nigeria for Amaranthus spinosus and A. hybridus L. (Agbaire, 2011; Akubugwo et al., 2007). Adedapo et al. (2011) reported phytic acid content of 5.59±0.02 mg/kg in B. pilosa which was higher compared to the value of 0.3264±0.0192 obtained in this study. In a related study, Essack (2017) reported that G. parviflora has 800 mg/kg phytic acid which is higher compared to the value of 0.5013±0.01130 mg/kg from this study. However, phytic acid values for Amaranthus spp., B. pilosa and G. parviflora determined in this study were below the value of 4 to 9 mg/100 g which results in 4 to 5 times reduction in iron absorption in humans.

Oxalic acid composition

Results on oxalic acid composition of the three indigenous vegetables are presented in Table 3. Oxalic acid consumption at 2 to 5 g/100 g levels has been reported to be toxic (Essack, 2017) because of the reduction in the bioavailability of minerals like calcium (Ladeji et al., 2004). Oxalic acid content in the indigenous leafy vegetables, in mg/100 g, ranged from 2141±81.7 to 2288±441 with *B. pilosa* and *G. parviflora* registering the highest and lowest values, respectively. *B. pilosa* had the highest oxalic acid concentration (p<0.05) compared to *Amaranthus* spp. (2250±111) and *G. parviflora*, respectively. The oxalic acid value for *Amaranthus* spp. obtained in this study was lower compared to the values of 5637, 3028 and 3325 mg/100 g for *Amaranthus viridis*,

Table 3. Anti-nutrient content of indigenous vegetables.

Indigenous vegetable	Phytic acid (mg/kg)	Oxalic acids (mg/100 g)
Amaranthus spp.	1.3504±0.0450 ^a	2250±111 ^a
Bidens pilosa	0.3264±0.0192 ^b	2288±441 ^b
Galinsoga parviflora	0.5013±0.0113 ^c	2141±81.7°

Data represent mean (±SE) of three separate measurements. Different letters in the same column represent significantly different values (P<0.05).

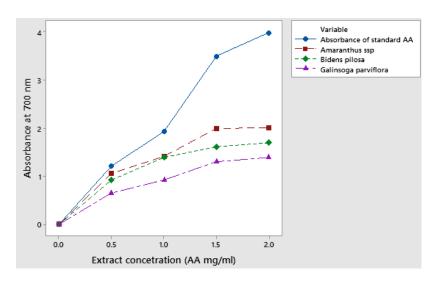


Figure 3. Reducing power of indigenous leafy vegetables.

Amaranthus spp. and A. spinosus previously reported by other authors (Sheela et al., 2004). On the other hand, the oxalic acid values of 2141±81.7 and 2288±441 mg/kg, for G. parviflora and B. pilosa reported in this study were comparatively lower to the values of 17600±1600 and 13100±400 mg/kg, respectively reported in South Africa (Essack, 2017). However, the oxalic acid concentration levels obtained in this current study were below the toxic levels and proper processing of vegetables such as cooking has been reported to further reduce the phytic acid concentration (Akwaowo et al., 2000) which further suggests that consumers are likely to be exposed to very low levels of oxalic acid making consumption of the three indigenous vegetables safe.

Reducing power

Results on the reducing power of the three indigenous vegetables are presented in Figure 3. The results have shown that the indigenous leafy vegetables extracts had high degree of electron-donating capacity with reference to the increasing sample extract concentration (Figure 3). *Amaranthus* subsp. 80% methanolic extracts had the highest (P<0.05) reducing power followed by *B. pilosa*

and *G. parviflora* extracts at all concentrations. However, at 1.0 mg/ml, extract concentration, *Amaranthus* subsp. and *B. pilosa* vegetable extracts had similar reducing power (Figure 3).

Conclusion

The findings from this study have shown that the three indigenous leafy vegetables contains high essential nutrients such as proteins, minerals, vitamin C and phenolic compounds which are important in improving human nutrition and health. The findings have further shown that the indigenous leafy vegetables exhibited high antioxidant properties and reducing power which is essential for their utilization as food as well as medicinal uses. The high nutrient content, high antioxidant capacity, reducing power and low phytic and oxalic acids present in the three indigenous vegetables suggest that low resource rural communities can get adequate nutrition and health through consumption of these indigenous vegetables. It is recommended that future studies on nutrient and phytochemical composition should target more indigenous vegetables consumed by communities in different parts of Malawi.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 14(9) pp. 274-284, October 2020

DOI: 10.5897/AJFS2020.1996 Article Number: B64B72564932

ISSN: 1996-0794 Copyright ©2020

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Full Length Research Paper

Consumers' acceptability of extruded maize-sorghum composite flours fortified with grain amaranth, baobab and orange fleshed sweet potatoes

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Received 13 July, 2020; Accepted 1 September, 2020

Porridge is a popular cereal flour-based food product for children in Sub-Saharan Africa. Compositing of cereal flours to improve their nutritional composition is done. However, the enrichment of such flours with naturally nutrient-rich plant products is poorly developed. A study was conducted to evaluate the acceptability and sensory attributes of newly formulated extruded composite flours containing maize, sorghum, grain amaranth, baobab and orange-fleshed sweet potatoes. Seven extruded formulations optimized for nutritional composition were developed. Twelve trained panellists evaluated the sensory attributes and overall acceptability of the composite flours determined on a 9point hedonic scale. Formulation of the composite flours significantly (p<0.05) affected scores of colour, flavour and overall acceptability with the mean score ranging between 5.7 and 7.4. There was no significant difference (p>0.05) on overall acceptability between extruded and non-extruded composite flours but extruded flours had significantly higher scores on texture (p<0.05). The comparison of the newly formulated composite flours with the conventional flours showed no significant difference (p>0.05) in the overall acceptability; therefore, they can be potentially adopted. All the sensory attributes contributed to the overall acceptability of the formulations, with mouthfeel and flavour having higher relationship with overall acceptability. It is concluded that these attributes are desirable characteristics of any new naturally fortified cereal formulation.

Key words: Extruded, composite flour, formulation, sensory evaluation.

INTRODUCTION

Micronutrient, especially iron, zinc, vitamin A and iodine deficiency is prevalent in developing countries despite the employment of different strategies to help reduce the

challenge (Faber et al., 2014; Osendarp et al., 2018). Food fortification methods employed to address the deficiencies include food to food fortification, industrial

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fortification with inorganic compounds, oral supplements and biofortification through plant breeding and genetic engineering (Mannar and Wesley, 2016). Food insecurity in developing countries and the high cost of commercially fortified food hinder the reduction of micronutrient deficiencies (Akinsola et al., 2018); therefore, there is a need to develop cost-effective and sustainable strategies for improving the nutritional status of food.

Food to food fortification is one of the cheapest interventions used to alleviate micronutrient malnutrition in developing countries (Chadare et al., 2019; De Groote et al., 2020; Morilla-Herrera et al., 2016). More attention is given to composite flours for making porridge because it is the most consumed food by low-income families in most African countries (Ajifolokun et al., 2019; Udomkun et al., 2019). Porridge is commonly prepared from cereals, which are low in most nutrients hence the rise in the formulation of composite flours from different locally available foods (Elina et al., 2016). Formulated products have different sensory characteristics which can be caused by the different ingredients and the changes they undergo during processing (Adedola et al., 2019; Gitau et al., 2019). Thus, there is need to assess the acceptability of the new formulations before releasing them to the market.

Flour blending has been adopted as one of the techniques for improving the micronutrient composition of cereal flours. In addition to augmentation of the nutrient composition, flour blending improves its rheological, physical and sensory properties (Adedola et al., 2019). However, some balance needs to be achieved in compositing: for example, composite flours containing sorghum are acceptable to a point but as the amount of sorghum increases, liking of the colour decreases because of dark pericarps found in red sorghum varieties that may cause undesirable dark colour in food products (De Groote et al., 2020; Omwamba and Mahungu, 2014). Baobab pulp is rich in ascorbic acid; it improves the flavour of food products and their acceptability (Mounjouenpou et al., 2018; Netshishivhe et al., 2019). In as much as amaranth grain flour flavour is not preferred on its own, Joshi et al. (2019) found that composite flours containing up to 25% of grain amaranth were acceptable. Orange fleshed sweet potatoes affect the colour and flavour of food which makes it desirable in composite flour formulations (Pereira et al., 2019).

Extrusion is a food processing method that has been widely used in the production of pasta, breakfast cereals and snacks (Leonard et al., 2020). It is currently being used in the production of nutritious instant composite flours with better sensory attributes (Otondi et al., 2020). Extrusion temperature and feed moisture are known to affect the colour and texture of extrudates (Gbenyi et al., 2016).

Composite flours containing maize, sorghum, amaranth grain, OFSP and baobab have been formulated, but the effect of formulation and extrusion on their sensory

attributes has not been studied. Therefore, the current study aimed at evaluating the sensory attributes and consumer acceptability of the newly formulated extruded composite flours.

MATERIALS AND METHODS

Sample acquisition

The raw materials were sourced from different parts of Kenya. Maize was obtained from Eldoret Market located in the Rift valley. Pale-red sorghum (E97) and pale cream amaranth grain were obtained from Busia and Bungoma, Western Kenya. Baobab powder was obtained from Mombasa and the sweet potato puree was obtained from Organi® Ltd in Homabay. The dry foodstuffs were stored at 25°C and the potato puree was stored in a deep freezer at -20°C.

Sample preparation

The grains were cleaned and dried in a forced-air draft oven at 60°C for 24 h to a moisture content of about 12%. Frozen orange-fleshed sweet potato (OFSP) puree was thawed in hot water, and oven-dried at 60°C for 6 h. The dry grains and OFSP were separately milled in an 800 μm sieve-hammer mill and whole-meal flours were obtained.

Formulation of the composite flours

The composite flour formulations were based on the findings of the nutritional profile of each of the ingredients (unpublished work) to meet the Recommended Dietary Allowance (RDA) of children below the age of five years based on World Health Organization (WHO) recommendations. Nutrisurvey linear programming software embedded with WHO RDA for children was used in the formulation of the flours (NutriSurvey, 2007). The formulations targeted 25% RDA of beta-carotene, iron and zinc contents and 15 % RDA of protein. Seven formulations were developed (Table 1).

The flours were thoroughly mixed and half of each mixture was stored separately as the control; whereas the other half was processed through extrusion. The moisture content of the other half was raised to 35% by adding water and mixing thoroughly and extruded at 160°C in a single screw extruder (TechnoChem, Indiana, USA) with a screw rotation of 800 rpm. The extruded products were dried at 50°C for 4 h, milled and vacuum-packed in polythene bags and transported in boxes to Nairobi for sensory evaluation. The best flour was selected for comparison with commercial flours.

Acquisition of commercial composite flours for comparison

Commercial composite flours containing maize and sorghum for comparison were obtained from different supermarkets in Nairobi Kenya as described in Table 2.

Porridge preparation

Thin porridge was prepared by modifying the method described by Onyango et al. (2020). Briefly, 40 g of the composite flour was added to 200 mL of cold water and thoroughly mixed with a wooden ladle. It was transferred to a stainless-steel pot containing 640 mL of boiling water and stirred continuously for 5 min. It was boiled for

Table 1. Composite flour formulations.

Committee	- Ir		Ingredient proportion (%)			- Description
Formulation	Maize	Sorghum	Amaranth	OFSP	Baobab	- Description
F1	30	35	20	10	5	Varying cereals and fortificants
F2	42.5	22.5	5	15	15	More maize than sorghum with constant fortificants
F3	22.5	42.5	5	15	15	More sorghum than maize with constant fortificants
F4	32.5	32.5	5	15	15	Equal maize and sorghum, constant fortificants
F5	65	0	5	15	15	Maize plus constant fortificants
F6	0	65	5	15	15	Sorghum plus constant fortificants
F7	20	45	5	15	15	A variant of formulation C

Table 2. Commercial composites for comparison.

Market composites	Composition
S1	Formulated flour containing sorghum, maize, baobab, grain amaranth and orange-fleshed sweet potato (the best formulation)
S2	Millet, sorghum, lemon and souring agent blended flour
S3	Finger millet, maize, wheat, amaranth, soya and sorghum cereal flour blend
S4	Finger millet, maize, sorghum and souring agent cereal flour blend
S5	Sorghum, maize, vitamins and minerals flour blend
S6	Soya beans, groundnuts, beans, finger millet, cassava, maize and silverfish flour blend
S7	Maize, millet, sorghum, soya and groundnuts flour blend
S8	Maize, sorghum and soy flour blend

10 min and transferred to vacuum flasks.

Sensory evaluation

Sensory evaluation was carried out at Kenya Bureau of standards laboratories in Nairobi by modifying the method described by Onyango et al. (2020). Twelve sensory evaluation panellists were recruited, trained on sensory attributes for 6 h using sample porridges with different attributes. The first 3 h involved attribute generation while the rest of the time was used to identify references that match with the attributes (Table 3) and how to rate them on a 100 mm unstructured scale.

During the actual evaluation, coded clear plastic cups with 50 g of porridge were served to the panellists. A cup of drinking water was also provided for the panellists to rinse their mouths between samples. All the attributes of each sample were fully evaluated using the provided questionnaire before the next sample was served and the results were recorded in duplicate.

Data analysis

The data were analysed using the *R* Project for *Statistical* Computing, R-3.6.3 (R Core Team, 2019). The normality of the data was first tested using Wilk's Shapiro test with the data that were not normal transformed to z-distribution before inferential analysis. Descriptive statistics, including mean and standard deviation, were determined for the formulations and treatments. Analysis of variance (ANOVA) was used to establish significant differences in the mean sensory scores of attributes with different means separated using Tukey's HSD test at p<0.05. The product with the

highest sensory scores was evaluated against commercial conventional blended flours retailed in the market and their means compared using ANOVA. Data exploration was done using clustering and principal component analysis.

RESULTS

Sensory attributes of blended cereal flours

Formulation, extrusion and their interaction affected sensory attributes of the formulated composite flours significantly (p<0.05). The scores of the new formulations in terms of the colour, texture, mouthfeel, flavour and overall acceptability were all acceptable (Table 4). Based on colour, F7 was the most acceptable (7.4±1.38) while F2 was the least acceptable (6.13±1.70). F7 was also the most acceptable for texture (6.88±1.54) as compared to F1 which scored the least (6.05±1.83). Based on the mouthfeel, F3 was the most acceptable (7.40±1.34) while F2 had the lowest score (6.25±2.26). F7 was the most acceptable based on flavour and overall acceptability (7.55±1.06 and 7.43±1.24). With a lower proportion of sorghum, the liking of the colour of the blended flour significantly (p<0.05) increased. Formulations with the highest proportion of amaranth grain powder had the least scores for flavour and overall acceptability (p<0.01).

Evaluation of extrusion as the main effect shows that

Table 3. Descriptive sensory lexicon developed by the sensory evaluation panel to evaluate the quality of porridge.

Attribute	Description	Reference and rating scale
Appearance		
Colour	Discernment of colour ranging from white to dark brown	Corn starch (10% w/v) stirred in hot water = 0 (white) ^a Dairy land dark compound chocolate = 10 (dark brown)
		Corn starch (10% w/v) stirred in hot water = 0 (no dark specks)
Brown and dark specks	Quantity of brown and dark specks in porridge when smeared on a white surface	Whole milled sorghum flour (30% w/v) stirred in hot water = 10 (many dark specks)
Flavour		
Maize flavour	Flavour characteristic of maize flour in hot water	Whole-milled maize flour (30% w/v) stirred in hot water = 10 (very intense)
Sorghum flavour	Flavour characteristic of sorghum flour in hot water	Whole-milled sorghum flour (30% w/v) stirred in hot water = 10 (very intense)
Grain amaranth flavour	Flavour characteristic of grain amaranth flour in hot water	Whole-milled grain amaranth flour (30% w/v) stirred in hot water = 10 (very intense)
Baobab powder flavour	Flavour characteristic of baobab fruit pulp flour in hot water	Whole-milled baobab pulp flour (30% w/v) stirred in hot water = 10 (very intense)
OFSP flavour	Flavour characteristic of OFSP flour in hot water	Whole-milled OFSP flour (30% w/v) stirred in hot water = 10 (very intense)
Taste		
Sour taste	The taste associated with lemon juice	^b Quencher mineral water = 0 (not sour) Whole-milled finger millet porridge (10% w/v) containing 1% w/v citric acid solution = 10
Texture		
Viscosity	Resistance to flow when the porridge is poured in another cup	^c KCC gold crown milk (fat content 3.5%) = 0 (thin) ^d Daima thick yoghurt= 10 (thick)
Coarseness	The extent to which particles are perceived in the mouth in the process of chewing	Honey = 0 (not perceived) Fresh blended, unsieved watermelon juice = 10 (intensely perceived)
Mouth feel		
	Physical sensation in the mouth	^e Krackles crisps= 0 (crispy)^d Daima thick yoghurt= 10 (smooth)
After swallow		
Sour aftertaste	Perception of lingering sourness in the mouth after chewing and swallowing	10= Strong after taste
Residual particles	Perception of particles in the mouth after swallowing porridge	Fresh blended, unsieved watermelon juice = 10 (many residual particles)

^aTopserve limited Kenya; ^bExcel chemicals limited, Nairobi; ^cNew Kenya co-operative creameries, Nairobi Kenya; ^dSameer Agriculture, Kenya and ^ePropack Kenya Limited, Nairobi.

extruded flour had significantly (p<0.05) higher scores for texture than the non-extruded flours (control) as shown in Figure 1. The extruded composite flours had slightly higher scores on colour (6.8), texture (6.9) and mouthfeel (6.8) compared to the non-extruded flours (control) which were better in flavour and overall acceptability with the scores ranging between 6.5 and 7.4.

Interaction of extrusion and the formulation as treatments resulted in significant (p<0.05) differences in the sensory scores of texture and flavour (Table 5). Control (non-extruded) formulations with higher amaranth content and those that were extruded with higher maize content had significantly (p<0.05) least scores for texture (5.35±1.42 and 5.65±1.69) respectively. For flavour, the extruded

Formulation	Colour	Texture	Mouthfeel	Flavour	Overall acceptability
F1	7.08±1.61 ^{ab}	6.05±1.83 ^a	6.30±2.05 ^a	5.90±2.01 ^a	5.65±1.63 ^b
F2	6.13±2.05 ^a	6.83±1.58 ^a	6.25±2.26 ^a	6.78±2.14 ^{ab}	6.98±1.73 ^a
F3	6.85±1.70 ^{ab}	7.08±1.31 ^a	7.40±1.34 ^a	6.95±1.48 ^{ab}	7.23±1.19 ^a
F4	6.68±1.51 ^{ab}	6.83±1.55 ^a	6.90±1.53 ^a	6.95±1.13 ^{ab}	6.75±1.43 ^a
F5	6.18±2.32 ^a	6.05±1.85 ^a	6.58±1.69 ^a	6.65±1.90 ^{ab}	7.03±1.46 ^a
F6	6.73±1.87 ^{ab}	6.78±1.85 ^a	6.78±1.87 ^a	6.70±1.76 ^{ab}	6.68±1.56 ^a
F7	7.43±1.38 ^b	6.88±1.54 ^a	6.88±1.80 ^a	7.55±1.06 ^b	7.43±1.24 ^a
%CV	27.3	25.3	27.3	25.1	22.8
p-value	0.013	0.116	0.083	0.002	< 0.001

Table 4. Main effect of formulation on the sensory attributes of non-extruded composite flours.

The values are mean ± SD. Values with the same superscript in a column are not statistically different at p>0.05. The samples (F1-F7) are as described in Table 1.

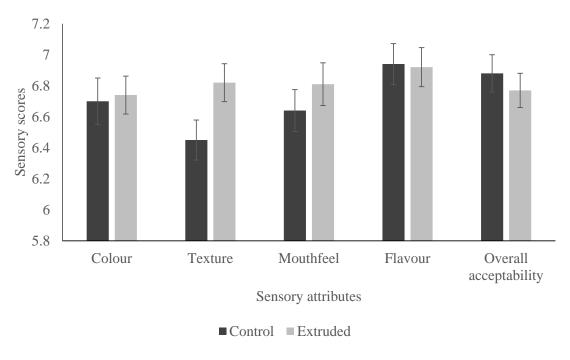


Figure 1. Main effects of extrusion on the sensory scores of blended cereal flours.

formulation with a higher content of amaranth had significantly (p<0.05) the least sensory scores. The principal component analysis showed that all the four attributes contributed to the overall acceptability of the formulations with mouthfeel and flavour having a higher relationship with the overall acceptability as shown in Figure 2.

Comparative sensory quality of blended flours to the conventional flours

The formulated flour (S1) had significantly (p<0.001) lower scores for colour compared to the commercial conventional flours (Table 6). The overall acceptability of the formulated cereal flours had no significant difference (p>0.05) with the most acceptable cereal flour blends in the market.

Clustering of sensory qualities of the blended flours

The sensory attributes of the flour blends are maximally explained by two clusters explaining 87.3% in the data variability. Cluster 1 had higher scores in all the four sensory attributes evaluated (Figure 3); colour, mouthfeel, flavour and overall acceptability. Except for the flour blends that had limited ingredients that majorly constituted

Table 5. Effect of the interaction between extrusion and formulation on sensory attributes of blended cereal flours.

Sample	Treatment	Colour	Texture	Mouth feel	Flavour	Overall acceptability
F4	Extruded	6.55±1.85 ^a	6.75±1.94 ^a	6.40±2.62 ^a	5.05±1.79 ^b	5.25±1.83 ^a
F1	Control	7.60±1.14 ^a	5.35±1.42 ^b	6.20±1.32 ^a	6.75±1.89 ^{ab}	6.05±1.32 ^a
F0	Extruded	6.26±1.48 ^a	6.50±1.82 ^a	6.20±2.04 ^a	6.70±2.00 ^{ab}	6.75±1.59 ^a
F2	Control	6.00±2.53 ^a	7.15±1.27 ^a	6.30±2.52 ^a	6.85±2.32 ^{ab}	7.20±1.88 ^a
5 0	Extruded	6.80±1.67 ^a	7.30±1.22 ^a	7.60±1.05 ^a	7.35±1.04 ^{ab}	7.55±0.94 ^a
F3	Control	6.90±1.77 ^a	6.85±1.39 ^a	7.20±1.58 ^a	6.55±1.76 ^{ab}	6.90±1.33 ^a
- 4	Extruded	7.05±1.36 ^a	7.40±1.14 ^a	7.10±1.45 ^a	6.95±1.10 ^{ab}	6.85±0.88 ^a
F4	Control	6.30±1.59 ^a	6.25±1.71 ^a	6.70±1.63 ^a	6.95±1.19 ^{ab}	6.65±1.84 ^a
	Extruded	6.15±1.69 ^a	5.65±1.69 ^b	6.25±1.68 ^a	6.30±1.49 ^{ab}	6.90±1.12 ^a
F5	Control	6.20±2.86 ^a	6.45±1.96 ^a	6.90±1.68 ^a	7.00±2.22 ^{ab}	7.15±1.76 ^a
5 0	Extruded	6.80±1.94 ^a	7.00±1.49 ^a	6.70±1.92 ^a	6.35±1.87 ^{ab}	6.55±1.61 ^a
F6	Control	6.65±1.84 ^a	6.55±2.16 ^a	6.85±1.87 ^a	7.05±1.61 ^{ab}	6.80±1.54 ^a
F-7	Extruded	7.60±1.14 ^a	7.15±1.31 ^a	7.40±1.50 ^a	7.65±0.99 ^a	7.45±1.10 ^a
F7	Control	7.25±1.59 ^a	6.60±1.73 ^a	6.35±1.95 ^a	7.45±1.15 ^{ab}	7.40±1.39 ^a
%CV		27.3	25.3	27.3	25.1	22.8
p-value		0.428	0.016	0.519	0.030	0.411

The values are mean ± SD. Values with the same superscript in a column are not statistically different at p>0.05. The samples (F1-F7) are as described in Table 1.

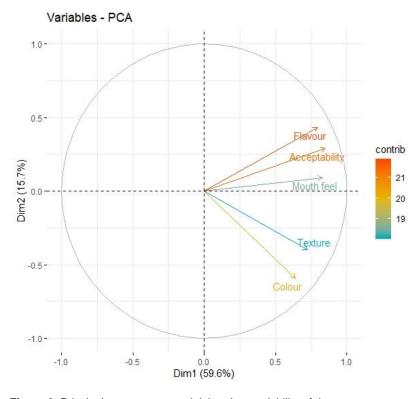


Figure 2. Principal components explaining data variability of the sensory scores for the formulated flour blends.

Table 6 . Comparison of the	formulated flour against	conventional flour	retailed in the market.

Sample	Colour	Mouthfeel	Flavour	Acceptability
S1	5.58±1.82 ^c	5.63±1.74 ^{ab}	6.21±1.89 ^a	6.33±1.66 ^{ab}
S2	8.04±0.81 ^a	6.42±2.02 ^a	6.21±1.86 ^a	7.25±1.26 ^a
S3	7.46±1.25 ^{ab}	6.75±1.59 ^a	6.63±1.53 ^a	7.29±1.55 ^a
S4	5.71±1.12 ^c	6.46±1.67 ^a	6.75±1.65 ^a	6.79±1.50 ^a
S5	3.25±1.75 ^d	4.42±1.86 ^b	4.33±2.06 ^b	4.46±2.13 ^c
S6	7.96±1.04 ^s	6.67±1.24 ^a	6.63±1.41 ^a	7.13±1.26 ^a
S7	6.04±2.37 ^{bc}	5.63±2.04 ^{ab}	6.04±1.90 ^a	6.46±1.98 ^a
S8	3.17±2.10 ^d	4.79±2.26 ^b	5.29±2.65 ^{ab}	4.88±2.27 ^{bc}
%CV	40.7	33.8	33.6	31.4
p-value	p<0.001	p<0.001	p<0.001	p<0.001

The values are mean ± sd. Values with the same superscript in a column are not statistically different at p>0.05. The samples (S1-S8) are as described in Table 2.

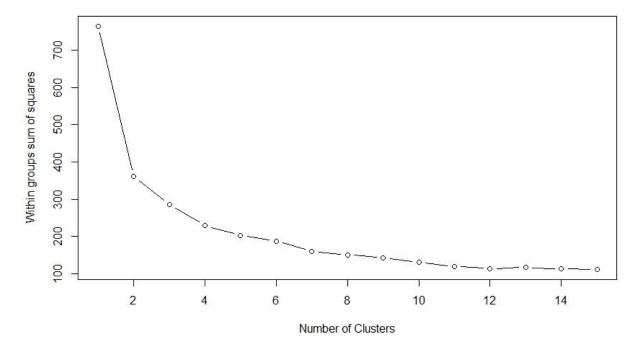


Figure 3. WSSplot for clustering of the sensory attributes of cereal flour blend.

cereals as the ingredients, all other flour blends were in cluster 1 (Figure 4). All the three attributes contributed towards determining the overall acceptability of the cereal flour blends (Figure 5). However, mouthfeel and flavour had a closer relationship with the overall acceptability (Figure 6).

DISCUSSION

The formulated composite flours varied in colour, flavour and overall acceptability. Flours with lower sorghum

contents had better scores in terms of colour. This finding is similar to that reported by Tegeye et al. (2019) who reported that increasing the proportion of fortificants tends to improve the liking of colour among the consumers. An increase in amaranth content in the formulations resulted in a decrease in flavour scores which agrees with other studies. Studies by Joshi et al. (2019) found that composite flours containing 25% amaranth were more acceptable. Akande et al. (2017), on the other hand, found that adding up to 35% amaranth grain in porridge composites left an aftertaste that was not liked by consumers.

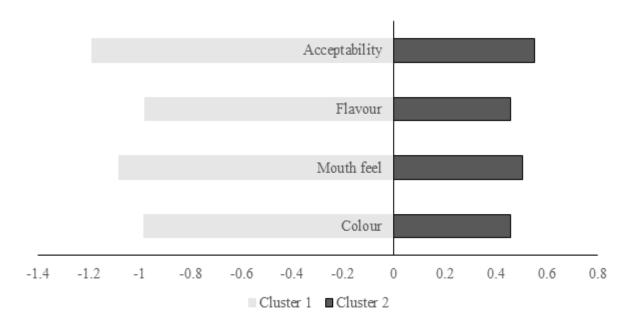


Figure 4. Clustering of the sensory attributes of blended flours (both formulated and conventional ones). The values were transformed into z-distribution where the mean is 0 and the standard deviation is 1.

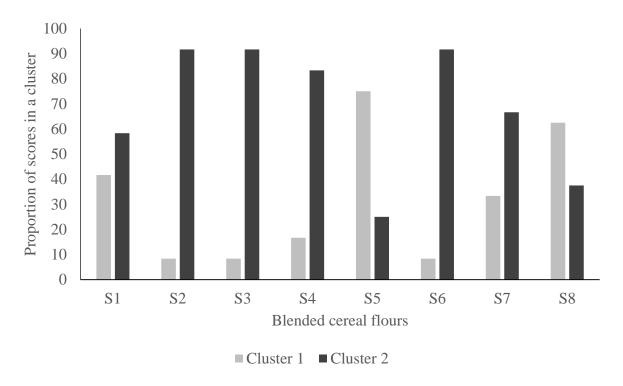


Figure 5. Proportion of the sensory scores of blended flours loaded into the clusters.

Extrusion affected the sensory scores in terms of colour and texture of the composites. This may be due to extrusion temperature and feed moisture which is known to affect the colour of the final product through mallards reaction, non-enzymatic browning and pigment destruction reactions (Adams et al., 2019; Gbenyi et al., 2016). Extrusion imparts a soft texture to extruded products hence the preference in texture (Patil et al.,

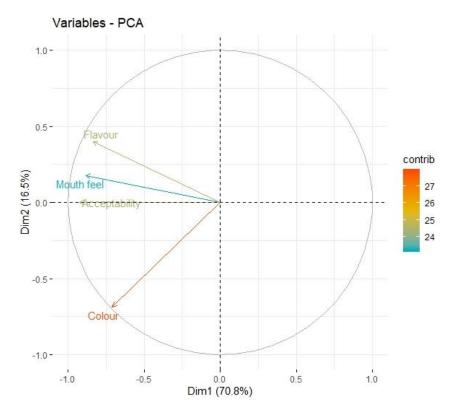


Figure 6. Principal Component Analysis plot of the sensory attributes of blended flours.

2016). This may also be attributed to texturisation that occurs during extrusion (Sun et al., 2019). Non-extruded flours had course texture because the flours were wholemeal and only milled once while the extruded ones were milled two times, before and after extrusion (Heiniö et al., 2016). Extrusion reduced the coarse properties of sorghum flour and improved overall acceptability and flavour of sorghum wheat composites (Jafari et al., 2018). This finding implies that consumers will tend to have a higher liking for the extruded flour with a softer texture than the non-extruded ones.

The study established that the attributes of colour, flavour and mouthfeel are essential sensory qualities that contribute towards acceptability of food products. These findings are in agreement with Eze et al. (2020) and Ramírez-Jiménez et al. (2018) who reported that aroma, taste, mouthfeel and colour were the major determinants of acceptability. However, the mouthfeel and flavour were found to be greater contributors in improving the acceptability of the products compared to colour. Therefore in developing product qualities, much more attention should be put in improving mouthfeel and sensory qualities (Elina et al., 2016).

There was no significant difference in the overall acceptability of the new formulation compared to the market blends. This means that all the flours were equally accepted which agrees with the study conducted by Elina

et al. (2016). However, it contradicts earlier studies that indicated that extruded products were disliked by consumers due to flavour changes that develop during extrusion (Muoki et al., 2012).

The newly formulated flours scored low on colour which can be attributed to the extrusion process which increases the intensity of the colour. Eze et al. (2020), in their study, found that extruded flours had more intense colour and better mouthfeel and liking compared to conventional flours. Thus, there is need to improve the colour attribute for enhanced acceptability. This also explains the low determinant level in overall acceptability of the cereal flour blends attributable to colour. Mouthfeel was highest for flours containing finger millet, maize, wheat, amaranth, soya and sorghum (6.75). This could be attributed to the use of roasted soya that is known to improve the taste of food (Gitau et al., 2019; Maria and Anuoluwapo, 2018).

Conclusion

Formulation affected the colour, flavour and the overall acceptability of maize, sorghum, amaranth grain, baobab and orange-fleshed sweet potato composite flours with the formulations containing higher sorghum amounts being the most acceptable while extrusion affected the

texture scores. The composite flours were all liked thus indicating the potential of the new formulations to be adopted. Colour, flavour, texture and mouthfeel contributed to overall acceptability of the composite flours with the major contributors being mouthfeel and flavour. Flavour and mouthfeel are therefore desirable characteristics of any new formulations of cereal flour blends in the food industry.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful for the financial support received from United States Agency for International Development (USAID) Bureau for Food Security under Agreement # AID-OAA-L-12-00003 as part of Feed the Future Innovation Lab for Food Processing and Post-harvest Handling and the Kenya Bureau of Standards for the technical support.

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Vol. 14(9) pp. 285-294, October 2020

DOI: 10.5897/AJFS2018.1744 Article Number: DFE5EFE64946

ISSN: 1996-0794 Copyright ©2020

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Full Length Research Paper

A review of explored uses and study of nutritional potential of tamarind (*Tamarindus indica* L.) in Northern Ghana

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Received 30 July, 2018; Accepted 1 September, 2020

Tamarind (*Tamarindus indica*) is increasingly becoming a commercially important underutilized tree crop worldwide. Due to its multi-purpose use and market demand the tree and its processed products are been traded in many towns and villages. Despite its potential, major setbacks are the lack of knowledge on its uses and nutritional potential within the Ghanaian context. This study reviews and exposes the beneficial potential of tamarind whiles studying with empirical data its nutritional composition for proximate analysis. Indigenous to tropical Africa and naturalized to many regions of the world, the tree is seen in over 50 countries. Within Ghana and other parts of the world, tamarind is distinctively called by different vernacular names either referring to the tree or its product. Almost every part of the tree is used in one way or the other from food including beverage drinks, jams, and curries, to pharmaceutical, textile, timber, fodder, and as a fuel source. It is rich in vitamins, minerals and other proximate elements. Proximate composition of locally sourced fruit pulp showed high levels of fats and oils 51.39% and fibre 15.10% while other parameters like protein, ash, vitamin C and moisture were similar to test results from other countries. The tamarind plant has undoubtedly great potential based on its benefits, and uses.

Key words: Tamarind, *Tamarindus indica*, Ghana, multipurpose, benefits, nutritional potential, medicinal, proximate composition.

INTRODUCTION

Tamarind (*Tamarindus indica*) is a leguminous tree that belongs to the family Fabaceae with Subfamily Caesalpiniaceae (Stege et al., 2011). The plant is believed to be indigenous to tropical Africa and also described by some botanist as a pan-tropical species which stretches from Senegal to Eritrea, from Sierra Leone to Cameroon, from Ethiopia and Somalia to

Mozambique (Bhadoriya et al., 2011). According to Abubakari and Muhammad (2013), the tamarind tree was long ago introduced into and adapted to India such that it has often been reported been indigenous from there. They added that, it was apparently from this Asiatic country that it reached the Persians and the Arabs who called it "Tamar Hindi" (Indian date, from the date-like

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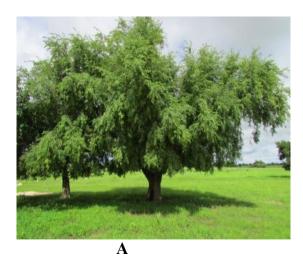




Figure 1. (A) The tamarind tree; (B) Tamarind shoot with hanging fruit pods and leaves. Source: Field survey (2019).

appearance of the dried pulp) traded in it, and thus giving rise to both its common and generic name. Unfortunately, the specific name "indica" also perpetuates the illusion of Indian origin. The genus, *Tamarindus* is monotypic in its taxon, and therefore has only one species (*indica*).

According to El-Siddig et al. (2006) the major production centres of tamarind are in the Asian countries like India, Sri Lanka, Thailand, Bangladesh, Indonesia and Thailand. Whiles in the Americas, Mexico and Costa Rica are the biggest producers. India is the major country that has broadly utilized the tree, with more than 250,000 tons of the fruit harvested each year. Of this, about 3,000 tons is exported into Europe and North America for use in the food and beverage industries (Abdelrahman and Mariod, 2019). Africa on the other hand does not produce tamarind on large scale basis, although it is widely utilized by locals in some minor producing countries mostly in West African.

The tamarind tree is well adapted to semi-arid regions of the tropics and can withstand drought conditions relatively well. It can tolerate a great diversity of soil types but does best in deep, well-drained soils, which are slightly acid or saline. The tree will not tolerate cold and continuous wet soils (Bhadoriya et al., 2011). The tree is fairly large in size (Figure 1A and B) and can produce up to 175 kg as fruit yield per year (Dassanayake and Fosberg, 1991).

Generally, there are sweet and sour types and this differ significantly in their morphological characteristics. Hailay et al. (2020) in a study in Ethiopia found that the sweet tamarind trees produced significantly more fruit pulp, seed, seed size and weight than the sour trees. They also observed that tamarind fruit vary from curved to straight. The colour of the sour variety pods and fresh pulp are a light brown, while the sweet variety pods and fresh pulp are usually deep brown. Ripe fruits are filled

with a yellowish or brown pulp, fibrous with an acid like but pleasant taste. The seeds are hard and shiny with the bark of pod been fragile and easily broken by hand (Okello et al., 2018).

As indicated by Shankaracharya (1998), the fruit pulp of tamarind comprises 30-50% of the ripe fruit while its shell and fibre account for 11-30% and the seed about 25-40%. The most outstanding characteristic of tamarind as indicated by El-Siddig et al. (2006) is its sweet acidic taste due to the presence of tartaric acid (10%). The author describes tamarind to be simultaneously the most acidic and sweetest fruit. According the World Health Organization (WHO) tamarind can be considered a source of all essential amino acids, with the exclusion of tryptophan. It contains also other organic acids as tartaric, succinic and malic acid (Ferrara, 2019).

The tree plays major and important roles in many aspects of life from food, pharmaceutical, and textile industries, to being used as timber, fodder, and as a source of fuel (Pugalenthi et al., 2004). The fruit pulp of tamarind is edible and is considered more appealing and palatable, as it becomes sweeter and less sour (acidic) as the fruit matures. It is rich in vitamins, minerals and other proximate elements (Emmy et al., 2010). According to El-Siddiq et al. (2006) in most growing areas, processed tamarind beverage drink is among the most popular flavoured drinks and the brand name "Jarritos" is a well-known tamarind export traded soda drink. As described by Arbonnier (2004), the tree's morphological features are distinct;

(i) Stems are more or less pubescent, fissured and scaly. Bark Scaly, with longitudinal and transverse fissures. Some pale brown-grey patches showing between the rectangular scales. Slash with yellow outer layer, pale red beneath.

- (ii) Leaves, are alternate compound, 7-15 cm long, pubescent, becoming glabrous, with 8-10 (-15) pairs of opposite leaflets, narrowly oblong, 2-3 cm long and 0.6-1 cm across. Leaf blade is rounded or notched apex and rounded asymmetrical base appearing glaucous green with the petiole channeled at the base.
- (iii) Flowers are pedicellate and appear attractive as pale yellow with red stripes, 2.5 cm in diameter, with 4 ovate sepals, green and yellow inside and brown outside, and 3 finely denticulate petals.
- (iv) Fruit appears as a sub-cylindrical pod, 10-18 cm long and 1.5-2.5 cm across, somewhat curved, more or less constricted and torulose, puberulous, brown-russet, persistent and turning blackish. Pulp is brownish, containing an astringent which sweet tasting and sticky. The shell of the fruit-pod is brittle and the seeds are embedded in the edible pulp.
- (v) Seeds are 3 to 10 per pod, approximately 1.6 cm long, unevenly shaped, with a hard, shiny, and smooth testa.

In Ghana, the tree is commonly found growing within the Savannah ecological zones. The tree starts flowering during the dry season and fruiting starts from January and stretches through to the beginning of rains (March/April). Its fruit is mainly used for food purposes with local beverage drink products being the major processed form that is seen marketed across towns and villages within Ghana. The tree is evergreen and mainly provides shade all year round. The tamarind tree in parts of northern Ghana is claimed to be a special tree for the elephant because of its constant shade and strength to lean on as a form of support when it is in labour. Locals claim, that in order to be safe from an attacking elephant, run to the tamarind tree, and you will be spared.

There are many published works about multipurpose fruit, El-Siddiq et al. (2006) and De Caluwé et al. (2009) has published a review on the traditional uses of tamarind with reference to sub-saharan Africa. However, very little is known of the local potential from the Ghanaian context as the tree plays major roles in local economies and landscapes where it is grown. Within Ghana, despite the wide usage of the tamarind tree, the tree can be termed as under-utilized since there is virtually no major industrial exploration of the potential of this important tree. There is also limited information on the nutrient content of the edible portions of many indigenous and underutilized fruits including tamarind in Ghana and this makes it difficult to make any substantial claim for their optimal use as a source of nutrients (WHO, 2005; Abebrese et al., 2007).

With emphasis on comparing and exploring the tree's nutritional potential and uses with already established facts, this study seeks to bring to bear from both primary and secondary data sources the potential of the tamarind tree. As a traditional food source plant in Africa, tamarind has a great potential to improve human nutrition, boost food security, promote rural development, enhance

revenue and support sustainable land care.

MATERIALS AND METHODS

Primary data for this study was solicited from personal interviews and interactions with local users, marketers and traders of tamarind within Northern Ghana. Secondary data was also compiled from an array of information search without time limitation in the World Wide Web across a wide range of search engines and databases using the main search keys; 'tamarind' and 'T. indica'. Data gathered was screened and synthesized for qualitative and quantitative outputs. Results from both primary and secondary sources are presented in a descriptive and narrative form and where applicable, tables used to compare data with previous work.

Study on proximate analysis for nutritional composition was done by randomly sampling ripe tamarind fruit pulps from a major local market (Aboabo market) in Tamale the capital of the northern region of Ghana. Each test parameter was replicated 3 times. Sampled pulps were tested using the methodologies as described in the Association of Official Analytic Chemistry (AOAC), International. 19th Edition.

As is conventional and ethical in reviews, authors respected originality of results as presented though very much aware that results presented are the views from respondents and also acknowledges the limitations in variation of results collated from other authors. And this could be based on test location, techniques and types of instruments used in the different laboratories. This paper does not in any way claim full proof of any responses as presented by respondents except in the case of analytical results obtained from the primary data from the study's laboratory test. All presented laboratory test parameter results are presented in same SI units to ensure homogeneity. Further details on analytical methodologies of reviewed work presented, could be obtained from the original articles as cited.

Vernacular names of tamarind tree within Ghana

The tamarind tree species though the same botanically across all growing areas in the world, it is known and called differently within the different spoken languages where it is grown or traded. Within northern Ghana it is known in some local dialects as shown in Tables 1 and 2.

POTENTIAL BENEFITS OF TAMARIND WITHIN THE GHANAIAN CONTEXT

Fruit pulp

Within the Ghanaian context, the fruit pulp of tamarind is

Tribe (language spoken)	Vernacular name	Tribe (language spoken)	Vernacular name
Builsa (Buili)	Pusik	Mamprusi (Mampruli)	Pussa
Kasena (Kasim)	Saana	Dagbani (Dagbanli)	Puhugu
Nankana (Nankam)	Pusika	Gonja	Kapaaleri/ Kapalud
Frafra (Gurune)	Pusa/Pusiga	Waala (Waali)	Puhee

Dagaaba (Dagare)

Sissala (Sissali)

Moshie (Moa)

Hausa

Table 1. Local names of tamarind in various spoken languages in northern Ghana.

Pussa

Putiye

Poses

Puah

Heeri/Fiiri

Talensi (Talen)
Source: Field survey (2020).

Kusasi (Kusaal)

Dagati (Dagao)

Bissa (Busanga)

Bimoba (Moar)

undoubtedly the most important part of the tree, as it is considered useful as a food source and traded in most local markets. The fruit pulp can be eaten raw to boost appetite, and the in most parts of northern Ghana the pulp is becoming increasingly popular in its use as a beverage drink commonly known in Hausa as "tankua beer" or within the Dagbon area (northern Ghana) as "poha". As suggested by Sadik (2010), the consumption of this drink could help reduce the prevalence of iron deficiency anaemia because of the vitamin C rich content of the pulp which enhances bioavailability of non-haem iron. Saha et al. (2010) and Abhijit et al. (2010) reported that tamarind fruit shell can be utilized as a low-cost biosorbent for the removal of malachite green from aqueous solutions.

The fruit pulp is processed into balls (Figure 1A), the extracted pulp is commonly and commercially marketed across Ghana. It is mainly processed by soaking it in water and straining out the extract which is mixed-up with sugar and other spices to make tamarind flavoured beverage drink. Among most tribes in the upper regions of Ghana, the pulp of tamarind is mixed with floor or water and used as flavouring in the preparation of "Tuo Zafe" (TZ) a traditional stable local food common in northern Ghana. It is also fermented and used in the preparation of porridge. The fruit pulp is also a good ingredient in making skin care products like soaps, as it enhances skin lightening or skin toning and treats skin irritation.

Seed

This has less uses in many processing areas within Ghana as it is often discarded as a by-product after extraction of the pulp from the fruit. However, some processors gather it and use as feed for animals especially pigs while in some parts of northern Ghana, the seeds are added during brewing of a local alcoholic beverage beer called "pito". Is has been shown in recent

times, that the almond composition of tamarind seeds is very similar to that of cereal seeds and is a good source of food (Okello et al., 2017).

Puree

Sunsuing

Samiya

Pussa

Leaves, flower, and pods

Among some tribes in northern Ghana, women use the leaves in preparing vegetable soups though this practice is not very common in recent times. The flowers, tinder leaves and dry pods after processing are very palatable and liked by ruminants including cattle, sheep and goats. The flowers are also attractive to bees and the honey produced from the tree is very tasteful and sweet. Within northern Ghana, tamarind tree comes with some useful non-food benefits in many societies. Some parts of the plant including the leaves, the bark, shoots and roots have various form of uses such as fuelwood, timber, charcoal making, chewing sticks among others (Figures 2 and 3).

OTHER POTENTIAL BENEFITS OF TAMARIND ACROSS THE WORLD

Fruit pulp

This is used for a variety of domestic and industrial purposes (Kulkarni et al., 1993). It is an important source ingredient in the making of marinades, curry, chutney, vindaloos and Worcestershire sauce (Fararra, 2019). In India, the pulp is eaten raw or sweetened with sugar and also used to make sweet meats mixed with sugar, commonly called tamarind balls. Commercially, it is used as a raw material for the manufacture of numerous industrial food products, such as tamarind juice concentrate, curries, tartaric acid, tamarind pulp powder, sauces, pectin, ice cream, and alcoholic beverages (Lotschert and Beese, 1994). The fruit pulp is also used as a fixative in dyeing when mixed with turmeric

Table 2. Local names of tamarind in various languages in other countries and regions.

Country/region	Language	Vernacular name (s)
Africa		
	Bemba	Mushishi
	Fula	Jammeth, Dabe, Jammi
	Jola	Budahar
	Mandinka	Timbingo, Tombi, Timbimb, Tomi
	Tigrina	Humer
	Wolof	Daharg, Dakhar, Dakah, Nclakhar
Ethiopia	Amharic	Hemor, Humar, Komar, Homor, Tommar
	Tigrina	Arabeb
	Gamo/Oromo	B'roka, Dereho, Racahu, Dindie,
Kenya	Swahili	Mkwaju
	Masai	Ol-masamburai
Malawi	Chewa	Ukwaju, Bwemba
	Yao	Mkwesu
Nigeria		Tsamiya
Somalia	Somali	Hamar
South Africa	Afrikaans	Tamarinde
Sudan	Arabic	Aradeib, Tamarihindi
	Nuba	Kuashi, Shekere, Danufi
Tanzania	Swahili	Ukwaju
Uganda	Teso	Esukuru, Esuguguru (leaves)
	Teso/Karamojong	E/apedyra (fruits)
Zambia	Bemba	Mushishi
	Nyanja	Mwemba
Asia		
China	Sino-Tibetan	Khaam, Mak kham
India	Hindi	Ambli, Amli, Imli
	Sanskrit	Amalika
	Bengali	Tintul, Tintiri, Tetul
	Marathi	Chinci, Chitz, Amli
Indonesia		Asam jawa, Tambaring, Assam
Malaysia		Asam jawa
Philippines	Tagalog	Sampalok
Sri Lanka	Sinhala	Makham
Elsewhere		
	Dutch	Tamarenn
	French	Tamarainer, Tamarin, Tamarindier
	German	Tamarinde
	Italian	Tamarindizio
	Portuguese	Tamarindo
	Spanish	Tamrin, Tamarindo

Source: Bhadoriya et al. (2011).

(*Curcuma longa*) and annatto (*Bixa orellana*), and it also serves to coagulate rubber latex (El-Siddig et al., 2006). It can also be used in the production of ethanol (Menon et al., 2010).

Processed tamarind pulp has several food uses, in some western cuisines, In parts of China, it is used in the manufacture of jams, syrups or chilled drink. In Mexico, tamarind is used in sauces or sold in various snack

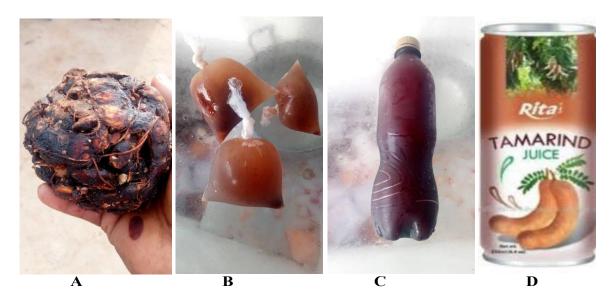


Figure 2. Different locally processed forms of the tamarind fruit pulp; (A) Fruit pulp ball, (B) Drink in polythene package, (C) Drink in plastic bottled container, and (D) Drink in canned container. Source: Field survey (2019).



Figure 3. Different kind of tamarind products across the world; syrup, juices, drinks, jam, candies, Sweets etc. Source: Emmy et al. (2010).

forms: usually dried and salted. In northern Nigeria, tamarind pulp is used with millet grain powder to prepare a traditional pap mostly used in breakfast and commonly eaten with bean cake (Sadiq et al., 2016). In other countries like in Jamaica, Trinidad and Tobago, Grenada,

Colombia, Mexico and other Latin American countries tamarind fruit pulp is made into rolled balls (around 5 cm in diameter) with white granulated sugar and blended with spices to create "tambran" balls. In southern Kenya, the Swahili's use it to garnish their legumes and also

make juices. In Somalia, it is used in cooking to give rice some sour flavour (El-Siddig et al., 2006).

Seeds and pods

Tamarind seed is considered a by-product of the commercial utilization of the fruit pulp. The seed comprises the seed coat or testa (20-30%) and the kernel or endosperm (70-75%) (Shankaracharya, 1998). The seed testa contains tannin, which is used in the preparation of ink and for fixing dyes (Storrs, 1995). In parts of India, the immature seed pod of tamarind is used to flavour foods ranging from meals and snacks with the flowers also pickled and used as side dishes. Tamarind is commercially marketed as a food additive for improving the viscosity and texture of processed foods (Sone and Sato, 1994). El-Siddig et al. (2006), explains that the name "jellose" has been recommended for the seed as it describes both its jelly forming properties and the carbohydrate character of the seed. Tamarind has also been recommended for use as a stabilizer in making ice cream, mayonnaise and cheese and as an ingredient in a number of pharmaceutical products whiles the seed oil is said to be palatable and of culinary quality (Morton, 1987). The oil is used for making varnish to paint idols, and light lamps (Salim et al., 1998). Krithika and Radhai (2007) reported in India, that the seeds are used as cattle feed due to its high protein content. Rosted seeds from the tamarind fruit are ground and used as substitutes for coffee and its flour is commonly used as animal feed for some ruminants and pigs (Khairunnuur et al., 2009). Among the numerous uses of the tamarind seed, Mokashi and Parlikar (2018) concluded that the tamarind seed powder could be a cheap bio-adsorbent for removal of fluoride in water.

Leaves and flowers

These are edible, characterized by a sour taste and are used to make salads, curries, stews and soups, especially in times of food scarcity. Coronel (1991) added that they are used in some Thai food recipes because of their sourness and definite aroma. Whilst Sozolnoki (1985), states that children in Gambia mix leaves with the acid leaves from the fig trees to make chewing gum. The leaves and flowers are useful as a mordant in dyeing. Yellow dye can be derived from the leaves and used to colour wool and can also turn indigo dyed silk to green (Salim et al., 1998).

Benefits derived from other parts of tamarind plant

The wood from the tamarind tree has many uses, in North America, it has been marketed under the name

"Madeira mahogany" and is used in the furniture and timber industry (Bhadoriya et al., 2011). It is valued for making gun-powder and the ash obtained from burning the bark or wood is used to remove hair from animal hides and can also be mixed with the fruit pulp for cleansing and brightening copper and brass vessels (Salim et al., 1998).

Coates-Palgrave (1988) listed a number of these uses from the hardwood of the tamarind tree which include making furniture, mortars, mallets, pestle, rice pounders, ploughs, tent pegs, canoes, side planks for boats, carts shafts and axles and naves of wheels, toys, oil pressers, printing blocks, sugar pressers, tools and tool handles, turnery and others. According to Storrs (1995) during a leather tanning test, tamarind tannins gave harsh and highly coloured leather, which can be used in making heavy soles, suitcases, ink and dyes.

MEDICINAL BENEFITS OF THE TAMARIND PLANT

The tamarind tree plays a major role in traditional medicine in Ghana. Also, it is reported in literature to be used in parts of Asia and Africa in the treatment and prevention of many human ailments and conditions ranging from internal disease conditions to external body wounds. Bhadoriya et al. (2011) concluded that many parts of the tamarind tree have been used in traditional medicines to treat diseases and other ailment conditions. The fruit pulp of tamarind has a laxative action due to the presence of malic and tartaric acid. It helps in relief of abdominal pain and diarrhea. It is digestive, acting on bile secretion and preventing liver disease (Rodriguez-Amado et al., 2016; De Caluwè 2009).

Havinga et al. (2009) widely reviewed in Africa, the ethno-pharmacology of tamarind tree and suggested differences in its use in local medicine. In detailing the medicinal uses of tamarind, Anon (2008) outlines the different medical properties of this tree. These include; anti-microbial, antiviral, anthelminthic (expels worms), antiseptic, sunscreen and astringent. Others are treatment of asthma, cholesterol metabolism disorders, boils, bacterial skin infections, chest pain, colds, colic, conjunctivitis, diabetes, constipation (chronic or acute), dysentery, indigestion, eyes, diarrhoea, inflammation, fever, gallbladder disorders, gastrointestinal disorders, gingivitis, haemorrhoids, jaundice, keratitis, leprosy, liver disorders, nausea and vomiting (pregnancyrelated), sore throat, sores, swelling (joints), sprains, and urinary stones.

Fruit pulp

In Ghana, the fruit when eaten raw helps relieve constipation and other stomach pains. It is a bilious substance and therefore stimulates the activity of the bile and aids in the faster dissolution of foods and fibre to speed up digestion (Razali et al., 2015). Nacoulma (1999) indicated that the tamarind pulp is valued for its medicinal properties, mainly for constipation, bowel obstruction, abdominal pains, pregnancy vomiting and intestinal disorders among many others. The fruit is used traditionally as a laxative, due to its high amounts of tartaric acids, malic and potassium acid. It is commonly used as a poultice in most parts of South Eastern Asia. To overcome constipation the whole tamarind fruit is eaten during breakfast in Madagascar while in Senegal, it is taken in the form of a sweet meat mixed with lime juice or honey, called Bengal by the Wolof people (Bhadoriya et al., 2011).

Tamarind fruit pulp helps with relief of abdominal pain and diarrhea and acts on bile secretion and preventing liver disease (Rodriguez et al., 2016). The tamarind pulp with lemon is used to treat diarrhea, and to relieve constipation and abdominal pains. The pulp in Mali is prepared as drinks, and in Burkina Faso and across rural Fulanis in Nigeria, it is crushed and soaked for half a day in water with a little salt before consumption (Lockett and Grivetti, 2000).

Leaves, roots and seeds

In most parts of Ghana, the leaves of tamarind is mainly added to other plant parts of the tree to treat malaria and also relieves body pains, weakness and the treatment of wounds (Asase et al., 2005). In some parts of Tanzania, the leaves have been known to be laxative whiles the root is used to treat dysentery and ankylostomiasis that is, hookworm (Bhat et al., 1990). The fiber-rich seed from the fruits, aids regulating the intestinal function and lower the level of cholesterol in the blood (Lim et al., 2013). A decoction of tamarind leaves are used in the extraction of Guinea worms and is one of the most important agents to clean wounds caused by Guinea worm infections (Fabiyi et al., 1993). The seed and pericarp contains phenolic antioxidant compound whiles the roots, prepared as an extract, is used in the treatment of stomach ache or abdominal pains, largely in East Africa, and also in Burkina Faso (Kristensen and Balslev, 2003). An extract of the seed pericarp is shown to provide anti-arthritic activity. counteracting bone degeneration degeneration of articular cartilage through the inhibition of the proteolytic enzymes (Sundaram et al., 2015).

Bark

In northern Ghana and among many tribes, the bark of the tree is mixed with the leaves and used to bath sick or weak children, or to treat chicken pox disease. According to Tignokpa et al. (1986) the fresh bark of young stems in Benin, is macerated for 24 h and taken orally as a purgative or for relieve of abdominal pains and in the medicinal plant market in Dakar, Senegal, tamarind bark is mainly sold and used for wound healing purposes.

NUTRITIONAL BENEFITS OF TAMARIND

Currently, consumers choose diets based on the associated nutritional and health benefits instead of taste (Katan and De Roos, 2004). This current study shows a good nutritional potential in tamarind pulps especially in fats, fibre and protein as compared to other test results. This could be attributed to the locations and maturity age of the tamarind trees selected for this study. Vitamin C content was however low in this current study as compared to a higher value obtained in a study in Nigeria by Sadiq et al. (2016). Most studies considered in this review did not test for Vitamin C content in the tamarind pulps. It has earlier been reported by Adekunle and Adenike (2012) that the content of vitamin C in tamarind pulps is very low.

Comparative analysis of the proximate composition of tamarind pulps

Table 3 compares the test results of this present study with others. Test results from Sokoto and Kaduna states in Nigeria by Adekunle and Adenike (2012), Sadiq et al. (2016), and Yusuf et al. (2007). From Sudan by El-Siddig et al. (2006) and from Bangolore in India by Shlini and Siddalinga (2015) were reviewed for various proximate analysis and Vitamin C.

According to an earlier study by El-Siddig et al. (2006) tamarind fruit pulp typically contains 20.6% water, 3.1% protein, 0.4% fat, 70.8% carbohydrates, 3.0% fibre and 2.1% ash. Nonetheless, the proximate composition of the tamarind pulp depends on locality of the plant. Tamarind pulps are a major source of sugars, vitamin C, minerals and exhibit high antioxidant capacity (Ajayi et al., 2006). From this study, proximate values obtained from the locally sourced tamarind pulps were 51.39% for fat/oils, 15.10% for fibre, 16.93% for moisture, 15.03% for protein and 2.71% for ash content while ascorbic acid (Vit. C) content was 2.42 q/100 mg (Table 3).

Due to tamarind's rich nutrient and chemical composition as reported in many studies, it could be adopted as a less expensive alternate protein source that can alleviate protein malnutrition among traditional people living in developing countries (Siddhuraju et al., 1995). Currently, most industries are interested in the development of nutraceutical products from waste products during the processing of tamarind, this includes the seeds, peels, stems, and leaves, generated by the food and agricultural processing industries. These waste products contain considerable quantities of phenols, flavonoids, anthocyanins, vitamin C and carotenoids which can be used as economic sources of natural

O	Fats/oils	Fibre	Moisture	Protein	Ash	Vit. C
Sources of pulps	(%)	(%)	(%)	(%)	(%)	(mg/100 ml)
Present study, Ghana	51.4	15.1	16.9	15.1	2.7	2.4
Sadiq et al. (2016) Nigeria	1.4	0.5	1.9	4.1	1.8	37.6
Shlini and Siddalinga (2015) India	3.7	3.7	4.2	15.0	3.7	N/A
Adekunle and Adenke (2012) Nigeria	1.0	17.5	13.8	7.1	1.5	N/A
Yusuf et al. (2007) Nigeria	10.7	3.6	10.9	20.7	6.8	N/A
El-Siddig et al. (2006) Sudan	0.4	3.0	20.0	3.1	2.1	N/A

Table 3. Proximate composition of tamarind pulp from different studies.

antioxidants for pharmaceutical, cosmetic and food applications (Natukunda et al., 2016; Ferrara 2019).

CONCLUSION

Many authors have recognized the tamarind tree as an underutilized crop with a high potential. The benefits derived from this tree and its products are promising and numerous as evident in this study. The tamarind plant is an all-round, beneficial and nutritious fruit with a great potential. Almost every part of plant (fruit pulp, leaves, bark, root, stems, and seeds) has either some nutritional benefit or medicinal value, and it widely used domestically in Ghana with a number of industrial and commercial applications across the world. This study has exposed in different ways through the collection and reviewing of primary and secondary data, that the tamarind tree comes with enormous benefits, uses and has a great potential.

From this study, it is clear that several authors have reported the use of this fruit tree for both local and industrial purposes. It can also be a very important remedy in parts of the world where malnutrition is a prevalent problem. From its use in local food, drinks and medicinal purposes across northern Ghana and other countries stretching from Africa through Asia and some Latin American countries, it can serve a source of lowcost nutrient supplement. Industrially, the pulp is commonly used in the making of chilled drinks, jams, syrups, juices and other localized products. In its nonfood use potential, this study has shown that tamarind non-fruit parts such as leaves, bark and roots have various uses such as a source of fuelwood, charcoal making, source of timber and as fodder for animals. Based on the high levels of fats and oils in sourced pulps in this study, it could be explored as an alternative source. The potential of the tamarind tree, its products and utilization forms should be further investigated to enhance human nutritional and medicinal needs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 14(9) pp. 295-303, October 2020

DOI: 10.5897/AJFS2020.1913 Article Number: FA1667264991

ISSN: 1996-0794 Copyright ©2020

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Full Length Research Paper

Relationship of household diversity dietary score with, caloric, nutriment adequacy levels and socio-demographic factors, a case of urban poor household members of charity, Constantine, Algeria

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Received 2 February, 2020; Accepted 11 September, 2020

This study was conducted to assess dietary diversity of Algerian urban poor household with household dietary diversity (HDDS) and to study its relationship with nutrient adequacy level and socio-demographic factors. A survey was followed by three 24 h dietary recalls during one year, with household's members of charity in Constantine. Qualitative method has been realised using household dietary diversity score and quantitative method was done by calculating ratio of caloric and nutritional intakes to household needs. The study showed that the mean HDDS was 6.8±0.7 food groups. Animal proteins percentage, caloric adequacy level and adequacy levels of fourteen vitamins and minerals had positive correlation with HDDS. Household with less than six persons and those with children less than five years had a higher HDDS (p<0.035 and p<0.0001, respectively).

Key words: Household dietary diversity (HDDS), poor, caloric, nutriment, adequacy level, constantine, Algeria.

INTRODUCTION

Quality diet encompasses adequate coverage of basic macro and micro nutrient needs and diet variety. Households living in urban areas are more prone to food insecurity, because they source the vast majority of their food through the market. Any decline in household income or increase in food prices can have catastrophic consequences on them. Available studies on food diversity were limited as far as Algeria is concerned.

In an urban setting, where inhabitants are usually

disconnected from the direct production and distribution of food, inability to access food resources can be the most immediate and critical manifestation of the many dimensions of poverty (Battersby and Watson, 2019). Algeria is the largest country on the Mediterranean and the second largest in Africa. The urban population in Algeria is estimated to account for more than 70% of the entire population (United Nations, 2016). Rate of poverty in Algeria was 10.5% in 2012 (World Bank report, 2013).

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Abbreviations: HDDS, Household Dietary Diversity Score; DZD: Algerian dinar; USD: American Dollar.

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Almost 75% of Algeria's poor live in urban areas, doing informal jobs or depend on subsistence agriculture (World Bank, 2016).

The survey MICS4-2012 (Multiple Indicator Cluster Survey) showed that 1% of urban Algerian population suffered from multiple deprivations. The intensity of poverty¹ in urban areas was 34.8% (CNES, 2016). By mid-1992, most of the food subsidies were eliminated except for semolina, flour for bread, milk, and the other goods like energy and public services were adjusted since 1990 (Louali, 2016). In 2011, sugar and oil were exempted from customs duties and value added tax. Since Households, especially those in urban areas, buy purchased food (Girma et al., 2015), poverty lines are commonly drawn because of how much money is required to meet basic food needs. There is therefore a clear correlation between income and food security. Urban households source the vast majority of their food through the market.

Studies have shown that an increase in dietary diversity is associated with socioeconomic status and household energy availability; lack of dietary diversity is a severe problem among poor populations in the developing world. The household dietary diversity score (HDDS) important in urban areas, where one of the important threats to food security is poor diets. HDDS was developed to measure household food access, one of the levels of food security. Previous research has shown dietary diversity is related to food security (Vellema et al., 2016). Thus, food may be available but not accessible to certain households during a given period of time, if they cannot acquire sufficient quantity or diversity of food through these mechanisms (Fanzo, 2017). The present study was conducted with general aim of assessment of dietary access of lowincome urban households, members of charity, from Constantine, Algerian, Eastern city and third largest city with HDDS. Secondary objective was to study the correlation of HDDS with nutritional adequacy levels and socio-economic factors.

MATERIALS AND METHODS

Sampling

The study was carried out with all members of the oldest charitable association of Constantine, urban commune in the northeast of Algeria, capital of the East of Algeria, and the third most populated city in the country. The association exists since 1984. It helps poor households, after verifying their situations.

Study and subjects

A cross-sectional study, repeated 3 times in one year was conducted among the beneficiaries of aid from Constantine.

Households included in the study are declared in need by a specialized organism, after establishing files administrative proving their needs. Households with dependent children without fixed incomes, members of charity, were included in the study.

Semi-structured questionnaire was used for collecting socioeconomic details and information regarding household dietary practices. Questionnaire was followed by three 24 h recalls during one year. The study was conducted with household heads, all of whom were responsible for family food's preparation. They were asked to recall all foods eaten and beverages taken by the whole household on the previous day. Inclusion criteria were that the participants were able to answer the questions and recall their diet from the previous day. Household heads who prepare the food for family were included in the study.

Socioeconomic characteristics

A questionnaire was used to capture information on socioeconomic characteristics of household, such as sex, age, education level, sources of income, habitation, connecting to electricity, gas, water, cleaning up, and possessions. The questionnaire was administered through face-to-face interview with respondents.

24 h recalls

In order to avoid seasonal differences, three 24h recalls dietary were conducted to determine household food consumption. Head of households were asking to recall food consumed for the past 24h, bearing in mind all foods consumed within their homes. The details included descriptions of all foods and beverages consumed, including cooking techniques. The quantities of food consumed were estimated using household measures. The amount of nutriment consumed was then estimated and calculated using CIQUAL (2015) Table. Mean nutriment intake was estimated from the three recalls for each household.

Caloric and nutriment adequacy levels

Caloric or specific nutriment adequacy levels are the ratio of household intakes to their needs. Intakes were assessed based on caloric or specific nutrient content of each food consumed in each household. Needs are the sum of caloric recommended dietary allowance or specific nutriment recommended dietary allowance by sex, age and physical activity of household members.

Household dietary diversity score

Food access was assessed using HDDS (FAO, 2013). Information on dietary diversification was extracted from 24h recalls, where the consumed food items were grouped into twelve food groups: cereals, roots and tubers, vegetables, fruits, legumes, nuts and seeds, meat, fish and other sea food, eggs, milk/milk products, oil/fats, sweets (sugar, honey, sugar cane), condiments and beverages. Total dietary diversity score was calculated by summing the number of food groups consumed at home. The households were divided into four classes, according to eating habits of Algerians:

Low dietary diversity: When households consume, less than six food groups;

Medium dietary diversity: When households consume, six to seven food groups;

High dietary diversity: When households consume, eight or more food groups;

¹ Is the average percentage of deprivation experienced by people in multidimensional poverty, which identifies multiple overlapping deprivations suffered by households in three dimensions: education, health and living standards.

Table 1. Household's social characteristics

	Number	Percentage
Gender		
Female	478	57.6
Male	352	42.4
Total	830	100
Age range [year	rs]	
0-2	7	0.8
3-6	28	3.4
6-9	61	7.3
10-18	276	33.3
19-65	445	53.6
> 65	13	1.6
Total	830	100
Household size	category	
< 6 *	80	52.3
6	31	20.3
> 6	42	27.4
Total	153	100

^{*} is the average household size of urban Algerian household.

Adequate dietary diversity: When households have high dietary diversity.

Analysis

Data was analysed using Epi-Info 3.5.1 (2008). Averages are expressed on mean (±standard deviation). Analyse of variance (ANOVA) was used to compare several means. The correlation between quantitative values was tested with Pearson test. t and z tests were used to compare two averages with N>30 and N<=30, respectively. All values were considered significant at p<0.05.

RESULTS AND DISCUSSION

There are few studies about diet and dietary quality among the Algerian, this study was conducted to enrich the literature with data from this country. Its objective is the evaluation of poor household dietary diversity, and its relationship with caloric, nutriments adequacy levels and socio-demographic factors.

Household characteristics

One hundred and fifty-three households were involved in the study. They include 830 individuals, among which 478 (57.6%) were females and 352 (42.4%) males. The majority (53.6%) of them were between 19 and 65 years old. About 52.3% of the households had less than six household members (Table 1).

The mean household income per person per day was

80 Algerian Dinars (DZD) (±37.1) equivalent to 0.701 USD (±0.325), the first quartile (Q1) have 53 DZD (0.64 USD) per capita per day and the last quartile have 200 DZD (1.74 USD) per capita per day. All respondents were females responsible for household food preparation, they have 47.0 (± 7.7 years) old, majority (46.4%) of theme were found in the age range of 40-50 years. Most (52.9%) are widowed, 23.5% are divorced, and 23.6% are married with sick or prisoned husband. More than a quarter was illiterate (27.5%) and 37.9% had previously completed primary education. The pension was the main (62.7%) source of income (Table 2).

The whole household heads in this study were widowed, divorced or alone women, the vulnerability of female household heads derived from the fact that many women are unemployed or employed in low paying jobs such as domestic work. Furthermore, most of them were also surviving from a single income, which was not adequate for household food sustenance. In both developed and developing countries, the emergence and strengthening of the phenomenon of single-parent families present remarkable analogies, similarity of causes (family breakdown, loss of responsibility for men, gender inequalities), socio-economic markers (restricted access to education, training and skilled jobs) and consequences (marginalisation, exclusion and feminisation of poverty) (Bessis, 1996).

Household dietary diversity score

Mean HDDS was 6.8 [±0.7] food groups. Households

Table 2. Socio-economic characteristics of respondents.

Variable	Number	%
Marital situation		
Widowed	81	52.9
Divorced	36	23.5
Married with	36	23.6
Disable husband	31	20.3
Prisoner husband	5	3.3
Total	153	100
Age range years		
< 30	1	0.65
30-40	26	17.0
40-50	71	46.4
50-60	46	30.1
≥ 60	9	5.9
Total	153	100
Education levels		
Illiterate	42	27.5
Primary	58	37.9
Medium	38	24.8
Secondary	15	98
Total	153	100
Family incomes		
Pension	99	64.7
Housemaid	30	19.6
Cook/preparing traditional dishes	15	9.8
Other	9	5.9
Total	153	100

consumed minimum 2 food groups, and maximum 11. HDDS was mainly between 6 and 8 food groups (Figure 1). The HDDS doesn't vary through the three recalls (Table 3).

About 7.2% households consumed up to five food groups (low dietary diversity), 86.9% consumed six to seven food groups (medium dietary diversity) and 5.9% of households consumed eight or more food groups (high dietary diversity) in their diet. From the twelve food groups, most of the households have consumed less than eight food groups (94.1%). The proportion of households with adequate dietary diversity in this study was therefore 5.9% (Figure 2). According to Petry et al. (2015), the dietary diversity score in all African countries is similar at minimum 6 points. This result was different from the ones found in Cambodia, 4.7 (±1.6) (McDonald et al., 2015); in Ethiopia, 5.2 (±1.9) (Geremew et al., 2019); in South Africa, 5.08 (South Africa, 2015). Also, in Tchad, 5.1 in sedentary households (Bechir et al., 2011); in Sahrawi refugees camp (Tindouf, Algeria), 3.8 (± 1.4) (SandsmarkMorseth et al., 2017); and in Indonesia: 9.1 (Trias et al., 2017). These findings are compared to those in Myanmar, 6 .2 (\pm 1.4) (Victoria, 2014), in Nioro 7 (\pm 1), in Nara 6 (\pm 2) (Welthungerhilfe, 2013) and in South Africa 6.63 (Grobler, 2015). Due to rarity of studies on urban household dietary diversity score based on 12 food groups, this study was compared to the studies above, which most of them were conducted with rural households. Differences in study area, study period, cultural beliefs and dietary patterns may be explained differently between studies.In this study, HDDS varied less by seasons, the same result was found in Hirvonen et al. (2016).

Description of food groups consumed

The details of the proportion of households who consumed various types of HDDS food groups, over one year (mean of three 24 h recalls distributed in one year) are presented in Figure 3. Cereals were the most commonly consumed food group by 100% of the households, followed by sugar (98.7%), milk (98.3%), vegetables (94.1%), oil and fats (89.5%), potatoes

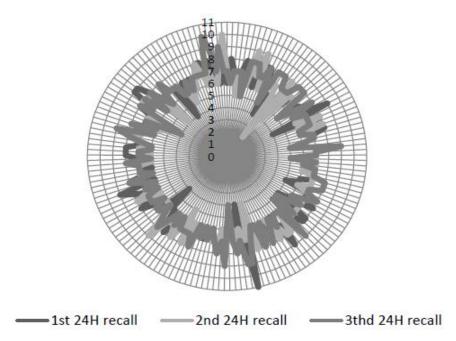


Figure 1. HDDS in the three dietary recalls.

 Table 3. Household dietary diversity score variation.

	HDDS				
	1 st Recall	2 nd Recall	3 rd Recall	Р	
Average value	6.90	6.70	6.93	0.099	
Standard Deviation	1.04	1.02	1.01		
Minimum	4.00	2.00	4.00		
Maximum	11.00	10.00	10.00		

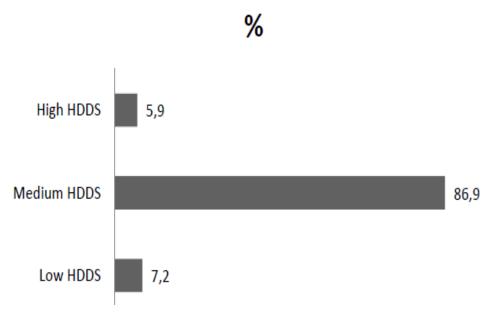


Figure 2. Household's distribution according to HDDS classes.

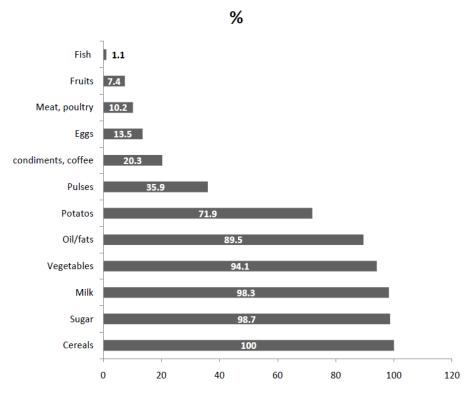


Figure 3. Household's distribution (%) according to HDDS food groups consumed.

(71.9%), pulses (35.9%), condiments and coffee (20.3%), eggs (13.5%), meat and poultry (10.2%), fruits (7.4%) and fish (1.1%).

The most commonly consumed food groups were cereals, sugar, milk and oil. The exemption from customs duties and value added tax for raw materials of sugar and oil and the subvention of milk and flour for bread have made it possible, to maintain these products at an affordable cost for all the Algerian population. On the other hand, these food groups are the staple of Algerian kitchen: cereals are consumed daily in all meals; sugar and milk are also consumed daily at breakfast and at afternoon snack. While vegetables (onion and garlic specially) with oil are presumably used at the beginning of sauce in Algerian cooking methods. Potatoes and pulses were also widely consumed; condiments are used in meals preparations while coffee is a stimulant drink and the most consumed in northern Algeria.

However, fish, meat, egg and fruits were the least consumed food groups. These food groups except eggs were the most expensive in markets. These results are consistent with the study of Padilla (2008), the Southern Mediterranean countries diets, which is mainly vegetarian as only a small proportion of calories is of animal origin; cereals are the basic ingredient and pulses the main protein source. Cereals are staple food in North Africa. The proportion of energy intake from cereals, roots and tubers is about 55% (Tanyeri-Abur, 2015).

Relationship of HDDS with socio-demographic factors

The study showed that HDDS did not vary between education level and marital status of household head. The HDDS was higher at the household with less than six persons and at those with under five years' children (Table 4). Several studies have shown that education was positively correlated to high dietary diversity (McCordic, 2016), schooling impact positively on dietary diversity. However, in this study, the level of education did not show any association with household dietary diversity; the same result was found in (Grobler, 2015; Mayanja et al., 2015). The HDDS was higher at the household with less than six persons compared to those with more than 6; this result is similar to Odusina Olanivi (2014), which found that households with larger sizes (p <0.1) had the propensity of being food insecure and Grobler (2015) which showed that an increase in household size will decrease household dietary diversity.

Relationship of HDDS with number of meals per day

Households consuming four or more meals a day had a higher HDDS compared to those taking three or less meals a day (6.99±6.63 versus 6.63±0.65 p <0.0001). The HDDS was positively correlated with the number of

Table 4. HDDS according to household socio-demographic factors.

Household socio-demographic factors	HDDS				
Household head education level	Mean±standard deviation	Р			
Illiterate	6.71±0.77	0.113			
Primary	6.68±1.03				
Medium	6.94±0.55				
Secondary	7.18±0.71				
Household head marital status					
Married	6.62±0.74	0.0651			
Divorced	6.83±0.67				
Widowed	6.95±0.67				
Household size category					
<6	6.79±0.78	0.035			
=6	7.13±0.74				
>6	6.74±0.70				
Household with under 5 years					
Yes	6,89±0.74	< 0,0001			
No	6.83±0.69				

Table 5. Correlation between HDDS and adequacy level intakes of nutriments.

	Energy	Total carbohydrates	Sugar	Fiber	Lipid	%Vegetable protein	%Animal protein	Vit B ₁
r	0.345	-0.123	0.032	0.004	0.336	-0.257	0.257	0.274
Р	< 0.0001	0.130	0.698	0.961	< 0.0001	0.001	0.001	0.001
	Vit B ₂	Vit B ₃	Vit B ₅	Vit B ₆	Vit B ₉	Vit B ₁₂	Vit A	Vit D
r	0.090	0.099	0.295	0.408	0.192	0.219	0.338	0.232
Р	0.268	0.223	<0.0001	< 0.0001	0.017	0.006	< 0.0001	0.004
	Vit E	Vit C	lodine	Iron	Zinc	Selenium	Magnesium	Calcium
r	0.426	0.268	0.144	0.241	0.313	0.173	0.134	0.197
Р	< 0.0001	0.001	0.076	0.003	< 0.0001	0.033	0.098	0.014

meals taken per day (r= 0.250, p=0.002). HDDS was correlated with the number of meal per day: the more the household takes meals, the more food groups are used and score increase. Food groups consumed increase with the number of meals.

Relationship of HDDS with caloric and nutriments adequacy levels

On twenty-two nutriments studied, HDDS was positively correlated with adequacy level intakes of energy and fourteen nutriments: lipids, vitamin B1, B5, B6, B9, B12, A, D, E, C, iron, zinc, selenium and calcium. HDDS increase with increasing of percentage of adequacy level

of these nutriments. In the other hand, HDDS was positively correlated with animal proteins percentage and negatively correlate with plant proteins percentage (Table 5).

Adequacy levels of nearly 70% of the nutriments studied, caloric and percentage of protein from animal sources were positively correlated with HDDS, as the percent of nutritional adequacy increases, the HDDS increases; which means that more households consume a balanced diet, more their HDDS increases.

According to Swindale and Bilinsky (2006) a more diversified household diet is associated with caloric and protein adequacy, percentage of protein from animal sources, as well as household income. Poor households frequently depend on a diet high in starchy foods with

limited protein and other nutrients (South Africa, 2015). The increase in animal protein share in diet increases the HDDS, while the increase in those of plant origin lowers the HDDS. This may be explained by the fact that HDDS contains four-food groups vector of animal protein: eggs, meal, milk and dairy, fish and seafood; while vegetable, mostly pulses group, represents proteins. Our results are consistent with Kennedy et al. (2010), HDDS increases with increase of food groups that provide micro- and macronutrients. Increased dietary diversity correlates with increased intake of micronutrients such as calcium, vitamin A, and iron with greater consumption of animal source foods, fruits and vegetables, and dairy.

Conclusion

Nutritional mistakes made during the growth period can be permanent and irreversible, hence the importance of nutritional studies on populations at food insecurity risk. The diet of Algerian poor urban household from Constantine was quite monotonous; they have less varied diet. They have poor quality diet, based on plant sources and very few animal sources; they tend to buy less meat, dairy products, fruits and fish. Cereals, milk, sugar, vegetables, oil and potatoes are their food staple. Except vegetables and potatoes, all of them are subsidized foods. Milk and pulses are their main source of protein; while meat, eggs and fish are scarcely consumed, which may reveal a possible under nutrition.

The study findings confirm that less varied diets are found in poor households from Algeria and more diversified household diet is associated with caloric and nutriments adequacy level, with a percentage of protein from animal sources. The score was easy to use, but requires some exactness in definition of universal class HDDS. Greater household access to varied food, nutrition education and development of household's chance to increase incomes should be considered. The nutrition education may include topics that can help increase maternal knowledge and improve practices related to feeding. On the other hand, the amount of fresh diet and animal food sources could increase with distribution vouchers.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Vol. 14(9) pp. 304-312, October 2020

DOI: 10.5897/AJFS2020.1993 Article Number: A57F28465016

ISSN: 1996-0794 Copyright ©2020

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African Journal of Food Science

Full Length Research Paper

Spoilage and microbial quality of crude palm oil from the North-west Region of Cameroon

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Received 9 July, 2020; Accepted 16 September, 2020

In Cameroon, palm oil is extensively used in its crude form for food. The present study was carried out to access stakeholders' perception of spoilage and the microbial quality of crude palm oil in the Northwest Region of Cameroon. A random survey was carried out on 148 stakeholders of the crude palm oil marketing channel about their perception of crude palm oil spoilage. 79 samples were collected from different market sites in the region. Handlings of crude palm oil by the stakeholders were unhygienic and they had poor knowledge of the causes and reasons associated with its spoilage. The microbial contaminants isolated were *Salmonella* sp., *Escherichia coli, Staphylococcus aureus*, yeast, and *Aspergillus niger*, *Aspergillus sulphureus* and *Aspergillus versicolor*. The estimated overall bacterial load ranged from 17.14 × 10⁴ to 36.41 ×10⁴cfu/ml. The bacterial load of crude palm oil samples from each market was far above the minimum acceptable range stipulated by NAFDAC. There is need for these stakeholders to be educated on the health implications and risks associated with palm oil production and post-production handling.

Key words: Crude palm oil, spoilage, microbiological quality, Northwest Region of Cameroon.

INTRODUCTION

Palm oil (PO) has become the most important and most traded vegetable oil globally, with a global production of 76 million tons (Nesaretnam, 2017; USDA, 2019; Gesteiro et al., 2019). Worldwide, PO is extracted by industrial, semi-industrial, or traditional methods, with the non-industrial sector representing about 30% of the total production of crude palm oil (CPO) (Dongho et al., 2016).

Seventy-seven percent of palm oil produced worldwide is consumed as food (Orinola, 2018; Nesaretnam, 2017).

In Africa, Cameroon is the fourth highest producer and is the world's 12th largest producer of PO (Palm oil production in 1000MT, 2019). In Cameroon, the production is done at individual and industrial levels (Nkeze, 2010). The major stakeholders involved in palm

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oil production, distribution and consumption include oil palm growers, palm oil processors wholesalers, retailers, investors, social or development Non-governmental organizations (NGOs) and environmental NGOs (Local and International Stakeholders, 2017) The major regions that produce palm oil in Cameroon are: Southwest, Littoral, Central and Part of Northwest Region ((Nkongho et al., 2014). In the Northwest region of Cameroon, palm oil is produced mostly in the Momo division (Nkongho et al., 2014). Palm oil accounts for about 90% of the edible oil needs of Cameroonians (Ngando et al., 2013).

Cameroonians (80%) consume red palm oil, with an estimated 30% produced by artisanal mills (Nkongho et al., 2014). Palm oil is extensively used in its crude form (crude palm oil) for food purposes because it is cheaper and had been a long-term eating habit (Ngando et al., 2013). It is nutritionally beneficial to being a rich source of some essential fatty acids, vitamin E, and carotenoids. Vitamin E has been extensively known for its nutritional and health benefits, including cholesterol-lowering, anticancer effects, antioxidant activities, and protection against atherosclerosis (Mukherjee and Mitra, 2009; Imoisi et al., 2015; Dongho et al., 2016). Moreover, because of its high provitamin A carotenoid content, Crude Palm oil (CPO) constitutes an important food that could be used to prevent Vitamin A deficiency (Dongho et al., 2016).

Though CPO is guite beneficial for human consumption, studies show that there are still problems related to its safety and quality (Ngando et al., 2013). Firstly, traditional methods of production employed for the extraction of palm oil are done by individuals who have little or no knowledge neither of modern aseptic production techniques nor of the microbiological implication of poor sanitation and storage methods (Okechalu et al., 2011; Madhusudhan et al., 2015). Secondly, change in oil quality during inappropriate storage conditions is still a major concern to public health as different packaging containers used under different storage conditions enhance oil spoilage (Viana et al., 2019). Microbial contamination from the environment, raw materials, equipment used for the processing, storage, and distribution, can also contribute to or enhance deterioration of the oil (Madhusudhan et al., 2015; Okechalu et al., 2011). The microbial quality of CPO plays a critical role in food, animal feed, and traditional medicine, as CPO is frequently used as a major ingredient in their preparations (Dongho et al., 2016). Frying and cooking of the oil can reduce the microbial load to the minimum level.

However, CPO is often consumed raw like in the yellow soup that is prepared cold and form one of the major meals consumed by the local people of the Northwest Region of Cameroon (Grimaldia et al., 2018). This calls for concern as this may cause health problems to consumers (Okechalu et al., 2011). This study was

carried to access the stakeholders' perception of spoilage and the microbial quality of crude palm oil in the Northwest Region of Cameroon.

MATERIALS AND METHODS

Study area and design

In the Northwest region of Cameroon (Figure 1), four market sites that sell crude palm oil were chosen for the study, namely Bamenda food market, Bafut Market, Mbengwi Tad market, and Widikum Market. A cross-sectional and experimental based study design was used, and the study was conducted from December 2017 to June 2018.

Data collection/survey

A survey on the stakeholder perceptions of crude palm oil spoilage was carried out where closed, and open-ended questionnaires were randomly administered to 148 stakeholders of the crude palm oil marketing channel from markets in Bamenda (52 consumers and 28 retailers), Widikum (23 producers), Mbengwi (16 retailers) and Bafut (29 retailers). Information on the respondents' awareness of crude palm oil spoilage, knowledge on the noticeable changes in organoleptic properties observed in spoiled palm oil, possible causes and reasons associated with CPO spoilage, appropriate methods of storage, signs of spoilage, appropriate mixing, knowledge on the microbiological quality of oil and effect of consumption of spoiled oil on human health were obtained.

Sample collection

A total number of 79 crude palm oil samples were collected in containers as bottled by the wholesalers and retailers in Mbengwi (2 wholesalers, 8 retailers), Bamenda (7 wholesalers, 15 retailers), Bafut (9 wholesalers, 10 retailers), and Widikum (28 wholesalers), markets and taken to the laboratory at the Institute of Agricultural Research for Development (IRAD) Bambui for microbiological analysis.

Laboratory analysis

Aerobic plate count of the samples

The aerobic plate count of the stock solution of each of the samples collected was made (Okechalu et al., 2011). This assay was done by dissolving 1 ml of each sample in 9 ml of sterile distilled water already emulsified with 10% v/v of Tween 80 solution, which acts as an emulsifying agent. Three more serial decimal dilutions were made from each stock solution. One milliliter of the last dilutions (10 $^{-3}$) of each sample was inoculated into Plate count agar, EMB agar, Salmonella-Shigella agar, Mannitol Salt agar in duplicates by the pour plate method. All plates were incubated at 37°C for 18-36 h. After incubation, the growing colonies were promptly counted, and the results expressed as colony forming units (CFU/ml).

Identification of bacterial isolates

All bacterial isolates on plates were identified based on biochemical characteristics, as described by Berge (Snealth et al., 1986).

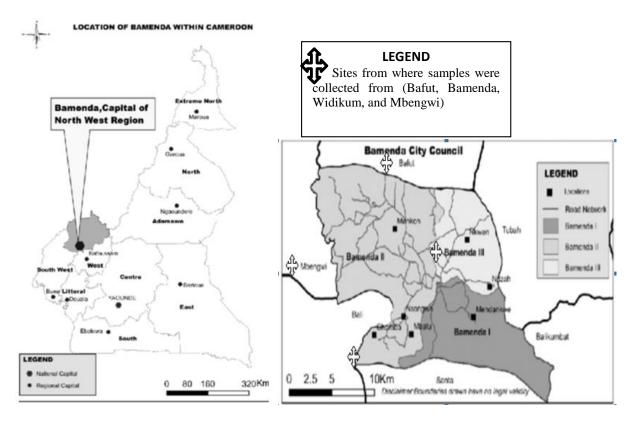


Figure 1. The map of North-West Region of Cameroon including the sites of sample collection. Source: Kimengsi and Fogwe (2017).

Isolation and identification of fungal Isolates

One milliliter of 10⁻³ dilutions of each sample was inoculated into Potato Dextrose Agar in duplicates by the pour plate method and incubated at room temperature for 3-7days. All fungal isolates were identified based on their macroscopic appearance regarding mycology online of Ellis (2006).

Data analysis

The data were analyzed using ANOVA with the SPSS version 22, and total plate count was analyzed after angular transformation. Significant means were separated using the Tukey's test.

RESULTS AND DISCUSSION

From stakeholders

Characteristics of the study population

The demographic characteristics of the 148 stakeholders investigated are shown in Table 1. Most (66.7%) of the respondents below 20 years were Bamenda consumers. The middle age group was mostly in Bamenda and Mbengwi. All of the respondents above 61 years of age

were Bafut retailers. Majority of the Bamenda consumers interviewed were students (66.7%). Most of the Bamenda retailers were doing the palm oil business only as a source of income compared to other stakeholders in other places that were doing many other things as a source of income. A majority of the respondents were female for all the sites except for Widikum producers/wholesalers (Table 1).

Knowledge of oil stakeholders on crude palm oil spoilage in the North-west Region

Out of all stakeholders surveyed, as shown in Table 2, 81% of them knew that palm oil gets bad. Majority (77%) of all the stakeholders knew that 'bad smell' is a sign of CPO spoilage, and meanwhile, just about 48.9, 33.8, and 37.2% in all sites knew the other three signs of CPO spoilage namely bad taste, soapiness, and bad colour respectively are signs of CPO spoilage. Out of the 4 major signs of CPO spoilage surveyed, more stakeholders knew about the bad smell, whereas many did not know about bad taste, soapiness, and bad colour hence a relatively poor knowledge and perception of stakeholders on CPO spoilage signs. Some authors attest

Table 1. Demographic characteristics of respondents for the five sites/stakeholders.

Variable	Bamenda consumers (%)	Bafut retailers (%)	Bamenda retailers (%)	Mbengwi retailers (%)	Widikum producers/ wholesalers (%)	X ²	Р
Gender							
Male	36.7	28.6	8.2	2.0	24.5	16.53	0.002
Female	34.3	15.2	24.2	15.2	15.4		
Age							
<20	66.7	0.0	8.3	8.3	16.7	37.62	0.000
21-30	46.7	15.0	8.3	5.0	25.0		
31-40	28.6	18.4	28.6	16.3	8.2		
41-50	11.8	29.4	29.4	23.5	5.9		
51-60	0.0	51.1	42.9	0.0	0.0		
>61	0.0	0.0	100	0.0	0.0		
Occupation							
Civil servants	44.2	1.8	22.2	0.0	0.0)	67.92	0.000
Farmer	33.3	14.8	7.4	3.7	40.7		
Business	18.5	16.7	38.9	22.2	3.7		
Student	66.7	7.4	3.7	7.9	14.8		
House wife	25.0	50.0	0.0	0.0	25.0		
Others	37.0	33.3	7.4	3.7	18.5		

Where: % = Percent.

to the fact that besides the rancidity of CPO, acidity, bitterness, soapiness and other off flavors may result from the lipolytic activity of microorganisms present in CPO that also leads to deterioration in their chemical quality (Ohimain and Izah, 2013; Okechalu et al., 2011).

Majority of the stakeholders in all the sites also knew the following related to the spoilage of CPO. 75.8% of the stakeholders in all the sites knew that bacteria cause CPO spoilage, 68.2% knew spoilt palm fruits cause CPO spoilage, 89.2% knew the quality of containers used and the activities involved in retailing (53.4%) are reasons associated with CPO spoilage respectively. However, just about 19.6 and 26.5% of all stakeholders in all sites knew that 'air and sunlight' and 'method of extraction' respectively are some reasons associated with CPO spoilage, respectively. In order words, most stakeholders did not know that 'air and sunlight' and 'method of extraction' are reasons associated with CPO spoilage. Furthermore, 56.1 and 66.5% for stakeholders in all sites recorded that 'mixing old oil and new oil' and 'readily eating oil at home' were measures they take to save and reuse bad CPO (Table 2). These results corroborate with the work done by Madhusudhan et al. (2015) in Gondar town Markets, North West Ethiopia, which showed that 36% expose oil to sunlight, 25% did oil measurement using clean jugs (retailing activities) and also as regarding responses to the knowledge of oil seller, 88% of them did not know the cause of oil spoilage.

Symptoms observed by stakeholders after palm oil consumption

Mbengwi retailers (68.8%) were the most who felt nauseated after eating CPO (Table 2). Nevertheless, a small proportion of all stakeholders in all sites felt other symptoms like diarrhea, head-ache, rash, and cold after consuming CPO. Furthermore, from observation, over 90% of stakeholders, especially wholesalers, use storage containers for as long as they can store oil without proper routine cleaning. In Widikum, it was observed that over 90% of wholesalers put their oil in very big drums (100L and more), which have only a small hole or opening that can only take a funnel for oil transfer and so the inner part of these containers are seldom washed or cleaned. Also, 60% of the stakeholders (retailers and wholesalers) who buy CPO from producers/wholesalers in Widikum do not have one supplier, but they buy from different producers and then mix in their containers until it reaches the quantity of oil they desired to buy. They do this without any concern for microbiological implications. As a result of the above local activities or in case of a health problem after CPO consumption, traceability in the food supply chain for CPO may be difficult.

Microbial contamination of CPO samples

The total plate count of the samples ranged from 17.14 x

Table 2. Responses from stakeholders in percentages.

0	Answer	Bamenda	Bafut	Bamenda	Mbengwi retailers	Widikum
Questions for respondents	Options	Consumers (n=52)	Retailers (n=29)	retailers (n=28)	(n=16)	Wholesalers (n=23)
Awareness of crude palm oil spoilage		85	75	85.7	81.2	65
	Bad smell	75	65.5	92,9	81.2	73.9
Knowledge on the noticeable changes	Bad taste	61.5	65.5	50	7.5	26.1
in organoleptic properties observed in palm oil	Bad color soapiness	46.2	24.1	42.9	7.5	26.1
paint on		40.4	6.9	25	6.2	21.7
	Air/sunlight	21.2	17.2	25	18.8	13
	Bacteria	65.4	86.2	75	93.8	75
	Fungi	46.2	34.5	82.1	81.2	56.5
Causes and reasons associated to	Spoilt fruits	69.2	62.1	78.6	81.2	52.2
CPO spoilage	Water	55.8	41.4	82.1	81.2	52.2
	Method extraction	6.2	37.9	50	43.8	17.4
	Storage conditions	73.1	100	100	100	91.3
	Retailing	46.2	69	17.9	6.2	87
	Closed containers	92.3	55.2	89.3	87.5	91.3
How they store oil	Open containers	1.9	0	0	0	0
How they store oil,	New containers	385	3.4	36	0	8.7
	Old containers	40.4	65.5	64.3	43.8	34.8
	1-3months	25.5	24.1	28.6	31.2	78.3
Length of Time before spoilage,	4-12 months	55.8	17.2	46.4	18.8	52.2
Length of Time before sponage,	2-5 years	11.5	13.8	39.4	12.5	21.7
	Re-cook it	61.5	65.5	50	62.5	34.8
Measures taken to safe palm oil	Mix old and new oil Readily eat at home	17.3	86.2	64.3	68.8	87
showing signs of spoilage	Feed animals	28.8	82.2	89.3	93.8	91.3
	Throw it	21.2	13.8	50	0	78.3
		46.1	10	28.6	43.8	21.7

n = number of stakeholders.

 $10^4~cfu/ml$ to $36.41~\times~10^4~cfu/ml$ (Table 4), with samples from Widikum market having the highest estimated overall bacterial load ($36.41~\times~10^4~cfu/ml$); while samples from Bafut market had the least estimated overall bacterial load ($17.14~\times~10^4~cfu/ml$). There is, however, a significant difference in the estimated bacterial load of samples from four markets at a 95% confidence level (P<0.05). This bacterial load of samples obtained from four markets were above the minimum acceptable range for oils as stipulated by Nigerian Agency of Food and Drug Administration and Control (NAFDAC) which states that, the maximum allowable number of organisms in a sample unit of oil should not be more than 2 with acceptable microbiological of $10^4~as$ reported by Okechalu

et al. (2011). This result is similar to the finding of Okogbenin et al. (2014) in Edo State, Nigeria who isolated food pathogens from palm oil and investigated the effect of sterilization on the oil quality. These microbial loads could be due to the habit of mixing old or bad oil with new oil, storage of oil in open and old containers, which are seldom washed and poor knowledge of causes and reason associated with palm oil spoilage (Table 2).

The wholesaler samples had a higher microbial load than retailer samples (Figure 2 and Table 5). The microbial isolates from the palm oil samples from four markets include Staphylococcus aureus, Escherichia coli, Samonella species, for bacteria, and Aspergillus niger,

Aspergillus flavus, Aspergillus candidus, yeast, sulphureus, Aspergillus Aspergillus versicolor and Penicillium species for fungi (Table 6). The presence of E. coli, Salmonella sp, S. aureus, A. niger, A. flavus, A. candidus, A. versicolor and Penicillium sp in samples is in concurrence with the findings of Okechalu et al. (2011) for palm oil obtained from Jos Metropolis, Plateau State, Nigeria. Yeast had the highest frequency of occurrence, while A. niger, A. versicolor, and Penicillium sp had the lowest frequency of occurrence.

The presence of Aspergillus sp was also similar to the findings of Odoh et al. (2016). The presence of E. coli could be indicative of fecal contamination or due to contamination from the environment or water (Okogbenin et al., 2014). The presence of S. aureus corroborates with the work of Madhusudhan et al. (2015) and Okechalu et al. (2011). Other studies and scientific review have identified other microbes like Enterobacter. Bacillus, Proteus, Micrococcus, Trichphyton scohoenleinii. Microsporum canis, Candida, and Mucor as microbes found in palm oil (Enyoh et al., 2018; Izah et al., 2018, Okogbenin et al., 2014). The presence of these microbes could be an indication of unhygienic handling of the oil by the stakeholders since most of them do expose oil to air and sunlight for long periods at their market sales point. Hence, contamination could be from the environment or water in containers used for retailing or cleaning (Table 2). In addition, when the wind blows, dust containing microorganisms can settle on CPO during the operations of packaging, storage, transport, or distribution; hence direct increase of microbial load of CPO contaminated by dust is evident (Ngono et al., 2016). This presence of S. aureus bacteria in the oil samples is a major health concern indicating unhygienic conditions of the oil (Gobena et al., 2018) and this bacterium is known to survive for extended periods in hostile environments and is capable of producing an enterotoxin; which can cause food poisoning (Madhusudhan et al., 2015).

It could also cause gastroenteritis in individuals if they consume the oil raw. It may cause common natural infections like arthritis (Madhusudhan et al., 2015). The presence of multiple microorganisms in CPO and their high loads are of great concern for the health of consumers. Although most Cameroonian CPO-based dishes are prepared hot, some like yellow soup/taro (which is one of the usual meals consumed by the local people of the North west Region of Cameroon) is prepared cold (Grimaldia et al., 2018). Likewise, CPO is sometimes used as an ingredient for traditional medicine. The nature of the microorganisms and microbial load is an essential marker of food quality. A food can have a low microbial load but contain a particular microorganism whose presence could be harmful to the consumer (Table 3) or that could contribute to the deterioration of that food (Ngono et al., 2016). Moreover, some of the isolates are

tolerant of high temperatures (thermo-tolerant); hence these pathogens can spoil oil easily. Some of these microorganisms could have a lipase activity, and so increasing level of microbial load of a sample may lead to high free fatty acid content which in turn leads to a further deterioration of the oil (Frank et al., 2011).

Samples obtained from the Widikum market had the highest microbial load, while samples from the Mbengwi market had the least microbial load (Table 4), and this could be attributed to the fact most of them mixed both 'old or bad oil' with newly made oil before selling (Table 2). The Partial Eta squared value for site 0.071 is slightly lower than that of the stakeholders 0.093, which implies that the relative impact of stakeholders on microbial load is relatively stronger than the impact of the site on microbial load. The R-squared value 0.294 or 29.4% of variance microbial load attributed to site stakeholders. Besides, the significant difference in the microbial load of samples from the four sites from the different stakeholders could be because, at marketing stage, the condition that oils are subjected to is quite different, as most oil marketers display their merchandise outside, under the sun daily (Kolapo and Oladimeji, 2011).

Conclusion

The palm oil microbial quality of samples from wholesalers and retailers at the four marketing sites in the North West Region of Cameroon was poor due to poor post handling practices and limited knowledge on microbial contamination and spoilage. The microbial loads were above the NAFDAC acceptable limit hence the overall poor quality of crude palm oil samples collected from different sites in this region. This could be suggestive of the quality of CPO sold in the markets in this region of the country. The inappropriate handling of CPO by stakeholders is still a call for concern in these sites as this could be a probable route for crosscontamination, presence of microorganisms, and hence poor-quality oil.

Recommendations

Since, all over worldwide, majority of palm oil produced is eaten as food, and in Cameroon it accounts for 90% of edible oil needs, it has become imperative that the highest food safety and quality standards be adopted in this regard. Based on the findings in this work, it is recommended that;

- (i) There should be a strong partnership between the government regulators and the stakeholders (producers/wholesalers, retailers) of the palm oil value chain.
- (ii) Further research could be done to provide urgent

Table 3. Symptoms observed after palm oil consumption.

	Stakeholders with positive response						
	Bamenda consumers (%)	Bafut retailers (%)	Bamenda retailers (%)	Mbengwi retailers (%)	Widikum producers (%)		
Nausea	23.1	10.3	50.0	68.8	8.7		
Diarrhea/abdominal pain	11.5	0.0	21.4	25.0	4.3		
Head ache	0.0	6.9	3.6	31.2	8.7		
Rash	9.6	6.9	3.6	12.5	13.0		
Cold	15.4	3.4	42.9	50.0	21.7		
Total	100	100	100	100	100		

Key: % = percent.

Table 4. Microbial population of palm oil sold in the different sites of the North West Region.

Sites	Total plate count × 10 ⁴ cfu/ml
Bamenda (n=22)	17.91±3.86
Bafut (n=19)	17.14±4.53
Widikum (n=28)	36.41±10.19
Mbengwi (n=10)	24.20±1.09
NAFDAC Limit	1.00

Key: n=number of samples collected from different sites.

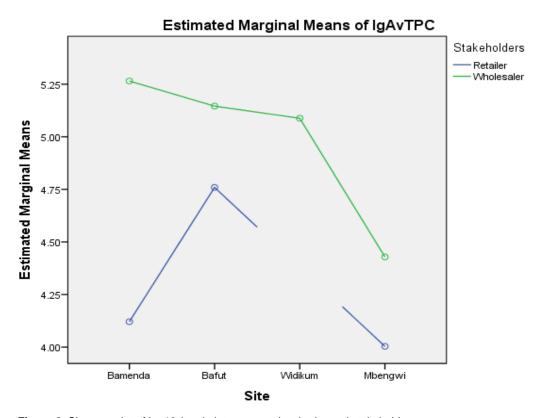


Figure 2. Shows a plot of log10 (total plate count values), site and stakeholders.

Table 5. Microbial population of palm oil for the different stakeholders of the North West Region, expressed as mean ± standard error.

Stakeholders	Total plate count ×10 ⁴ cfu/ml
Retailers	8.791±2.19
Wholesalers	34.50±7.17
NAFDAC Limit	1.00

Table 6. Tentative microbial isolates with frequencies of occurrence of oil samples from different sites in the North west Region.

	Sites								
Microbial isolates —	Baf	ut	Ban	enda	Widi	kum	Mbe	ngwi	
present	n=19		n=22		n=28		n=10		
	WH	RT	WH	RT	WH	RT	WH	RT	
	n=10	n=9	n=7	n=15	n=28	n=0	n=2	n=8	
E. coli	9	3	5	4	12	/	0	3	
Overall	+			+	+		-	ŀ	
Staphilococcusaureus	0	5	1	3	8	/	0	3	
Overall	+			+	+		-	ŀ	
Salmonella sp	7	2	0	0	13	/	0	0	
	+			-	+		•	-	
Yeast	10	7	7	15	28	/	2	8	
	+			+	+		-	ŀ	
A. flavus	0	2	2	8	6	/	0	4	
	+			+	+		-	ŀ	
A. candidus	3	5	2	8	16	/	1	3	
	+			+	+		-	ŀ	
Peniciliumsp	1	0	0	2	2	/	0	2	
	+			+	+		-	ŀ	
A. sulpureus	6	1	2	2	8	/	1	2	
	+			+	+		-	ŀ	
A. versicolor	0	0	0	0	0	/	1	2	
	-			-	-			ŀ	
A. niger	0	0	0	0	1	/	0	0	
	-			-	+			-	

Presence; - = absence; n=number of samples collected from different sites; WH=wholesalers, RT=retailers; /=no data: n=number of samples collected from different sites

alternatives to avoid the consumption of contaminated crude palm oil.

(ii) Further research could be done to urgently provide processing alternatives that include sanitation methods, in order to achieve minimum quality in palm oil for human consumption.

Frequently, there should be a public enlightenment in the form of workshops and seminars through which palm oil stakeholders should be educated on hygienic post handling methods and new methods of improving the quality of palm oil.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This study was supported by the Department of Food Science and Nutrition, College of Technology, the University of Bamenda Cameroon and the Laboratory at the Institute of Agricultural Research for Development (IRAD) Bambui, Cameroon.

ABBREVIATIONS

CPO, Crude Palm oil; NAFDAC, Nigerian Agency for Food and Drug Administration and Control; EMB Agar, Eosin Methylene blue Agar; NGO, Non-governmental organizations; *E. coli, Escherichia coli; S. aureus, Staphilococcus aureus; A. niger, Aspergillus niger; A. sulphureus, Aspergillus sulphureus; A. versicolor, Aspergillus versicolor.*

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