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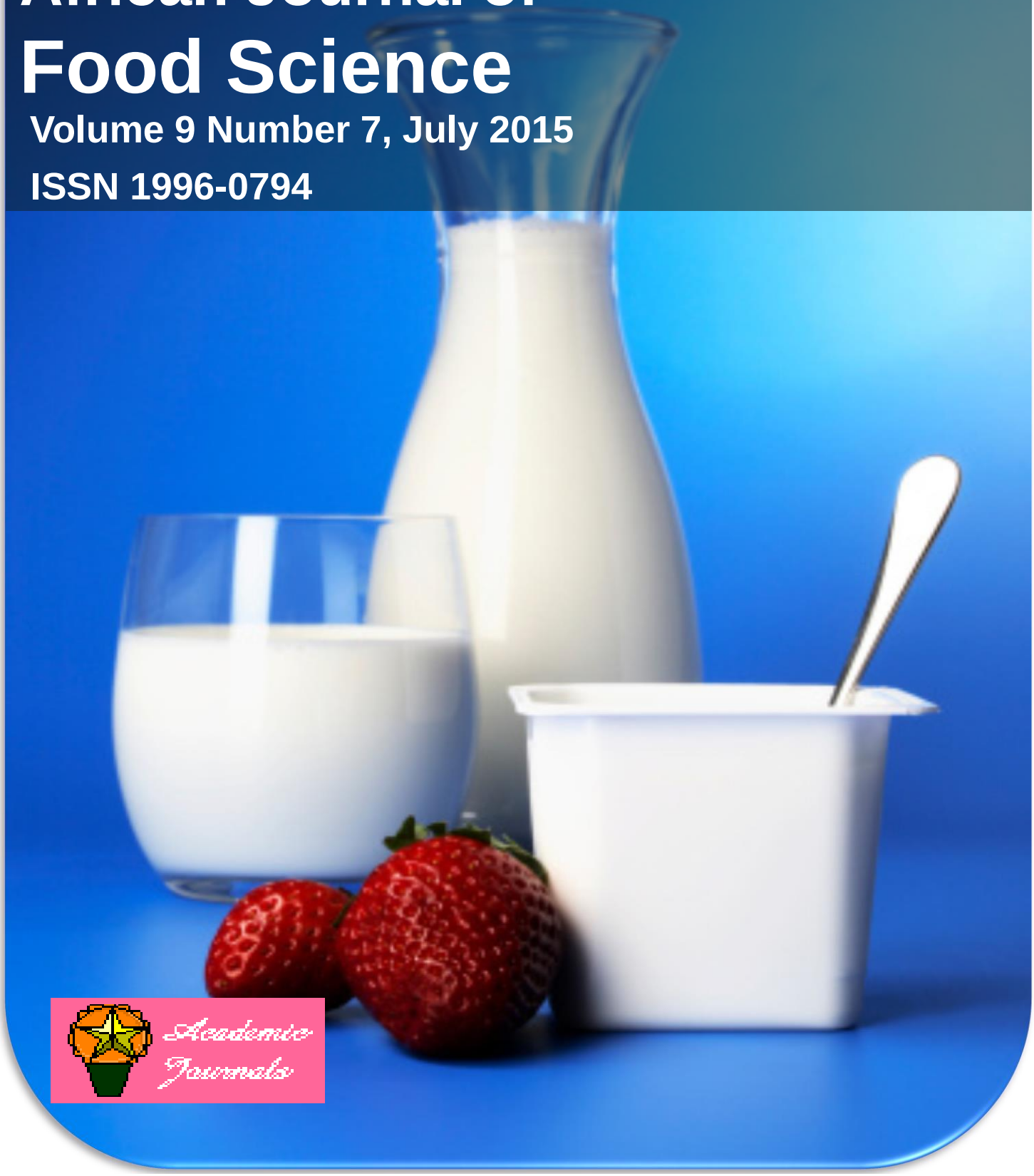

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: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

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Full Length Research Paper

Chemical composition and fatty acid profile of kernels from different Brazilian cashew tree genotypes

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Kernels from nine cashew tree genotypes were characterized with respect to their chemical composition and fatty acids profile, peroxide value and free fatty acids content of the extracted oil. Their proximate composition ranged from 2.69 to 8.37% for moisture, 17.50 to 24.49% for proteins, 39.88 to 47.10% for lipids, 27.14 to 34.94% for total carbohydrate and 2.74 to 4.14% for ash. The amounts of free fatty acids in the oils were smaller than 0.55% for all genotypes tested and no peroxides were detected. Oleic acid was the most abundant fatty acid in kernel oils, ranging from 57.66 to 67.12%. The genotypes that showed higher lipid contents and smaller carbohydrate contents among all genotypes tested were CCP09, EMB51, BRS226 and CCP1001. Those genotypes can be regarded as potential sources of high quality vegetable oil.

Key words: *Anacardium occidentale* L., cashew nut, edible oil, lipids, proximate composition.

INTRODUCTION

Nuts in general are being regarded as healthy foodstuff because their regular consumption has been reported to decrease the risk of coronary heart disease. The health benefits of nuts are usually attributed to their chemical composition, mainly unsaturated fatty acids, and their relation with total and LDL-cholesterol decrease as well as with HDL-cholesterol increase (Mexis and Kontominas, 2009; Yang, 2009; Yang et al., 2009).

There is an increasing interest in trials for the cultivation of high-yielding cashew tree genotypes that are adapted to specific locations and highly resistant to plant diseases. Furthermore, cultivation with a single genotype is not advisable because the orchards without genetic variability can be exterminated by new diseases and pests. However, knowledge from new tree genotypes is limited to aspects such as plant height, nut yield, nut weight, kernel weight and weight of kernel/weight of nut ratio (Barros et al., 2000; Cavalcanti et al., 2000; Paiva et al., 2004). The chemical composition and nutritional aspects of kernels from new genotypes have been mostly disregarded. Furthermore, the nut's chemical composition has been found to vary significantly among different tree genotypes and environmental conditions (Barros et al., 2000; Cavalcanti et al., 2000).

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On the other hand, cashew nut processing involves a number of steps that yields about 40% of broken kernels with lower commercial value than that of the whole kernel. Broken kernels have lipid content ranging from 35.7 to 45.5% (Lima et al., 2012) that can be extracted and commercialized as high quality oil. Moreover, as the technology involved in the oil extraction is simple, it can be used by small producers in order to improve their income. Lafont et al. (2011) studied different methods for oil extraction from cashew kernel and their influence on oil quality. The authors reported that the oil obtained from pressing presented better characteristics than oils obtained from different solvent extractions. Extraction by pressing yield was 68%.

Therefore, the objective of this study was to determine the chemical composition and fatty acid profile of kernels from nine cashew tree genotypes cultivated in Northeast Brazil. There was a special focus on determining which genotypes are more suitable for oil extraction. The cashew kernels' composition should be taken into account for genotype selection along with the advantages and disadvantages for their cultivation.

MATERIALS AND METHODS

Cashew kernels

Nuts from nine cashew tree genotypes (CCP76, CCP09, Embrapa 51, BRS189, BRS226, BRS265, BRS274, BRS275 and CP1001) were obtained from production areas located at Embrapa Tropical Agroindustry's Experimental Station, in Pacajus, Ceará, Northeastern Brazil (4°11'26.2" S and 38°29'50.78" W). All tree genotypes were cultivated under the same conditions. Cashew nuts were processed to obtain the kernels according to the following processing steps:

i) Manual harvesting and apple (peduncle) detaching;
ii) Manual cleaning to eliminate strange materials, such as leaves, stones, sand and pieces of cashew apples;
iii) Sun drying for 2-3 days to reduce the moisture to 7-8%;
iv) Storing in gunny bags piled up on stands in airy cleaned dry place (nuts can be stored up to one year, but for our experiment, they were stored for one month);
v) Heating/steaming for 20 min at 2 Kgf/cm^2 by direct vapour injection;
vi) Cooling at room temperature (~28°C) for 10-12 h to bring the steamed nut to equilibrium with the atmospheric conditions;
vii) Shelling to remove kernels with the help of a hand cum pedal operated cutter (hand built);
viii) Drying of cashew kernels with hot air at 65-70°C for 6-8 h, until 5-8% moisture content;
ix) Manual peeling with knifes to remove the brown testa;
x) Packing in 50 pounds (22.68 kg) aluminium bags and a secondary card board packing, making up the total of 50 pounds net weight. In our experiment, 5 kg of kernels from every genotypes were packed in high density polyethylene bags and transported to the laboratory for analysis.

Proximate composition of kernels

Cashew kernels were ground and analysed for moisture (method 925.40), ash (method 950.49), lipid (method 948.22) and protein (method 950.48) contents (AOAC, 1997). Total carbohydrate content was estimated by the difference from other components using the modified formula from AOAC, since water and alcohol were not considered: 100 - (weight in grams [protein + lipids + ash] in 100 g of food). Results were reported in dry weight.

Chemical analysis of kernel oils

Oil was extracted from dried and triturated cashew kernels through cold press extraction with a home built hydraulic press and filtered in qualitative paper (ø = 12.5 cm). Oils were analysed for free fatty acids (method Ca 5a-40) and peroxide values by iodometric titration with 0.01 N sodium thiosulfate solution (method Cd8-53) (AOCS, 1988).

Fatty acid profile of kernel oils

Fatty acids obtained from the oils, as described below, were converted to their methyl esters (FAMEs) and following the method described by Hartmann and Lago (1973). FAMEs were determined by gas chromatography using a GC CP3380 (Varian) equipped with a flame ionization detector (FID), a split/splitless capillary inlet system and a SP2560 (100% bis-cyanopropyl polysiloxane; Supelco Bellefonte, USA) column with dimensions of 100 m x 0.25 mm id x 0.20 µm df. The carrier gas (hydrogen) flow rate was 1.5 mL/min. The temperatures of the injection port and detector were 220 and 230°C, respectively. The GC oven was programmed as follows: column initial temperature of 80°C, increasing at the rate of 11.0°C/min to 180°C, then at 5.0°C/min to 220°C and held for 9 min. FAMEs were identified by comparing the retention time of samples and the appropriate fatty acids methyl esters standards purchased from Supelco (Bellefonte, USA). Each fatty acid was expressed in percentages of relative area, obtained by area normalization (fatty acid peak area relative to the chromatogram's total area).

Statistical analysis

Results were submitted to analysis of variance (ANOVA) and Tukey test was applied (α=0.05) for the comparison of mean values. Proximate composition and chemical analysis in kernel oils were performed in three repetitions and fatty acids profile in four repetitions. Statistical analyses were performed using the SAS statistical program for Windows System (SAS, 2009).

RESULTS AND DISCUSSION

Chemical characteristics of kernels and oils

The chemical characteristics of kernels from different cashew tree genotypes and the free fatty acids values of the extracted oils are shown in Table 1. Peroxides are not reported since they were not detected for oils from all genotypes tested.

Kernel moisture varied from 2.69 to 8.37%, which are acceptable values for the commercialization of cashew kernels. Actually, drying to appropriate moisture content is an important factor to ensure good kernel quality. Moisture is not an intrinsic characteristic of the cashew genotype, although it can influence the kernel's characteristics. Low moisture (< 2%) renders kernels more

Table 1. Chemical composition (% dry weigh) of kernels from different cashew tree genotypes and acid values (g/100 g) of cold pressed oils (mean ± SD, n=3).

Genotype	Moisture	Ash	Lipid	Protein	Carbohydrate	Free fatty acids, as oleic
CCP76	7.04^{cd} ± 0.46	2.98^{b} ± 0.03	43.34^{bcd} ± 0.41	21.83^{c} ± 0.63	31.86^{cd} ± 1.03	0.07^{d} ± 0.01
CCP09	7.62^{abc} ± 0.47	4.14^{a} ± 0.31	45.40^{ab} ± 0.14	23.29^{b} ± 0.66	29.78^{e} ± 1.01	0.31^{c} ± 0.02
EMB 51	6.71^{d} ± 0.16	2.74^{b} ± 0.04	45.33^{ab} ± 0.99	22.15^{c} ± 0.25	29.78^{e} ± 1.18	0.30^{c} ± 0.04
BRS189	7.50^{bc} ± 0.10	2.86^{b} ± 0.26	42.43^{d} ± 1.43	19.77^{d} ± 0.10	34.94^{a} ± 1.37	0.34^{c} ± 0.01
BRS226	7.73^{abc} ± 0.09	2.75^{b} ± 0.04	45.21^{ab} ± 0.94	17.50^{e} ± 0.02	34.54^{ab} ± 1.00	0.32^{c} ± 0.02
BRS265	6.54^{d} ± 0.17	2.85^{b} ± 0.23	42.86^{cd} ± 0.33	22.15^{c} ± 0.16	32.14^{bcd} ± 0.50	0.29^{c} ± 0.01
BRS274	8.37^{a} ± 0.17	2.87^{b} ± 0.22	44.80^{bc} ± 1.08	21.64^{c} ± 0.13	30.69^{cd} ± 1.19	1.34^{a} ± 0.05
BRS275	7.94^{ab} ± 0.16	2.86^{b} ± 0.06	39.88^{e} ± 0.57	24.49^{a} ± 0.30	32.77^{abc} ± 0.32	0.55^{b} ± 0.02
CP1001	2.69^{e} ± 0.21	3.14^{b} ± 0.05	47.10^{a} ± 0.48	22.61^{bc} ± 0.38	27.14^{e} ± 0.87	0.29^{c} ± 0.00
Mean	6.91 ± 1.63	3.02 ± 0.44	44.04 ± 2.16	21.71 ± 1.97	31.23 ± 2.84	0.42 ± 035

In each row, means with the same letter are not significantly different (Tukey, α=0.05).

breakable, whereas an excess of moisture (> 10%) can be a problem for storage, making kernels elastic and not sensory acceptable (Lima et al., 2000; Cárcel et al., 2012).

Minor differences (α=0.05) were observed in ash content among kernels from different cashew tree genotypes, ranging from 2.74 to 4.14%. Kernels showed lipid content ranging from 39.88 to 47.10%, protein content ranging from 17.50 to 24.49%, and total carbohydrate content ranging from 27.14 to 34.94%. Results are in good agreement with those of USDA National Nutrient Database for Standard Reference (2010), regarding cashew kernel proximate composition. Many reports were found on cashew kernel proximate composition, but authors usually report only the geographic production area. Kosoko et al. (2009) reported lipid content ranging from 44.58 to 47.01% in cashew kernels from Nigeria, Oladimeji and Kolapo (2008) reported lipid content of 42.1%, protein of 19.5% and carbohydrate of 23.8%, also for kernels from Nigeria.

Considering that oil extraction from kernels is one of the goals of this project, high oil content is an important kernel characteristic. The cashew tree genotypes that produced the highest lipids levels were CCP09, EMB51, BRS226 and CCP1001.

High total carbohydrate content can be a technological problem for oil extraction, since during extraction by mechanical pressing the material's temperature is raised significantly, which can lead to starch gelatinization. During continuous pressing starch gelatinization promotes the formation of a film which impairs the oil flow. Even if the oil is cold pressed, cooking before pressing generally improves oil yield and can also result in starch gelatinization (Singh et al., 2002; Venter et al., 2007). The cashew tree genotypes that produced the smallest total carbohydrate content were CCP09, EMB51 and CP1001. Yang (2009) reported that cashew nuts possess the maximum total carbohydrate content (>20%) among various nuts tested (almonds, brazil nuts, hazelnuts, macadamia, peanuts, pecans, pine nuts, pistachios and walnuts).

Free fatty acid amounts found in the extracted oils were smaller than 0.55% for all cashew kernels studied and peroxides were not detected. Those results are within the Codex General Standard for Fats and Oils that state the cold pressed oil maximum values of 4.0 mg KOH/g oil for acid value (2.01% free fatty acids, as oleic) and 15meq/kg oil for peroxide values (FAO/WHO, 2001).

Adeigbe et al. (2015) on a review of cashew production in Nigeria, reported existence of narrow genetic base within Nigeria cashew germplasm and within geographic cashew variety groups in Tanzania and India. The existence and exploration of different genetic materials from Brazil can broaden the genetic base in those countries.

Fatty acid profile of kernel oils

Fatty acid compositions of the extracted oils are shown in Table 2. The fatty acids profile was constituted by 12 fatty acids, and significant (α=0.05) differences among the kernel oils from different cashew tree genotypes were observed for palmitic, stearic, oleic and linoleic acids. Oleic acid was the most abundant one in all kernel oils ranging from 57.66 to 67.12%. Linoleic acid was the second in order of importance, ranging from 17.57 to 21.95%. As to the remaining fatty acids, only palmitic, ranging from 7.31 to 9.70%, and stearic acids, ranging from 6.33 to 9.32 %, showed considerable amounts.

Considering the importance of essential fatty acids in the human diet, the CCP76, CCP09, BRS226, BRS265, BRS274, BRS275 and BRS189 cashew tree genotypes showed higher linoleic acid content in kernel oil than the other genotypes. Linoleic acid is known as a dietary essential fatty acid because it cannot be synthesized by humans.

Unsaturated fatty acids, due to the high content of oleic

Table 2. Fatty acid composition (expressed as percentage of total fatty acid) of the oil extracted from kernels of different cashew tree genotypes (means ± SD, n=4).

Fatty acid	CCP09	EMB51	CCP76	BRS189	BRS226	BRS265	BRS274	BRS275	CP1001	Mean
Myristic (C14:0)	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.02^{a} ± 0.01	ND	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.37^{a} ± 0.04	-	0.02^{a} ± 0.00	0.05 ± 0.01
Palmitic (C16:0)	8.38^{ab} ± 0.94	7.31^{b} ± 0.11	8.28^{ab} ± 0.16	9.70^{a} ± 0.57	7.93^{b} ± 0.23	8.23^{ab} ± 0.11	8.75^{ab} ± 0.10	8.20^{ab} ± 0.11	8.58^{ab} ± 0.05	8.37 ± 0.65
Palmitoleic (C16:1)	0.26^{a} ± 0.08	0.28^{a} ± 0.02	0.27^{a} ± 0.01	0.43^{a} ± 0.24	0.25^{a} ± 0.01	0.28^{a} ± 0.02	0.26^{a} ± 0.03	0.24^{a} ± 0.00	0.30^{a} ± 0.02	0.28 ± 0.06
Margaric (C17:0)	0.13^{a} ± 0.02	0.12^{a} ± 0.01	0.12^{a} ± 0.02	0.26^{a} ± 0.02	0.08^{a} ± 0.05	0.08^{a} ± 0.05	0.13^{a} ± 0.01	0.12^{a} ± 0.01	0.17^{a} ± 0.01	0.13 ± 0.05
Stearic (C18:0)	8.67^{a} ± 0.80	6.33^{b} ± 0.28	9.32^{a} ± 0.20	8.50^{a} ± 1.47	7.85^{ab} ± 0.41	8.53^{a} ± 1.16	8.62^{a} ± 0.84	8.68^{a} ± 0.05	7.47^{ab} ± 0.01	8.22 ± 0.88
Oleic (C18:1)	60.63^{bc} ± 2.02	67.12^{a} ± 0.21	58.81^{c} ± 0.10	57.66^{c} ± 5.02	62.03^{abc} ± 0.20	61.96^{abc} ± 0.04	60.90^{bc} ± 1.26	62.13^{abc} ± 0.03	64.73^{ab} ± 0.00	61.77 ± 2.86
Linoleic (C18:2)	20.83^{ab} ± 1.99	17.57^{b} ± 0.76	21.95^{a} ± 0.20	18.61^{ab} ± 1.52	20.62^{ab} ± 0.71	19.73^{ab} ± 1.66	19.44^{ab} ± 1.83	19.43^{ab} ± 0.08	17.79^{b} ± 0.03	19.55 ± 1.44
Linolenic (C18:3)	0.20^{a} ± 0.06	0.19^{a} ± 0.01	0.17^{a} ± 0.04	0.87^{a} ± 0.02	0.19^{a} ± 0.02	0.11^{a} ± 0.04	0.22a ± 0.02	0.20^{a} ± 0.00	0.11^{a} ± 0.00	0.71 ± 0.54
Arachidic (C20:0)	0.53^{a} ± 0.02	0.64^{a} ± 0.02	0.52^{a} ± 0.01	2.15^{a} ± 0.06	0.50^{a} ± 0.04	0.54^{a} ± 0.08	0.58^{a} ± 0.02	0.53^{a} ± 0.01	0.43^{a} ± 0.00	0.34 ± 0.26
Gondoic (C20:1)	0.18^{a} ± 0.08	0.26^{a} ± 0.04	0.35^{a} ± 0.03	1.02^{a} ± 0.01	0.33^{a} ± 0.09	0.33^{a} ± 0.06	0.20^{a} ± 0.01	0.21^{a} ± 0.01	0.20^{a} ± 0.01	0.25 ± 0.24
Behenic (C22:00)	0.10^{a} ± 0.01	0.11^{a} ± 0.02	0.09^{a} ± 0.01	0.51^{a} ± 0.02	0.17^{a} ±0.01	0.10^{a} ± 0.02	0.46^{a} ± 0.06	0.15^{a} ± 0.05	0.08^{a} ± 0.00	0.20 ± 0.17
Lignoceric (24:00)	0.09^{a} ± 0.01	0.07^{a} ± 0.01	0.10^{a} ± 0.01	0.30^{a} ± 0.03	0.06^{a} ± 0.01	0.10^{a} ± 0.02	0.08^{a} ± 0.01	0.14^{a} ± 0.04	-	0.10 ± 0.08
SFA	17.90^{ab} ± 0.19	14.58^{a} ± 0.72	18.44^{ab} ± 0.23	21.43^{b} ± 0.46	16.59^{ab} ± 0.72	17.59^{ab} ± 1.50	18.99^{ab} ± 0.29	17.80^{ab} ± 0.06	16.74^{ab} ± 0.04	17.78 ± 1.87
USFA	82.09^{ab} ± 0.19	85.41^{a} ± 0.73	81.56^{ab} ± 0.23	78.58^{b} ± 0.46	83.42^{ab} ± 0.72	82.40^{ab} ± 1.50	81.01^{ab} ± 0.29	82.21^{ab} ± 0.07	83.12^{ab} ± 0.04	82.20 ± 1.86

SFA = Saturated fatty acids, USFA = unsaturated fatty acids. In each row, means with the same letter are not significantly different (Tukey, α=0.05).

and linoleic acid, were the main component of the total oil that was extracted from the genotypes studied, representing values around 82% of total oil. Unsaturated fatty acids have been associated with beneficial effects on health promotion and disease prevention (Mexis and Kontominas, 2009; Yang, 2009; Yang et al., 2009).

Conclusions

Results show that most kernels' characteristics measured were significantly affected by cashew tree genotypes, but regardless the differences, kernels can be considered good sources of proteins (21.71%), lipid (44.04%) and total carbohydrate (31.23%) for the human diet. The CCP09, EMB51 and CP1001 genotypes showed higher lipid content and smaller total carbohydrate content than the other genotypes tested which are desirable characteristics for oil production.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Effect of drying temperature on the nutritional quality of *Moringa oleifera* leaves

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Moringa oleifera **leaves are generally consumed in the dry powdered form. Therefore, this research was carried out to investigate the effect of temperature on the nutritional quality of** ***M. oleifera*** **leaves powder. The leaves were dried under the shade at 30°C for two weeks and in the oven at temperature range from 40 to 70°C for 2 h. The results show that temperature affects nutrient composition of the leaf powder. As the drying temperature increased, crude protein decreased significantly (p≤0.05). The samples from shade drying had protein content value of 28.44 g/100 g, while samples those dried at 70°C had 19.89 g/100 g. Similar trends were observed for fat content which was 2.69 g/100 g for shade drying and 2.46 g/100 g at 70°C. The ash, fibre and carbohydrate contents of leaf powder increased with drying temperature. Samples from shade drying had 4.55, 16.33, and 32.75 g/100g for ash, fibre and carbohydrate respectively. However, the amounts of ash, fibre and carbohydrate increased significantly to 5.20, 17.66 and 52.30 g/100 g, respectively when dried at 70°C. The beta-carotene which is the precursor of vitamin A significantly (p<0.05) decreased from 5,220.20 mg/100 g in shade dried leaves to 4,946.20 mg/100 g in oven dried at 70 °C. Vitamin C content decreased slightly from 27.39 mg/100 g for shade dried to 25.70 mg/100 g, dried at 70°C. The minerals investigated generally showed significant increase with temperature. In general, the nutritional parameters of** ***M. oleifera*** **leaves dried in the shade varied closely with those dried in the oven at 40 and 50°C for 2 h.**

Key words: *Moringa oleifera*, drying, temperature effect, nutritional quality.

INTRODUCTION

Moringa oleifera (*Moringa pterygosperma*) is the most widely cultivated specie of the genus Moringa (Fuglie, 2001). Other English common names are benzolive tree and West Indian ben. It is also known as drumstick tree, from the appearance of long, slender, triangular seed pods (Mishra et al., 2012; Jed and Fahey, 2005). The tree is slender and with drooping branches that grow to approximately 10 m in height. In cultivation, it is often cut-back annually to 1-2 m and is allowed to re-grow, so the pods and leaves remain within arm's reach (Fuglie,

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2001). *M. oleiferatree* is rich in iron, potassium, calcium, zinc, magnesium, and produces man with useful vitamins, and vitamin A, four times the amount in carrots (Willis, 2003). The beta-carotene found in *M. oleifera* is a precursor of retinol.

Fahey (2005) considered *M. oleifera* leaves to contain significant source of essential nutrients such as Beta-carotene, Vitamin C, protein, iron, potassium, calcium and phosphorus and are commonly dried and crushed into a powder and stored without refrigeration for months without loss of nutritional values). The leaves contain 7.5 mg water, 6.7 mg protein, 1.7 mg fat, 14.3 mg total carbohydrate, 0.9 mg Fibre, 2.0 mg ash, 440 mg Calcium, 70 mg Phosphorous, 7 mg Iron, 110 mg Copper, 5.1 mg/l, 11.300 mg vitamin A, 120 mg vitamin B, 0.8 mg nicotinic acid, 220 mg ascorbic acid and 7.4 mg tocopherol per100 mg. Estrogenic substances, including the anti-tumor compound, beta-sitosterol, and a pectine-sterase are also reported. Leave amino acid include 6.0 mg arginine, 2.0 mg methionine, 4.9 mg threonine, 9.3 mg leucine, 6.3 mg isoleucine and 7.1 mg valine (Olushola, 2006).

Almost every part of *M. oleifera* is of food value, no part of the plant is useless as both human beings and animals have one thing or the other to gain from the plant (Adeniyi, 2007). Foliage is eaten as green in salad, in vegetable curries, as pickles and for seasoning. The seeds yield 38-40% of non-drying oil, known as Ben oil, and is used in arts for lubricating machines and other delicate machinery. The oil is clear and odourless, never becoming rancid. Consequently, it is edible and is used in the manufacture of perfumes and hairdressings. Wood yields blue dye (Duke, 1982).

The roots are shredded and used as a condiment; however, it contains the alkaloid called spirochim, a potentially fatal nerve paralyzing agent. The flowers can be processed and used for the production of pesticides because they contain certain natural chemical for which insects and other pest cannot withstand (Adeniyi, 2007).

M. oleifera leaves have been used successfully in its dried state or powdered form to augment and make delicious meals and porridge diets for pregnant expectant mothers, nursing mothers, infants and young children, as well as adults of all age groups. In Africa, nursing mothers have been shown statistically to produce far more milk when they add *M. oleifera* leaves to their daily diets and malnourished children have made significant weight gains when nursing mothers and care-givers add them to their diets as well (Duke, 1982).

For pregnant and breast feeding-nursing women, the leaves can do much to preserve the mothers' health and pass on strength to the foetus or nursed child. 100g portion of the leaves could provide a woman with over one-third of her daily requirement of calcium and gives her important quantities of iron, protein, copper, sulphur, B-vitamin (Price, 2000). The leaves are used in areas of nutrition, water purification; livestock feed, vegetable dyes, herbal medicine and oil production (Adeniyi, 2007).

For purposes of preservation, packaging, transportation and distribution, *M. oleifera* leaves are most commonly available and consumed in the dried form. It is generally believed and advised that *M. oleifera* leaves dried under shade is the way to preserve nutrient content (Olushola, 2006). This method is generally adopted by local processors of the material. However, with the renewed campaign and interest in *M. oleifera* consumption, it may become increasingly difficult to produce sufficient leaf powder by drying naturally under the shade to meet the growing demand. Therefore, it is needed to conduct a laboratory study on the effect of drying temperature on the nutrient content of moringa leaves. This study will be useful when considering industrial drying of the leaves for large scale production of *M. oleifera* leaf powder. It will serve as a guide to industrialist to select optimum drying temperature to maximize nutrient retention.

The objective of this work was therefore to evaluate the effect of drying temperatures on the nutritional value of *M. oleifera* leaves.

MATERIALS AND METHODS

Sample preparation

The preparation of the oven dried *M. oleifera* leaves and room dried *M. oleifera* leaves were carried. The stalks were cut from the tree and brought to the laboratory, where the leaves were removed from the stalks. The leaves were immersed in a large volume of clean potable water and shaken in order to remove dirt and impurities on the leaf surfaces. The washed leaves were spread out on racks for 20 minto drain out water. Four portions of the leaves were then dried successively in an electric oven for 2 h at 40, 50, 60 and 70°C. Another portion which served as control was dried for two weeks under the shade. The dried leaves were milled using kitchen blender, packaged in a translucent or coloured polythene bag and kept in a plastic container with cover and stored at room temperature of 30 ± 2°C for chemical analysis.

Chemical analysis

The crude protein and water soluble and insoluble ash contents were determined using the method of AOAC (2000). The moisture, fat and crude fibre contents were determined using the method described by AOAC (2005), while carbohydrate was by difference (Ihekoronye and Ngoddy 1985). Vitamin C content was determined titrimetrically using the method of British pharmacopoeia (2000). While total carotenoid content was determined using the method described by Akpapunam and Ibiama (1985). The mineral composition was determined using the atomic absorption spectrophotometer (UNICAM 960 series) as described by AOAC (2005).

Statistical analysis

Data obtained was analyzed by analysis of variance (ANOVA) using a split-split plot model according to the methods of Gomez and Gomez (1984). When significant ($P<0.05$), Duncan's new multiple range test (Duncan, 1975) was used to separate means.

Table 1. Effect of drying temperature on proximate composition (%) of *M. oleifera* leaves.

Parameters	Fresh leaves	Samples (dried leave powder)					LSD
		A	B	C	D	E	
		(30±2°C)	40°C	50°C	60°C	70°C	
Moisture	80.04^{a}±0.03	15.01^{b}±0.01	12.60^{c}±0.06	10.33^{d}±0.33	5.00^{e}±0.00	2.50^{f}±0.00	0.43
Fat	1.52^{a}±0.01	2.67^{b}±0.08	2.71^{b}±0.01	2.65^{c}±0.03	2.47^{b}±0.03	2.46^{b}±0.00	0.13
Ash	2.00^{a}±0.00	4.55^{b}±0.06	4.60^{b}±0.06	4.70^{b}±.0.06	5.22^{c}±0.00	5.20^{c}±0.00	0.13
Fibre	3.51^{a}±0.01	16.33^{b}±0.33	17.46^{c}±0.03	17.40^{c}±0.06	17.61^{c}±0.01	17.66^{c}±0.01	0.43
Protein	10.93^{a}±0.02	28.44^{b}±0.01	26.24^{c}±0.01	21.18^{d}±0.33	20.75^{e}±0.03	19.89^{f}±0.00	0.42
Carbohydrate	6.42^{a}±0.00	32.57^{b}±0.21	36.44^{c}±0.01	46.34^{d}±0.33	49.08^{e}±0.00	52.30^{f}±0.00	0.50

Values are means of triplicate determinations. Means with different superscripts within the same column are significantly different from each other (p>0.05). A = Shade dried, B = oven dried at 40°C, C = oven dried at 50°C, D = oven dried at 60°C, E = oven dried at 70°C. lime spray drying (LSD).

RESULTS AND DISCUSSION

Effect of drying temperature on proximate composition

The proximate composition of fresh, shade dried and oven dried *M. oleifera* leaves samples is presented in Table 1. The results shows that all the parameters significantly (p<0.05) changed when the leaves were dried either under the shade or in oven.

As expected, Table 1 shows that the moisture content of the leaves decreased with increase in drying temperature. The moisture content of the wet leaves was higher than samples dried in the shade and at 40 to 70°C. Moisture content decreased significantly (p<0.05) from 80.0% for fresh leaves to 15.00% for shade dried leaves. This further decreased significantly (p<0.05) in oven dried samples as the temperatures increased. Sample B showed the highest moisture content (12.50%) while C, D and E had 10.00, 5.00 and 2.50% respectively.

Crude protein of fresh leaves was 10.93 g/100 g, which is more than double that of cow's milk (3.20 g/100 g), and was about three times the amount obtainable in spinach (Gernah and Sengev, 2011). As the drying temperature increased from shade drying (30±2°C) to 70°C, crude protein decreased significantly (p<0.05). While samples dried in the shade had protein content value of 28.44 g/100 g, samples dried at 40°C had 26.24 g/100 g and that dried at 70°C had the value of 19.89 g/100 g. Similar trends were observed for fat content which had values of 2.69 g/100 g at 30°C, 2.46 g/100 g at 40°C and 2.46 g/100 g at 70^{o}C. As drying temperature increased there was corresponding increase in protein denaturalization resulting to significant decrease in both protein and fat. These results are in agreement with the report of Gernah and Sengev (2011) and Sengev et al. (2013). According to the authors and the report of Muller (1988), during pregnancy and breastfeeding a woman should consume about 38.00 g of protein daily. A meal of 100 g fresh and dry leaves will provide a good percentage of her protein requirements.

Table 1 also shows that the ash, fibre and carbohydrate content increased significantly (p<0.05) with drying temperature of *M. oleifera* leaves. While samples dried in the shade (30±2°C) had ash content of 4.55 g/100 g, fibre content of 16.33 g/100 g and carbohydrate content of 32.75 g/100 g, the values increased significantly to 5.20, 17.66 and 52.30 g/100 g respectively when the leaves were dried at 70°C. This is in agreement with what was reported by Gernah and Sengev (2011). Kumar et al. (2014), reported that mild drying conditions with lower temperature may improve the product quality but decrease the drying rate.

The crude fibre of all samples, ranging from 16.33to 17.66 g/100 g, is lower than the recommended average daily requirement for an adult (16-32 g). Similarly, the carbohydrate content of samples ranging from 32.75 to 52 g/100 g is lower than the recommended daily allowance (Gamman and Sherrington, 1990). In general, 100 g of all samples can supply more than one quarter of nutrient requirement of the body. The result shows that dry leaves are better source of fat, fibre, protein and carbohydrate than the fresh leaves. The trend in change in the proximate composition of the *M. oleifera* leaves at different drying conditions agrees with the work of Adeyemi et al. (2014).

Effect of drying temperatures on vitamins

Table 2 shows the carotenoid and vitamin C contents of *M. oleifera* leaves. The beta-carotene of fresh leaves was 6,010 mg/100 g, in the range reported by Gernah and Sengev (2011) and Olushola (2006). Beta-carotene of sample A was 5,220.20 mg/100 g, which was higher than that of sample B (5,150.25 mg/100 g). This further decreased in sample C to 5,025.20 mg/100 g in sample D to 4,958.53 mg/100 g at 60°C and finally decreased to 4,946.25 mg/100 g in sample E dried at 70°C.

The reductions in total carotenoids from 6,010 mg/100 g in fresh leaves to 4,946.25 mg/100 g in sample E of oven dried leaves at 70°C for a constant drying time of 2

Table 2. Effect of drying temperature on vitamin content (mg/100g) of *M. oleifera* leaves.

Parameters	Fresh leaves	Samples (dried leave powder)					LSD
		A	B	C	D	E	
		(30±2°C)	40°C	50°C	60°C	70°C	
Carotenoids	6010.00^{a}±5.77	5223.50^{b}±3.33	5140.20^{c}±0.33	5024.90^{d}±0.33	4824.50^{e}±3.40	4946.20^{f}±00	168.94
Vitamin C	220.00^{a}±5.77.	27.39^{b}±0.33	27.85^{b}±0.33	26.53^{c}±0.33	25.70^{d}±0.33	25.70^{e}±0.00	0.75

Values are means of triplicate determinations. Means with different superscripts within the same column are significantly different from each other (p>0.05). A = Shade dried, B = oven dried at 40°C, C = oven dried at 50°C, D = oven dried at 60°C, E = oven dried at 70°C. lime spray drying (LSD).

Table 3. Effect of drying temperature on mineral content (mg/100 g) of M. oleifera leaves.

Parameters	Fresh leaves	Samples (dried leave powder)					LSD
		A	B	C	D	E	
		(30±2°C)	40°C	50°C	60°C	70°C	
Ca	0.81^{a}±0.01	1.37^{b}±0.01	1.80 ^{c}±0.10	2.44^{d}±0.01	3.17^{e}±0.06	3.67^{f}±0.02	0.08
Mg	0.46 ^{a}±0.01	0.69^{b}±0.01	0.78 ^{c}±0.01	0.88^{d}±0.01	0.94^{e}±0.06	1.13^{f}±0.06	0.06
K	0.31 ^{a}±0.01	0.46^{b}±0.06	0.63^{c}±0.01	1.15^{d}±0.01	1.37^{e}±0.01	1.79^{f}±0.01	0.06
P	0.07^{a}±0.01	0.09^{b}±0.00	0.09^{b}±0.01	0.13^{a}±0.01	0.18^{d}±0.01	0.18^{d}±0.01	-
Fe	3.17^{a}±0.01	4.29^{b}±0.60	4.23^{b}±0.01	4.71^{bc}±0.01	5.08^{c}±0.01	5.40^{c}±0.01	0.15
Zn	4.65^{a}±0.01	5.13^{b}±0.01	5.60^{c}±0.10	6.20±0.10	6.86^{e}±0.01	7.34^{f}±0.01	0.10

Values are means of triplicate determinations. Means with different superscripts within the same column are significantly different from each other (p>0.05). A = Shade dried, B = oven dried at 40°C, C = oven dried at 50°C, D = oven dried at 60°C, E = oven dried at 70°C. lime spray drying (LSD).

h indicated the effect of temperatures on the vitamins, and was in agreement with Eskin (1979) and Goodwin (1980). Olushola (2006) reported that only 20 – 40% of carotenoid in leaves were probably retained when the dried by solar drying and 50 – 80% by shade drying.

Vitamin C of fresh leaves was 220.45 mg/100 g in agreement with the value of 220.00 mg/100 g reported by Duke (1983). Moreover, it was higher than values reported by Muller (1988) for orange being 20.00 mg/100 g. Vitamin C content of shade dried leaves decreased slightly from 27.72 to 27.52 mg/100 g for leaves dried at 40°C sample B, and further decreased to 26.08 mg/100 g in sample C, dried at 50°C, and finally to 25.75 mg/100 g in sample E dried at 70°C. The vitamin C content of all samples is adequate for the daily need of adults based on the recommended daily allowance (Oslon and Hodges (1987).

The reduction in vitamin C content of the leaves could be due to drying temperature. This is consistent with the reports of Duke (1983), Olushola (2006), Mbah et al., 2012 and Gernah and Ajir (2007), that high temperature can cause huge losses of vitamin C.

Effect of drying temperature on mineral content of *M. oleifera* leaves

Table 3 presents the results of the effects of drying temperatures on the mineral composition of *M. oleifera* leaves. The mineral contents of fresh leaves were generally lower than dried samples and generally showed significant (p<0.05) increase with temperature.

The calcium content of fresh leaves was significantly (p<0.05) lower than the shade dried samples. However, there was significant difference (p≥0.05) in calcium content of samples dried at 40 – 70°C. The calcium content of fresh leaves was higher than value reported by Gernah and Sengev (2011), many times more than that of cow milk (Gordon, 1999) and higher than the recommended daily allowance (Olson and Hodges, 1987). Calcium builds healthy, strong bones and teeth and also assists blood clotting (Gordon, 1999). Deficiency can cause rickets, bone pain and muscle weakness. Taking *M. oleifera* fresh leaves and that dried in the shade and at 40 -70°C will meet the calcium need of adults and children based on RDA of 0.6 – 0.8 g (Glewe et al., 2001).

Table 3 reveals that other minerals studied especially magnesium, potassium, phosphorous, iron and zinc showed significant difference (p<0.05) between the fresh and shade dried leaves and increased significantly (p<0.05) with drying temperature. The general increase in mineral contents with increase with drying temperature is attributable to concentration factor due to moisture removal, which resulted in higher level of total soluble solid. The finding is in agreement with those of Gamman and Sherrington (1990).

Table 3 shows that increase in drying temperature did not deplete the mineral content of samples and therefore

did not have negative effect on the nutritional content of the samples. In general, the mineral content was above recommended daily allowance (Gamman and Sherrington, 1990).

Conclusion

In summary, it can be concluded that fresh *M. oleifera* leaves, which are normally consumed as vegetables and as food supplement, are rich in macronutrients and micronutrients required for proper growth and good health for human. Temperature affects the nutrient content of *M. oreifera*. *M. oleifera* leaves dried at 40 and 50°C compares favourably with that dried in the shade for two weeks (672 h). Oven drying should not exceed 50°C for optimum nutrient quality.

Conflict of interests

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

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Physiochemical and fatty acid analysis of *Virescens* (Ojukwu) oil and *Nigrescens* (ordinary) palm oil of *Eleaisguineensis*

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Traditionally in Igbo land folklore medicine, *Virescens* (Ojukwu) palm oil of *Eleaisguineensis* is of value as anti-poison and miracle oil. The objective of the study was to evaluate the physiochemical properties with the identification of the fatty acids of the *Virescens* oil in comparison to *Nigrescens* oil of *Eleaisguineensis*. The result of the physiochemical properties shows that the values for meeting point (slip point) of the oils were found to be the same (33°C) while solidification (titer) point, 22°C, viscosity, 51.20 centistokes and moisture content, 1.6% of *Nigrescens* (ordinary) palm oil are higher than that of *Virescens* palm oil with solidification point (titer) point, 15°C; viscosity 29.89 centistokes and moisture content, 0.2%. Both *Virescens* oil, 83.82 and *Nigrescens* oil, 53.98 are non-drying oil (low iodine value) and have high saponification values (*Virescens*, 222.3 and *Nigrescens*, 223.7). The result of the peroxide value revealed that there are more peroxides in Virescens oil (15 and 18) than in Nigrescens oil (8 and 12.3) for a week and 4 weeks oil respectively. Results on Ester value revealed high ester value (*Virescens*, 265.78 and *Nigrescens*, 263.16) with percent Ester purity of 21.11% for *Virescens* and 21.32% for *Nigrescens*. *Nigrescens* have higher acid value (40.67) than Virescens (29.73). The fatty acid analysis result revealed the presence of oleic, stearic acid, tocopherol in both the *Virescens* and *Nigrescens* palm oil with R_F values of 0.50 (oleic), 0.40 (stearic) and 0.29 (tocopherol) while lecithin was only observed in Virescens palm oil with R_F value of 0.34. Some values of *Virescens* were found to agree with the same value of olive oil and the presence of lecithin suggests why *Virescens* is anti-poison and medicinal.

Key words: *Virescens* and *Nigrescens* palm oil, physiochemical properties, anti-poison, medicinal.

INTRODUCTION

Palm oil is an edible vegetable oil derived from the mesocarp (reddish pulp) of the fruit of the oil palms, primarily the African oil palm *Elaeisguineensis* (Reeves and Weihrauch, 1979). It is naturally reddish in colour due to high beta–carotene content. Palm mesocarp oil is 41% saturated and semisolid at room temperature and contain several saturated and unsaturated fats in the forms of glyceryllaurate (0.1% saturated), myristate (1%

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saturated), palmitate (44% saturated), stearate (5% saturated), oleate (39% monounsaturated) and linoleate (10% polysaturated) (Cottrell, 1991). Like all vegetable oils, palm oil does not contain cholesterol (US Federal Food, Drug and Cosmetic Act, 1990; UK Food Lebelling Regulation, 1984) although saturated fat intake increase both LDL and HDL (Mensink and Katan, 1992) cholesterols (Cha Sook et al., 2002; Tan, 1991). Palm oil is largely the cooking oil in the tropical belt of Africa, South East Asia, part of Brazil and South America. Its use in the commercial food industry in other parts of the world is due to its low cost and the high oxidative stability of the refined product when used for frying (Che Man et al., 1999; Matthaus, 2007). A large proportion of the oil is also consumed in the manufacture of soaps including native black soap, candles, lubricants and in tin plating industry (Onyegbado et al., 2002; Edgar, 1985). The folklore nutritional and healing properties have been recognized for generations. Red palm oil was the remedy of choice for nearly every illness in many parts of African. The taking of spoonful of palm oil when someone sick was common.

Red palm is rich in vitamin A precursors and can be used in place of cod-lover oil (Zeba et al., 2006; ChaSooket al., 2002). It was reported in some research work that oleic acid, a monounsaturated fatty acid in palm oil is as effective as the polyunsaturated fatty acids in lowering blood cholesterol (Mattson and Grundy, 1985; Qureshi et al., 1995). Palm oil can not only improve coronary blood flow and remove plaque buildup in arteries, it also reverses the process of atherosclerosis and improves cholesterol values and also helps maintain proper blood pressure (Homstia, 1987; Yuen et al., 2011; Edem, 2002). Both crude and refined palm oil helps to maintain proper blood pressure due to the high antioxidant content of the oil. These antioxidants quenches free radicals and keep inflammation that causes swelling that narrows artery passage way restricting blood flow to vital organs such as the heart under control (Esterhuyseet al., 2005). Tocopherol one of the phyto nutrients of palm oil are beneficial to consumers who want to maintain healthy brain (neuroprotection), blood lipid level, arterial compliance (reducing arterial stiffness), liver health, skin nutrition, immune protection and inhibit the growth of skin, stomach pancreas, liver, lung, colon, prostate, breast and other cancers (Rink et al., 2011; Patel et al., 2012; Qureshi et al., 1995; Yano et al., 2005).

The antioxidant power of red palm oil is of help in protecting against a variety of health problems including osteoporosis, asthma, cataract, macular degeneration, arthritis and liver diseases. It also stunts the processes that promote premature aging (Khanna et al., 2003).

MATERIALS AND METHODS

Sample collection

The fresh samples of *Nigrescens* (ordinary) and *Virescens* (Ojukwu) types of *E. guneensis* palm fruit were collected from Oghe community in Ezeagu LGA, Enugu state on March 10, 2014 and authenticated by Prof. JC Okafor of Applied Biology and Biotechnology Department, Enugu State University of Science and Technology.

Isolation of oil from the samples

The riped fruits of *Nigrescens* and *Virescens* types of *E. guineensis* were each boiled in water and pounded to disintegrate the pulp, thus freeing the nut. The traditional method of oil expression in some parts of West African was used. The fresh pulp was re-cooked with a large volume of water (1:5 v/v). The oil floated on top and was skimmed off and stored in different container for analysis, respectively.

Physical characterization

Ubbbelohde melting point determination

Each oil in a capillary tube was allowed to freeze in a freezer for 1 h and heated slowing in a water bath. The temperature at which the oil began to slip in the capillary tube was recorded as the slip point or melting point (Ubbolohde melting point method).

Solidification point (titre value) determination

The oil in a capillary tube was allowed to flat in watch glass on a water trough. Blocks of ice were added continuously until the oil solidified and the temperature recorded.

Viscometric studies

The time taken for the oil to fall between the two graduation Marks on Oswards viscometer at room temperature was recorded (Ikhuoria and Maliki, 2007).

Moisture content

The percent loss in weight of oil sample heated in a drying oven at 105°C for 2 h and weighed at an interval of 30 min till a constant weight was obtained. Initial weight of oil sample = a gm, Final weight of oil sample after drying = b gm, the dry weight percent = (a/b)*100.

Moisture content (x %) = (1-(a/b))*100 (AOAC, 1980).

Chemical characterization

Determination of iodine value

Twenty five (25) milliliters of iodine monochloride was added to 1 01 g of the oil, stoppered and left to stand in the dark alongside a blank without the oil sample and 10 mL of chloroform added instead; for 1 h. The flask was rinsed with 50 mL of distilled water and 10 ml of 10% KI solution was added. The liberated Iodine was immediately titrated with 0.1 M $Na_2S_2O_3$ until the iodine solution was brownish yellow then 1 mL of starch solution indicator was added. The titration was continued until the developed blue colour disappeared. The volume of the $Na_2S_2O_3$ was used to calculate the iodine value.

$$\text{Iodine value} = \frac{(\text{Blank- Titre value}) * \text{molarities of } Na_2S_2O_3 * 12.69}{\text{Weight of sample gm}}$$

Determination of saponification value (JIS K 007- 1992)

Two (2) grams of the oil was refluxed with 25 mL of alcoholic potassium hydroxide solution (0.5 M) for 1 h with frequent shaking. The excess alkali was titrated with 0.5 M hydrochloric acid and 1 mL of phenolphthalein indicator. A Blank titration was carried out alongside and the Saponification value calculated thus:

$$\text{Saponification value} = \frac{(\text{Blank-Titre value})*28.05}{\text{Weight of oil (g)}}$$

Acid value determination (Ejim and Kamen, 2013)

Two grams of the oil sample was dissolved in 25mL diethyl either with 25mL ethanol was titrated with 0.1M NaOH solution and 1mL of phenolphthalein indicator until a faint pink colour persisted for 15 seconds.

Acid value = %FFA (as Oleic)* 1.99

Peroxide value determination (Eddy et al., 2011)

One (1) gram of the oil sample was allowed to boiled with 1 g potassium iodide and 20 mL of solvent mixture (Glacial acetic acid and chloroform [2:1] v/v) for 30 s and then vigorously for another 30 s. This was poured into 20 mL of 5% potassium iodide and the boiling tube washed twice with 25 mL of distilled water. This was titrated with 0.002 M of the $Na_2S_2O_3$ using starch indicator, a blank was similarly titrated. Calculation

$$\text{Peroxide value} = \frac{1000(V_2-V_1)T}{M}$$

Where M = mass of oil taken (1 g); V_2 = volume of 0.1 N $Na_2S_2O_3$; V_1 = volume of 0.1 N $Na_2S_2O_3$ used I blank and T = nomlity of $Na_2s_2O_3$ (0.1 N)

Determination of ester value (JIS K 0070-1992)

Two (2) grams of the oil sample was refluxed for 1 h with 25 mL of aqueous sodium hydroxide in a water bath. The condenser was washed down with 5 mL of distilled water and the content allowed to cool down to room temperature. The excess alkali was titrated with 0.5 M HCl using phenolphthalein as indicator. A blank titration was repeated without the oil sample.

Ester value = Saponification value – Acid value.

Protein determination (nitrogen) through Kjedahls method

Half a gram of the oil sample was digested by heating in an inclined position with 1 g of digestion catalyst mixture and 5 mL of concentrated sulphuric acid. The flask was stoppered loosely with cotton wool. After fronting subsided, then it was heated vigorously until the solution became clear. The digest was allowed to cool down to room temperature, then 25 mL of distilled water was added with 20 mL of 10 M NaOH through a funnel with tap which was closed after the addition to act as a seal with little water. The kjedahls flash content was heated and the stream absorbed into 25 mL of 0.04 M HCl for 3 min. This was titrated with 0.02 M sodium hydroxide using phenolphthalein as indicator. A blank determination was carried out side by side using glucose in place of the oil sample. The value obtained in the computation was multiplied by 6.25 (Chaco et al., 1993).

Extimation of hydroxyl group

Half a gram of the oil sample was refluxed with 10 mL of acetylating mixture (pyridine and acetic anhydride [3:1v/v]) in a water bath for 1 h. The condenser was washed with 20 mL of distilled water into the mixture and gently shaken. This was allowed to cool for 10 min and titrated with 1 M NaOH using phenolphthalein as indicator. A black was similarly titrated (Chaco et al., 1993).

Protein hydrolysis

Half a gram of the oil sample was refluxed with 20 mL of 20% hydrochloric acid with two pieces of anti-bump for 45 min using a very low flame. Three milliliter of the hydrolysates was carefully neutralized with 10% sodium hydroxide. The solution was made alkaline with 1 mL of 2 M NaOH (Linstronberg and Ballmgaten, 1966).

Protein hydrolysis

Half a gram of the oil sample with 5 mL water and 5 mL hydrochloric acid was sealed in 25 mL ampoule. The ample was wrapped in a cotton wool and heated in an electric over at 135°C for 5 h. After cooling down to room temperature the ampoule was opened and the content heated in a crucible to dryness until the odor of hydrochloric acid is no longer detectable in water bath. Then the residue was dissolved in 1 mL of distilled water (Beckett and Stenlake, 1974).

Separation of α- amino acid by paper chromatography

A rectangular piece of Whatman no.1 chromatography paper (30 cm x 10 cm) was used to separate the α-amino acid present in the hydrolysate using 80% phenol solution as solvent system (Linstromberg and Ballmgaten, 1966). The chromatogram after development are visualized after washing with acetone and allowed to dry with 2% solution of ninhydrin in 95% ethanol. The R_F values computed were compared with those given in literature (Linstromberg and Ballmgaten, 1966).

Two dimensional separation of amino acid by paper chromatography

The chromatogram, developed with 6% acetic acid solvent system by the method of ascending chromatography in one dimension using 6% acetic acid. The paper was dried and turned at right angles to the first and developed with a second solvent system either n-butanol- acetic acid - water (4:1:5). The chromatogram developed was dried and sprayed with 0.1% nihydrin in n-butanol saturated with water and heated at 90°C for 10 min. Each spot was circled (Hartley, 1988).

TLC analysis of fatty acids

TLC plate (F-254 type E) was used to separated the fatty acids in the *Virescens* and *Nigrescens* oils in comparison to standard fatty acids using n-hexane: ethyl ether: acetic acids [80:20:1] solvent system. The chromatogram after development is visualized by

Table 1. The results of the physicals and chemical properties of Nigrescens and Virescens palm oils.

Property	*Nigrescens*	Virescens
Physical property		
Specific gravity	0.9002	0.9116
Melting point [slip point]	33°C	33°C
Solidification [titre] point	22°C	15°C
Viscosity [centistokes]	51.20	29.89
Moisture content [%]	1.6	0.2
Chemical property		
Iodine value	53.98	83.82
Saponification value	223.7	222.3
Acid value	40.46	29.73
Peroxide value [1 wk old]	8.0	15.0
Peroxide value [4 wk old]	12.3	18.0
Hydroxide group [%]	2.55	3.23
Ester value	263.16	265.78
%purity Ester	21.32	21.11
Phospholipids [µg/ml]	0.35	0.76

spraying with 10% phosphomolybdic acid solution in a fume hood and heated in an electric oven at 70°C for 20 min (Moran et al., 1994; Moran and Serimgeour, 1994; Plumer, 1971).

Quantitative determination of phospholipids

Colorimetric determination through an acidic digestion method was used. One milliliter of the oil sample was digested by heating with 0.65 mL of 70% perchloric acid until the yellow colour disappeared alongside the standard (0.10 to 0.90 µg/mL KH_2PO_4). The digests were diluted with 3.5 mL of distilled water followed by 0.5 mL of ammonium molybdate solution and 0.5 mL of ascorbic acid solution. The content was shaked very well and heated in a boiling water for 30 min for colour development. The absorbances of cool samples (including the standards) are read at 800 mm. (Rouser et al., 1970).

RESULTS AND DISCUSSION

Table 1 shows the results of the physical and chemical parameters of *Nigrescens* and *Virescens* palm oils as determined. The result of the iodine value presented in this study shows that *Virescens* and *Nigrescens* (ordinary) palm oil belongs to a class of oil known as non-drying oil. This evident in the iodine number of the oils which is less than 110 for non-drying oils. However, the *Virescens*oil can be grouped with olive oil because the iodine value and peroxide value are relatively within the same range (80 to 90 and 10 to 20), respectively. They are also both liquid below room temperature, the specific gravity of both are within the same range (0.910 to 0.916) (Thomas, 2002; Codex Stan 33 to 1981; 2001). Furthermore, the rather very high iodine number than *Nigrescens* (ordinary) palm oil well suggested that *Virescens* palm oil have components which may have higher degree of unsaturation than *Nigrescens* (Ordinary) palm oil. Moreover, it also suggested that oleic acid may be more than palmitic acid in *Virescens* palm oil. It becomes possible that *Virescens* palm oil could be very easily hydrogenated and used in cosmetics and creams than *Nigrescens.* The degree of unsaturation of the two oil suggested that the melting point will not be low. This is true because unsaturation lowers melting point, *Virescens* and *Nigrescens* palm oils are only a little unsaturated. This much agrees with the fact that palmitic and oleic acid have boiling and melting points which are generally high. These oils cannot therefore be used in making paints and varnishes. Interestingly, *Virescens* and *Nigrescens* palm oils have high saponification values. In agreement with many previous studies, this implies that the oil contains few carbon chains and produces very large acid per gram of fatty acid (Chemical and Process Technology Encyclopedia, 1974). It contains very large glycerides and can be easily sapoinfied-for use in soap production. This is further supported by the titre (solidification) value of the oils which is 22°C for *Nigrescens* and 15°C for *Virescens*; for oils that are good for making soap must have high titre value.

Generally, acid value gives idea about the purity of oil. High value implies high content of fatty acids which in turn implies low purity. On the other hand, low value means that the oil contains low amount of fatty acids and is pure. Acid value may also indicate the age of oil. The acid value obtained for *Nigrescens*palm oil is high (40.67) while that of *Virescens* palm oil is low (29.73) and is therefore of high purity than *Nigrescens*palm oil. From the specific gravity determination it may be inferred that the density of *Virescens* palm oil (0.9116) is within the range of specific gravity of olive oil which is between 0.910 to 0.916 while that of *Nigrescens* palm oil is 0.9002. The peroxide value of the *Nigrescens* palm oil is 8 within the first week old and 12.3 after four weeks. While that of the *Virescens*palm oil is 15 with one week old and 18 after four weeks old. This is probably due to the age and degree of unsaturation (higher iodine value) of *Virescens* palm oil, because the greater the degree of unsaturation, the greater is the liability of the oil or fat to oxidative rancidity (Pearson, 1976). The peroxide value of *Virescens* palm oil is closer to that of olive oil which is always less than 20 (Tayeb, 2013). It is worthy to note that both oils have high ester value probably due to the high saponifiable ester content of the oils. They have also the same percent purity ester. Both oils have alanine amino acid while *Nigrescens*palm oil has proline amino acidand *Virescens* palm oil has cystine and phenylalanine amino acids Viscosity of the *Nigrescens* (51.20 Centistokes) palm oil (Table 1) appears to be nearly double that of *Virescens* palm oil (29.89 centistokes). This is probably due to the high titre (solidification value of the *Nigrescens*palm oil than *Virescens* palm oil.

Table 2. TLC analysis of Fatty acids/other lipids in *Nigrescens* and *Virescens* palm oils with standards.

Fatty acids/ other lipids		
Oleic acid	+	+
Stearic acid	+	+
Palmitic acid	+	+
Lecithin	-	+
Tocopherol	+	+

Table 3. Amino acids present in Nigrescens and Virescens palm oils.

α-Amino acid		
Cystine	-	+
Alanine	+	+
Proline	+	-
Phenylalanine	-	+

The result of the thin layer chromatographic identification of fatty acids and other lipids (Tables 2 and 3) with standards revealed that *Nigrescens* palm oil contain Tocopherol, Oleic acid, stearic acid, palmitic acid, while *Virescens*palm oil contain tocopherol, oleic acid, stearic acid, palmitic acid and lecithin. Cholesterol was not identified in the two oil samples. The estimation of the total amount of phospholipids in the oil samples revealed that *Virescens* palm oil contains 0.76 mg/ml while *Nigrescens* (ordinary) palm oil contains 0.35 mg/ml. With the results of the study, it becomes obvious that *Virescens* palm oil is more healthy oil than *Nigrescens* palm oil. This is probably due to the large proportion of unsaturated fatty acids that is heart friendly. Finally, it can be concluded that the relationship of the *Virescens*palm oil and olive oil, more so, the presence of cystine amino acid in both of them and the amount of phospholipids in *Virescens* palm oil accounts for the anti-poison and medicinal characteristics of the Virescens palm oil.

Conflict of interests

Theauthor(s) did not declare any conflict of interest.

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Isolation and identification of fungi associated with some Libyan foods

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Cereal and its products can be contaminated with fungi in the field, during drying, processing, transportation and subsequent storage, which may lead to secretion of mycotoxins under favourable condition. The aim of this study was the isolation and identification of some fungi associated with four kinds of Libyan food products of different trademarks. Twenty four (24) samples of couscous, macaroni, wheat flour and rice regularly used for human consumption by Libyan family were collected from local markets in the city of Alzawia, west of Tripoli, Libya. The results reveal isolation of 113 isolates belonging to nine genera: *Penicillium*, *Aspergillus, Fusarium, Paecilomyces, Alternaria, Rhizopus*, *Mucor, Scopulariopsis* and *Cladosporum.* Approximately 24 species were identified to belong to those isolated genera, several of which are known as main producer of mycotoxins especially *A. flavus* which are known to produce aflatoxins, *Aspergillus niger, Aspergillus carbonarious*, *Penicillium chrysogenum* and *Penicillium verrucosum* known to produce ochratoxin and *Fusarium oxysporum* and *Fusarium chlamydosporum* known to produce fumonisins and trichothecenes. Certainly, the occurrence of such types of mycotoxins can pose a health threatening risk for the consumer of those food items. Presence of these fungi in food products could be due to lack of good agriculture and food manufacturing practices throughout the food chain.

Key words: Couscous, macaroni, wheat flour, rice, fungi, Libya.

INTRODUCTION

Cereals and derived products represent an important nutrient source for mankind world-wide. In addition they are the most important dietary food for North African populations (Riba et al., 2010). Wheat is the most important small-grain cereal crop in the world with a total production of almost 700 million tonnes per year (Anonymous, 2010). Unfortunately, cereals are naturally contaminated with fungi in the field, during drying, processing, transportation and subsequent storage and it may be difficult to completely prevent mycotoxins formation in contaminated commodities, particularly those that are produced in tropical and subtropical climates, in countries where high temperature and humidity promote the growth and proliferation of fungi (Kumar et al., 2008).

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Thus, they are often colonised by fungi, including species from the genus *Aspergillus*, *Penicillium* and *Fusarium*, which cause significant reductions in crop yield, quality and safety due to their ability to produce mycotoxins. Mycotoxins commonly occurring in cereals and cereal products include zearalenone, fumonisins, trichothecenes (as deoxynivalenol and T2-HT2), ochratoxin and aflatoxins (Miller, 2008). It was reported that 25-50% of harvested world crops have been contaminated with mycotoxins (Ricciardi et al., 2012). A number of surveys have been carried out to identify a general pattern of toxigenic fungi and mycotoxins contamination in crops that are dried prone to contamination. In a research work by (Shaker et al., 2013) for isolation and identification of some fungi that produce mycotoxins in 80 different food samples that included rice in Iraqi market showed presence of *A. niger, A. parasiticus, A. veriscolar, A. ochraceus, P. citirinum, P. verrucom, Mucor* spp. and *Rhizopus. stolonifer.* In study by Belkacem-Hanfi et al. (2013) using a total of 127 durum wheat samples intended for food production that were collected during 2010-2011-2012 season in Tunisia indicated the presence of a total of 6035 postharvest fungi strains. The most pre-dominant post-harvest fungi genera isolates were *Alternaria* (28%), *Fusarium* (19%), *Penicillium* (19%), *Aspergillus* (14%), *Rhizopus* (7%), *Mucor* (6%) and other fungi (6%). The study also revealed that all stored grains were highly contaminated by field fungi and storage fungi. A recent study in Egypt revealed the occurrence of toxigenic fungi in 10 samples of wheat and 9 samples of rice from markets in central delta provinces. The researchers reported isolation of eight fungal genera that belongs to *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor*, *Rhizopus*, *Trichoderma*, *Alternaria* and *Cladosporium* (El-Shanshoury et al., 2014). Similarly, in India, a study for the fungal associated with 36 samples of stored rice showed isolation of 96 isolates belonging to 16 genera of *Aspergillus* (50%)*, Penicillium* (25%) and *Fusarium* (25%) (Sawane and Sawane, 2014). Evaluation of fungal contamination of consumed wheat flour for 89 samples carried by Rezazadeh et al. (2013) in Tabriz, Iran, reported that 31.5% of the samples contaminated with fungal genera belong to *Aspergillus* (50%), *Mucor* (7%)*, Penicillium* (3.5%)*, Acremonium* (14.5%), *Cladosporium* (3.5%) and *Alternaria* (3.5%). Survey for isolation and identification of fungi were conducted in two well-known markets in Ogun state-Nigeria on January 2014 using a total of 21 samples of different kinds and trade marks (Ezekiel and Sombie, 2014). The study revealed that 11 samples (52.4%) contaminated with fungi belong to genera of *Aspergillus* (56.3%), *Fusarium* (6.3%), *Penicillium* (6.3%) and other fungi (31.3%). In North African countries, foods that are most susceptible to contamination by fungi are locally produced or imported cereals, such as wheat. This crop is usually staple in dry Mediterranean regions of North Africa, where its consumption is in the form of couscous, pasta, macaroni, and bread. The mycobiota of wheat and wheat products was found to be dominated by *Aspergillus, Penicillium* and *Fusarium* species (Riba et al., 2008). The aim of this study was to isolate and identify some fungi associated with several major food products destined for human consumption and regularly used by most of the Libyan families.

MATERIALS AND METHODS

Food samples

Twenty four (24) food samples of several kinds and trade marks were randomly collected few days before analysis from different markets and bakery places in the area of Alzawia city with valid expiration date for consumption. They consist of 6 samples of wheat flour (National production), 6 samples of couscous (National and imported production), 6 samples of rice (Imported production) and 6 samples of macaroni (National and imported production).

Isolation of fungi

Isolation of fungi from rice and macaroni were carried out based on method described by Samson et al. (2010). Food samples were initially subjected for surface sterilization with 0.2% sodium hypochlorite solution for 2 min and rinsed three times with sterile distilled water and excess water on the sample was mopped using sterile filter paper. The samples were directly placed on Potato dextrose agar (PDA) media supplemented with chloramphenicol (in triplicates) at a plating rate of 5 pieces per plate and incubated at 25°C for 5 - 7 days. Each fungal colony obtained was then sub cultured on PDA and incubated at 25°C for 5 - 7 days for subsequent characterization and taxonomic identification. In case of wheat flour and couscous samples, dilution plating technique was used by taking10 gof each sample and added to 90 ml of sterilized distilled water. This mixture was then shaken and 1 ml aliquots were spread on the surface of PDA media supplemented with chloromphenicol (in triplicates), and incubated at 25°C for 5 - 7 days. Each fungal colony obtained was then subcultured on PDA and incubated at 25°C for 5 to 7 days for subsequent characterization and taxonomic identification.

Identification of fungi

Isolated fungi were identified on the basis of their micro and macro-morphological characteristics using standard taxonomic key used prevoiusly (Samson et al., 2010; Pitt, 1979; Pitt and Hocking, 2009; Raper and Fennel, 1965; Ellis et al., 2007).

RESULTS AND DISCUSSION

The results of this study indicate the isolation of 113 fungal strains that were found to belong to 9 genera of *Penicillium, Aspergillus, Fusarium, Paecilomyces, Alternaria, Rhizopus*, *Mucor, Scopulariopsis* and *Cladosporum,* covering 24 species (Table 1) and the most common genera isolated were *Penicillium* and *Aspergillus* (Table 2). Some of these genera are known to produce mycotoxins in food products such as aflatoxins, ochratoxin, trichothecenes (as deoxynivalenol

Table 1. Isolation and identification of fungi associated with some Libyan foods.

Genus	Species	Food samples			
		Couscous	Macaroni	Wheat flour	Rice
Penicillium	*P. citirinum*	+	+	-	-
	P. corylophilum	+	+	+	+
	P. conescens	-	+	+	-
	P. vinaceum	-	-	+	+
	P. duclauxii	-	-	-	+
	P. expansum	-	-	+	+
	P. verrucosum	-	+	+	-
	P. chrysogenum	-	+	+	-
Aspergillus	*A. flavus*	+	+	+	+
	A. carbonarious	+	+	+	+
	A. candidus	-	-	-	+
	A. niger	-	-	+	+
	A. ustus	-	+	-	-
	A. veriscolar	-	+	+	-
	A. fumigatus	+	-	-	-
Fusarium	*F. chlamydosporum*	-	+	+	-
	F. oxysporum	-	-	+	-
Paecilomyces	*P. lilacinus*	+	-	-	-
	P. variotil	+	-	-	+
Alternaria	*A.alternata*	+	+	-	-
Rhizopus	*R. stolonifer*	-	-	+	+
Mucor	*M. hiemalts*	+	-	-	-
Scopulariopsis	*S. brevicaulis*	-	-	-	+
Cladosporium	*C. cladosporioides*	-	-	-	+

+, Presence of fungi; **-,** absence of fungi.

Table 2. Percent (%) of isolated and identified fungi associated with some Libyan foods

Fungal genera isolated	%
Penicillium	33.33
Aspergillus	29.16
Fusarium	8.33
Paecilomyces	8.33
Alternaria	4.17
Rhizopus	4.17
Mucor	4.17
Scopulariopsis	4.17
Cladosporium	4.17

and T2-HT2) and fumonisins which could pose a risk for consumer health. The distribution of the above stated genera in the 4 food items under study is shown in Table 1. As could be seen from the table, some species were detected in all food items, while some were present in only one or two items. For instance, *A. flavus* and *A. carbonius* were detected in all food items, while the other *Aspergillus* species were present in only 1 or 2 items. Similar findings could be seen for the rest of the isolated fungal species. That was true for *Fusarium, Alternaria, Rhizopus*, *Scopulariopsis*, *Paecilomyces* and *Cladosporium.*

Results of this study are in conformity with several studies around the world. In a study for the presence of toxigenic fungi associated with 48 samples of marketed rice grain collected from different markets in Uganda revealed presence of 8 *Penicillium* species including *P. chrysogenum* and *P. citrinum* which are considered as ochratoxin A producers, and presence of 8 *Fusarium* species included *F. solani, F. graminearum, F. oxysporum* and *F. verticillioides* which are able to produce

fumonisins and trichothecenes (as deoxynivalenol and T2-HT2), and presence of 12 *Aspergillus* species that include *A. flavu* and *A. parasiticus*, which are able to produce aflatoxins and *A. ochraceus* and *A. niger* strains known to produce ochratoxin A (Taligoola et al., 2010). A total of 125 samples of winter and spring wheat harvested in 2009, 2010 and 2011collected in southern Sweden (Lindblad et al., 2013) and the isolation and identification result showed that *F. poae* and *F. avenaceum* were present in almost all samples, other common *Fusarium* species were *F. graminearum* and *F. culmorum,* present in more than 70% of samples. Mycological survey carried out (Embaby et al., 2012) on freshly harvested wheat grains from the main production regions in Egypt resulted in eight fungal genera isolates and identified as: *Alternaria* (36.9%)*, Aspergillus* (12.4%)*, Drechslera* (1.3%)*, Epicoccum* (0.7%)*, Fusarium* (5.2%)*, Mucor* (0.2%)*, Penicillium* (18.3%) and *Rhizopus* (25.0%).

Mycotoxins analysis revealed that 3 fungal isolates were reported to produce aflatoxins and one of *F. moniliforme* isolate was able to produce fumonisin B_1. A study of fungal mycoflora and mycotoxins for 88 polished rice samples harvested in 2002 intended for human consumption were obtained from several grain wholesale markets in Seoul, Korea (Park et al., 2005): isolation of 63 strains belong to genera of *Aspergillus*, *Penicillum* and *Fusarium* and 18 strains have the ability to produce aflatoxins, ochratoxin, fumonisin B_1, deoxynivalenol, nivalenol and zearalenone. Research work to explore fungi and mycotoxins associated with rice grains during storage for 25 rice samples collected from different locations of district Mandi in India revealed that all the samples were found to be contaminated with one or more fungal genera of *Aspergillus* (41.6%), *Fusarium* (8.3%), *Penicillium* (16.6%) and other genera (41.5%). In addition, aflatoxins B_1 and B_2 were detected in 72% of the total samples used in the study. The researchers stated that the presence of these toxigenic fungi and aflatoxins poses a risk for consumer's health and it is necessary to check the rice grains prior to final distribution for public use (Gautam et al., 2012). The mycotoxins contamination of 123 samples of imported rice and its producing fungi in Zabol, (Iran) investigated by Amanloo et al. (2014), showed that 34 (27.6%) of the samples contaminated with *A. flavus, A. parasiticus*, *A. niger, A. fumigatus,* and fungal genera of *Penicillium, Fusarium* and *Rhizopus,* and presence of aflatoxins.

Presence of several species of possible toxigenic fungi reported in this study is an alarming indictor for the possibility of the presence of mycotoxins in food samples. Incidence of ochratoxin A in rice used for human consumption for 100 samples in Morocco showed that 26 (26%) samples contain ochratoxin A at concentration range from 0.08-47 ng/g (Juan et al., 2008). In Algeria, 108 samples of wheat and its derived products, intended for human consumption, were collected during pre-harvest in a state of storage and from flour and semolina mills by (Riba et al., 2010) from two regions: during the seasons of 2004 and 2006 the results of isolation and identification revealed that 150 isolates belong to *Aspergillus* genera (64.5%) representing 144 strains of *A. flavus* and 6 strain of *A. tamarii*, also the result showed presence of aflatoxins B_1 in 56.6% of the wheat samples and derived products (flour, semolina and bran) with concentration levels ranging from 0.13 to 37.42 ng/g. In a study by Raiola et al. (2012) for 27 samples of Italian commercial pasta indicated presence of ochratoxin A in 26 (96.30%) samples and deoxynivalenol in 22 (81.48%) samples.

In conclusion, presence of different species of fungi associated with food products used in this study could represent a serious problem by secretion of different kinds of mycotoxins which could affect human health, and all relevant authorities should work together in reducing the risk of contamination and risk of mycotoxins exposure from consumption of cereals and derived products. In fact, it is important to obtain assurance that the products are safe and of high quality. Food inspection during the food chain production plays an important role in food control. High risk products, such as with mycotoxins contaminated foodstuffs, shall be subject to an increased level of official control.

Also in the same time, immediate action should be taken to insure the application of good agriculture and manufacturing practices to prevent the fungal contamination and growth in cereals and cereal products during the entire food chain by using different methods to minimize fungal infection, including seed treatment, bio control and possibly cropping sequence. Other crop production practices that can be managed to reduce mycotoxins may include irrigation, fertilization and plant density. Another potential strategy to reduce mycotoxins contamination is to grow cultivars resistant to fungal invasion and subsequent mycotoxins production and final and most important factor after the above mention practice is to control the storage condition such as moisture and temperature of the row material and final product.

Conflict of interests

The authors did not declare any conflict of interest.

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Composition of sorghum-millet flour, *Aframomum danielli* essential oil and their effect on mycotoxins in kunu zaki

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This paper determines the composition of sorghum-millet flour, *Aframomum danielli* essential oil and its effect on mycotoxins in kunu zaki. Sorghum and millet grains in ratio 1:2 were cleaned, soaked for 12 h, washed, drained and dried in the hot air oven. The dried sorghum-millet grains were milled and packed for kunu zaki production. Kunu zaki was produced and different concentrations of *Aframomum danielli* essential oil (0.25, 0.50, 1.0, 2.5, 5.0 and 10%) were added. The bulk density, swelling index, water absorption capacity, solubility and oil absorption capacity of the flour were 0.75 g/cm^3, 1.16, 2.49 ml H_2O/g, 2.56% and 0.79 ml oil/g, respectively. The flour had low phenols, oxalate, phytate and saponins contents. Peak viscosity of the flour was 1234 Cp while the holding viscosity was 811 Cp. Breakdown and final viscosities were 423 and 1824.5 Cp, respectively. The components detected in the essential oil of *A. danielli* seed were dominated by 1, 8-Cineole (56.16%). There were gradual reductions in the mycotoxins contents of kunu zaki with addition of *Aframomum danielli* essential oil. Kunun zaki with 10 % *A. danielli* essential oil resulted in 76% reduction in fumonisin B_1. *A. danielli* exhibited ability to reduce FB_1, FB_2 and Ochratoxin A in kunu zaki.

Key words: *Aframomum danielli,* anti-nutrients, essential oil, kunu zaki, millet, sorghum.

INTRODUCTION

Cereals belong to the grass family and constitute important crops which serve as industrial raw materials and staple for the world (Enwere, 1998). The geographical location and climatic conditions of each area determine the kind of cereals that can be grown and utilized (FAO, 1989). The three most important cereals in the world are wheat, rice and corn. Other important cereal grains include sorghum, oats, barley, millet and rye. In Nigeria, the cereals cultivated are sorghum, millet, maize, rice, wheat and hungry rice to a lesser extent (Okon, 1998). Cereals have high carbohydrate, low fat and fair protein content (Enwere, 1998). Various food products are obtained from grains based on the processing techniques and the type of cereals employed (Gaffa et al., 2002). Cereals apart from being eaten directly are use largely to make various other products

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like flour, gruel, porridge and drinks especially alcoholic and non-alcoholic beverage.

Sorghum [*Sorghum bicolour* (L) Moench] locally called guinea corn is the most extensively grown cereal grain in the country (Aba et al., 2004). It is considered as one of the most important food crops in the world, following wheat, rice, maize and barley (FAO, 2006). Sorghum is the most amenable cereals grain to different processing technologies including primary, secondary and tertiary methods (Obilana and Manyasa, 2005). Primary processing involves: fermentation, malting, wet and dry milling, boiling, roasting and popping. Secondary processing involves: brewing, beverages and drinks production, baking and confectionery making, steaming, extrusion (for paste and noodles) while tertiary processing involves composite flour, biofortification and chemical fortification with additives. Sorghum drinks are also high in minerals, vitamins and some essential amino acids which are further enhanced through biofortification thus making them superior to other cereal foods. They contribute more energy and digestible protein in the diets of the majority of the people in the sub-Saharan regions than those obtained from root and tuber crops (Aba et al., 2004). In addition, its polyphenol content are used as antioxidants just as the slow digestibility of sorghum starch and protein makes its food useful in diabetic treatments. However, millets also have high starch, fiber content and poor digestibility of nutrients which severely limits their values in nutrition and influence their consumers acceptability (FAO, 1995). This paper determined the composition of sorghum-millet flour, *A. danielli* essential oil and its effect on mycotoxins in kunu zaki.

MATERIALS AND METHODS

Sorghum and millet grains were purchased at Bodija market, Ibadan while the fresh pods of *A. danielli* spice was obtained at Ibode market in Ibadan, Nigeria.

Raw material preparation

Sorghum and millet grains in ratio 1:2 were cleaned and soaked for 12 h. The grains were washed, drained and dried in the hot air oven (50°C) for 18 h. The dried sorghum-millet grains were milled and packed for kunu zaki production.

Production process of kunu zaki

The traditional method described by Gaffa et al. (2002) was adopted for use in the production of kunu zaki with slight modification. Four hundred grams of the sorghum-millet flour was mixed with 600 ml of water to form paste. The slurry was then divided into two portions (¾ and ¼). The larger portion was gelatinized by addition of boiling water. The two portions (gelatinized and ungelatinized) were then mixed together and then left overnight at room temperature for chance fermentation. It was then filtered using a muslin cloth. Spices were not added to the mixture intentionally. This is to ensure that no other spices interfere with the study.

Extraction of the essential oil

The essential oil extraction was done by hydro-distillation method using Clevenger's apparatus. The flask with weighed samples, condenser and other gadgets were connected to complete the hydro-distillation arrangement using Clevenger-type apparatus. The crushed sample in the flask was entirely covered with de-ionized water as suspension and placed on the heating mantle. The water was allowed to boil in the flask and the essential oil is carried over to the condenser along with the steam. The essential oil and the steam are separated below the condenser through a separator. The procedure was repeated until a sufficient amount of oil was obtained for the analysis. The essential oil was dried over anhydrous sodium sulphate and stored in a 2 ml sealed Agilent vial protected from light at 4°C before analysis.

Functional, pasting and anti-nutritional properties sorghum-millet flour

Bulk density was done using Udensi and Okaka (2000) method, water absorption and swelling index using method of Iwuoha (2004). Oil absorption capacity was done using the method of Balogun and Olatidoye (2010) method. The pasting property of the flour was determined by RVA (RVA-4, Newport Scientific, Australia) according to Vongsawasdi et al. (2009). Method of Dairo (2008) was used for phytate determination while oxalate content was determined using the method of Nwinuka et al. (2005). Total phenol, flavonoid, saponin and alkaloid determination were done using method of Obadoni and Ochuko (2001) and Sahoré and Amani (2012), respectively.

Determination of the composition of essential oil

Gas chromatograph HP 6890 Powered with HP ChemStation Rev. A 09.01 [1206] software with the following condition was used for the determination of the essential oil component. The Injection method was split injection, the split ratio of 20.1, Carrier Gas is Hydrogen, Flow Rate: 1.0 ml/min. The inlet temperature of 150°C, Column Type of HP 5MS. Column Dimensions were: 30 m x 0.25 mm x 0.25 µm. Oven Program was: Initial at 40°C; ramped was from 5°C/min to 200°C, run at 200°C for 2 min. Detector was FID; detector temperature was 300°C; hydrogen pressure was 22 psi and Compressed air was 28 psi.

Mycotoxin detection and quantification

All analytical procedure for mycotoxin includes three steps. These include: extraction, purification and quantification. Gas chromatograph used for the mycotoxin analysis was HP 6890 powered with HP Chem Station Rev. A 09.01 [1206] software. The Gas chromatograph conditions were as follow: Carrier gas was hydrogen gas; detector used was PFPD. The simultaneous analysis of the fumonisins and ochratoxins in the sample with gas chromatograph was of good sensitivity. The reagent grade solvents and salts were used for sample extraction, cleanup and derivations; Deionised water was used where applicable. Standards were obtained from Sigma Aldrich. The modified AOAC method originally developed for corn and its products for mycotoxin analysis was followed for the extraction of the fumonisin in the sample. The pH was adjusted to 6.0 before filtration into the clean borosilicate beaker. 50 ml of the sample was applied to the anion exchange SPE column previously conditioned with 10 ml methanol followed by 10 ml of the methanol and water ratio 3 to 1 and followed by another 6 ml of ethanol. The fumonisins was eluted with 20 ml of the methanol and acetic acid ratio 95 to 5.

The eluents was concentrated using a stream of the nitrogen gas in a 60°C water bath. The modified method from R-Biopharm as follows, the sample was free of gas by mixing with a magnetic stirrer at 100rpm for 6ominutes. The pH was adjusted to 7.2. The column was washed with 20 ml of the deionised water at the flow rate of 5 ml per minute. An aliquot of 30 ml was applied to the column (Ochraprep, R-Biopharm). The sample was eluted into the vial with 3 ml of the methanol. The eluents was concentrated using a stream of the nitrogen gas in a 60°C water bath. The concentrated extract was combined and derivatised for the injection into the gas chromatography. 1.0 µl of the derivatised extract was injected into the chromatograph.

Statistical analysis

The mean and standard deviation of the triplicate data were calculated and subjected to analysis of variance (ANOVA) and a difference was considered to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Properties of sorghum-milet flour

Table 1 shows the physical and functional properties of sorghum-millet flour, the major raw materials in the production of kunu zaki beverage. The bulk density, swelling index, water absorption capacity, solubility and oil absorption capacity were 0.75 g/cm^3, 1.16, 2.49 ml H_2O/g, 2.56% and 0.79 ml oil/g, respectively. Bulk density of flour is important in determining the packaging requirement and material handling (Ezeocha et al., 2011). Swelling index is an indication of the water absorption index of the granules and reflects the extent of the associative forces within the granules (Moorthy and Ramanuhan, 1986). Water absorption capacity is the ability of flow properties to entrap large amount of water and also refers to total amount of water held by starch gel under a defined state of condition (Pinnavaia and Pizzirani, 1998; Chen and Lin, 2002). The physico-chemical properties of flour affects the textural characteristics of the food preparations made from grain. The behavior of starch in water is temperature and concentration dependent. Grain starches shows low uptake of water and swelling power at room temperature.

The pasting properties of sorghum millet flour are as shown in the Table 2. Peak viscosity of the flour was 1234 Cp while the holding viscosity was 811 Cp.

Breakdown and final viscosities were 423 and 1824.5 Cp respectively. Final viscosity showed the ability of the flour to form viscous gel after cooking and cooling. Setback value was 1013.5 Cp. Setback viscosity is a process that occurs during cooling in which the starch molecules start to re-order and subsequently form a gel structure. The higher setback value is indicative of higher rate of starch retrogradation. The viscosities observed by Liu et al. (2012) for sorghum flours were higher than the viscosities value observed for sorghum-millet flour. Peak time was 4.97 mm while peak temperature was 79.53 Cp.

Table 1. Functional properties of sorghum-millet flour.

Functional properties	Value
Bulk density (g/cm^3)	0.75±0.11
Swelling index (%)	1.16±0.09
Water absorption capacity (ml H_2O/g)	2.49±0.11
Solubility (%)	2.56±0.23
Oil absorption (ml oil/g)	0.79±0.17

Table 2. The Pasting properties of sorghum millet flour.

Pasting properties	Value
Peak (Cp)	1234±0.32
Holding strength (Cp)	811±0.53
Breakdown (Cp)	423±0.19
Final viscosity (Cp)	1824.5±0.20
Setback (Cp)	1013.5±0.23
Peak time (min)	4.97±0.017
Pasting temp (°C)	79.53±0.37

Table 3. Anti-nutritional composition of sorghum-millet flour.

Anti-nutritional factor	Concentration
Phenols %	0.11
Oxalate %	0.01
Phytate (mg/100 g)	0.05
Saponins (mg/100 g)	0.83
Flavonoid (mg/100g)	2.31
Alkaloid (mg/100 g)	2.22
Tannin (mg/100 g)	1.69

Peak time is the time required to achieve peak viscosity (Liu et al., 2003). The anti-nutritional compositions of the flour are shown in Table 3. The flour had low phenols (0.11%), oxalate (0.01%), phytate (0.05 mg/100 g) and saponins (0.83 mg/100 g). The values for flavonoid, alkaloid and tannin were 2.31, 2.22 and 1.69%, respectively.

Composition of *A. danielli* essential oil

Essential oil compositions of *A. daniellii* seed are shown in Table 4. The essential oil of *A. daniellii* contained 56 components. The components detected in the essential oil of *A. danielli* seed were dominated by 1, 8-Cineole (56.16 %), and five other main component being, β – Pinene (14.77 %), Alpha Terpineol (11.46 %), Alpha

Table 4. Composition of the major content of the essential oil of *Aframomum danielli*.

Component	Retention time (min)	%
Alpha pinene	9.70	4.28
β - Pinene	11.38	14.77
Cis-ocimene	12.26	1.59
Pinene-2-oL	13.84	1.16
Gama terpinene	14.89	0.05
Geranial (Neral)	15.40	0.01
1,8-Cineole	17.71	56.16
Citronellal	18.21	0.04
Alpha Terpineol	18.69	11.46
Terpinen-4-ol	18.79	3.05
Citronellol	19.27	0.04
Alpha terpinenyl acetate	21.10	4.24
Neryl Acetate	21.72	0.02
Humulene (alpha Caryophylene)	27.78	0.04

Table 5. Concentration of mycotoxins in grains and kunun zaki.

Mycotoxin	Concentration (µg/kg)	
	Millet	Sorghum
Fumonisin B_1	7.87	12.55
Fumonisin B_2	0.92	1.27
Ochratoxin A	12.13	21.27

Pinene (4.28 %), Alpha Terpinenyl Acetate (4.24 %) and Terpinen-4-ol (3.05 %). The result is in agreement with the findings of Adegoke and Krishna (1998). Though, Adegoke and Krishna (1998) isolated 41 essential oil components but 1,8-cineole (59.8%), Alpha pinene (4.3%) and Alpha terpinyl acetate (3.2%) were also among the major compounds detected in the essential oil. The composition of essential oil vary depending on genetic variability inside plant species, the geographical region of plant cultivation, the occurrence of biotic and abiotic stresses, the phonological stage of the plant and its chemotype, the method of drying and the method of extraction of the oils (Cosentino et al., 1999; Jerkovic et al., 2001; Labra et al., 2004; Rota et al., 2008; Zheljazkov et al., 2008). It had been reported that spices owe their antimicrobial properties mostly to the presence of alkaloids, phenols, glycosides, steroids, essential oils, coumarins and tannins (Ebana et al., 1991).

Concentration of mycotoxins in cereal grains and beverages

The concentration of mycotoxin in cereal grains are shown in Table 5. Ochratoxin A and Fumonisin B_1 have higher values of 12.13 and 7.87 ug/Kg, respectively in millet. These values were increased in sorghum with 21.27 and 12.55 ug/Kg in Ochratoxin A and Fumonisin B_1 respectively. These results show that the cereal grains were contaminated during storage with sorghum being more susceptible to mycotoxin contamination. Likewise, Aroyeun et al. (2011) detected ochratoxin A in cocoa powder during storage. Therefore, susceptibility of cereal grains to mycotoxin contamination depend on storage conditions, climatic conditions and handling of the cereals after harvest. The level of FB_1 which was 7.09 µ/l is well above the provisional maximum tolerable daily intake of 2 µg/g set up by the Joint Food and Agricultural organisation and World Health Organisation (FAO/WHO) expert Committee on Food Additives (JECFA) (WHO, 2002). The presence of mycotoxins in cereal beverages like kunun zaki is expected due to poor handling of grains both in the field and during storage in developing countries like Nigeria. Amina et al. (2012) reported the occurrence of fumonisin and deoxynivalenol in stored maize used in industrial production in Zaria, Nigeria. This is a matter of concern because high cost of soft drinks and increased consumers awareness about synthetic products brought about high increase in the consumption of cereal beverages.

The initial concentration of FB_1 in kunu zaki is 7.09 µ/l, with the application of 0.25 % *A. danielli* essential oil. The value reduced to 6.56 signifying 8% reduction of FB_1 while the highest dose of 10 % resulted in 76% reduction (Table 6). Sumalan (2013) also recorded that reduction of fumonisin in wheat grain was dose dependent. The results revealed higher reductions in FB_1 than FB_2. *A. danielli* exhibited ability to reduce FB_1, FB_2 and Ochratoxin A, although in varying amount. Aroyeun et al. (2011) observed reduction in the level of ochratoxins in cocoa powder treated with different concentrations

Table 6. Effect of *Aframomum danielli* essential oil on the concentration of mycotoxin in kunun zaki.

Concentration of essential oil (%)	Concentration (µ/l)		
	FB1	FB2	Ochratoxin A
0	7.09±0.11^{b}	0.96±0.05^{c}	12.10±0.09^{a}
0.25	6.56 ±0.02^{f}	0.72 ± 0.01^{d}	10.66 ± 0.01^{f}
0.50	5.56 ±0.01^{e}	0.70 ± 0.01^{d}	9.93 ± 0.01^{e}
1.00	3.38 ± 0.01^{d}	0.42 ± 0.01^{c}	5.49 ± 0.01^{d}
2.5	2.67 ± 0.01^{c}	0.38 ± 0.01^{bc}	4.14 ± 0.01^{c}
5.0	2.15 ±0.01^{b}	0.36 ± 0.01^{d}	3.38 ± 0.01^{b}
10.0	1.67 ± 0.01^{a}	0.33 ± 0.01^{a}	2.81 ± 0.01^{a}

of *A. daniellii*.

It has been reported that *A. danielli* possesses broad-spectrum antimicrobial properties as it had been found to inhibit the growth of some microorganisms such as *Salmonella enteriditis, Streptococcus aureus*, *Aspergillus niger* and *Pseudomonas fragii* (Adegoke et al., 2002; Ashaye et al., 2006). The essential oil of *A. danielli* is able to penetrate into the amine group and commence the process of deamination. This may be responsible for the reduction in the toxins as the doses of essential oil increases. Yoon et al. (2000) describe that the essential oil is capable of depolarizing the mitochondrial membrane and decreasing the membrane potential, this affect the Ca^{2+} and other ion channels, resulting in the reduction of pH and also affecting the proto pump and ATP pool. The change of the fluidity of the membrane resulted into the leakage of radicals, cytochrome C, calcium ions and protein. Thus, permeabilization of outer and inner mitochondrial membrane leads to cell death by apoptosis and necrosis.

Conclusions

The combination of raw materials (sorghum and millet) for kunu zaki beverage showed high viscosities with high setback value which indicate its ability to retrogradate after cooling. Introduction of different doses of *A. danielli* essential oil into kunu zaki reduced mycotoxins in the beverage. Percentage reduction of mycotoxins in kunu zaki depends on the doses of *A. danielli* essential oil used.

Conflict of interests

The authors did not declare any conflict of interest.

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Assessment of aflatoxin B1 and ochratoxin A levels in sorghum malts and beer in Ouagadougou

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Mycotoxins are natural metabolites produced by fungi that can cause disease and death in human and animals alike, even in low concentrations. More than thirty mycotoxins that contaminate sorghum (*Sorghum bicolor* L.) and its derived products are known. This work aimed to investigate the presence of aflatoxin B1 and ochratoxin A in sorghum malt and beer, sampled between January and April 2012 in Ouagadougou. Mycotoxins in fifty samples of sorghum beer and twenty samples of sorghum malts have been purified using immunoaffinity columns, and later identified and quantified using high performance liquid chromatography (HPLC). Aflatoxin B1 and ochratoxin A were no found in any of the sorghum beer samples. However, 25% of the sorghum malt samples contained aflatoxin B1 with an average of 97.6 ± 88.2 ppb ($p < 0.05$), while none contained ochratoxin A above the level of 0.8 ppb (ochratoxin A limit of detection). From the samples collected in this study, consumption of sorghum beer seems to be safe concerning contamination in aflatoxin B1 and ochratoxin A but a dietary assessment of exposure should be conducted.

Key words: Aflatoxin B1, ochratoxin A, sorghum beer, sorghum malt, HPLC.

INTRODUCTION

Cereals, such as sorghum (*Sorghum bicolor L.*) constitute an important part of the daily diet in Burkina Faso. Sorghum is used in the preparation of daily meals, and has been used additionally in the preparation of traditional beer commonly called "dolo" (Table 1). Dolo is the most commonly consumed alcoholic beverage (60% of population) in Burkina Faso where 75% of the total sorghum grain production is used for its preparation (Sawadogo-Lingani et al., 2007). This beverage is also used during some traditional ceremonies within the country. Sorghum ranks fifth in total area planted in the world after rice, wheat, corn and barley. It is the main cereal food for over 750 million people living in the semi-arid tropical regions of Africa, Asia and Latin America (Codex Committee on Food Contaminants, 2011). Sorghum is an annual crop, but some varieties are perennial. Sorghum grows in hot areas, with a minimum temperature of 25°C for maximum productivity, and

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Table 1. Types of dolos.

Ethnie	Mossé %	Gourounsi %	Bissa %	Bwaba %	Dagara %
Percentage	90	2	4	2	2

requires less water compared to maize (Codex Committee on Food Contaminants, 2011).Burkina Faso is the eleventh world largest sorghum producer with a production of 1.875 million tonnes in 2010 (FAO Statistics Division, 2010).

Malting is the process of germinating seed under favourable controlled conditions, followed by a drying step. Malted grain is used to make a series of products such as beer and whisky. In Burkina Faso, malting is a common activity, which is usually performed by women at home and necessitates a great expertise (Agropolis International, 2010). However, traditional techniques may result in products of questionable microbiological quality and are not suited to the requirements of large urban markets. *Enterobacteriaceae* and moulds which may produce mycotoxins can affect malt quality (Bennett, 1987; Lefyedi and Taylor, 2007). Several fungi species have been identified in sorghum and its derivatives (Comité du Codex sur les Contaminants dans les Aliments, 2011). The toxigenic moulds contaminate and grow on a wide variety of food and crops and are involved in many human and animal diseases (Fung and Clark, 2004; IARC, 1993; Hendrickse, 1997). Exposure to mycotoxins can cause both acute and chronic toxicities such as adverse effects on the central nervous system, the cardiovascular and pulmonary systems, gastro-intestinal tract, and on the liver. Mycotoxins can be carcinogenic, mutagenic, teratogenic and immune-suppressive (Castegnaro et al., 1998; Pfohl-Leszkowicz et al., 1998; IARC, 1993).

Given the importance of sorghum, this study focuses on malt and beer derived products. Depending on the malt processing and storage conditions, both products can be potentially contaminated by two main mycotoxins: aflatoxin B1 and ochratoxin A. Aflatoxin B1 and ochratoxin A are of special importance because, in addition to their adverse effects on humans and animals, they are resistant to traditional cooking conditions (Müller, 1982; Turcotte et al., 2013; Baldi et al., 2003). The aim of this study was to investigate the occurrence of aflatoxin B1 and ochratoxin A in different traditional sorghum' malt and beer (dolo) sold in the Ouagadougou area.

MATERIALS AND METHODS

Sampling

Sampling was conducted from January 2012 to June 2012.
Sampling was done at the level of dolo producers of the five districts of the city of Ouagadougou, which constitute the cluster unit of sampling.

Sorghum beer

Ten sorghum beer samples (0.5 L / sample) were collected in each district. A total of 50 samples were collected in this study. Sorghum beer samples were filtered, degassed using an ultrasonic, and then neutralized with NaOH (4 N) for a final pH of about 7.2. 50 mL of this sample were stored at 4°C until analysis.

Malt

Four malt samples were collected in each district, constituting a total of 20 malt samples at the end. About 100 g of sorghum malt sample were collected and stored in a transparent plastic bag at 4°C until analysis.

Survey sheet

An information sheet was given to each sorghum beer producer. This fact sheet was used to determine the origin of sorghum and evaluate producer's knowledge about potential risks associated with sorghum beer consumption. The forms were filled by a researcher who assisted the beer producer.

Equipment

The following equipment was used: HEIDOLPH UNIMAX 2010 for homogenization; vacuum VARIAN with pump; Agilent model 1100 HPLC with an Agilent fluorescence detector; derivatization module for the analysis of aflatoxins; Aflatest® and Ochratest® cleanup columns, stored at room temperature, were from Vicam (Watertown, MA, USA), with 60 ml polypropylene reservoirs and adapters for IAC chromatography; silanized 4 ml amber glass vials and 2 ml amber deactivated autosampler vials, with Teflon (PTFE) lined septa and threaded caps; Nitrogen gas was Ultra High Purity.

HPLC analysis

Ochratoxin A and Aflatoxin were separated by reversed-phase HPLC. Fifty µl of extracted sample were injected twice into an Agilent Zorbax HPLC, fitted with a C18 250 mm × 4.6 mm × 5 µm column, with a flow of 1 ml/min. Detection of the Aflatoxin B1 was performed with a post-column photochemical reactor, while Ochratoxin A was detected by fluorescence detector with excitation wavelengths (λex) of 360 nm for Aflatoxin B1 and 225 nm for Ochratoxin A, and at emission wavelengths (λem) at 440 nm for Aflatoxin B1 and 470 nm Ochratoxin A. A calibration curve was constructed for each mycotoxin, with correlation coefficients of 0.98 for both Aflatoxin B1 and Ochratoxin A. The limits of detection were 0.8 ppb for Ochratoxin A and 0.2 ppb for Aflatoxin B1. The purification procedure used for Aflatoxin B1 is described in the manual "Aflatest® Instruction Manual. Massachusetts: VICAM." And for ochratoxin A the procedure is detailled in ISO 15141-2. Immunoaffinity columns (Aflatest®, Ochratest®). A quality control test was performed for sorghum beer as well as for malt to determine the immunoaffinity column recovery rate for each type of mycotoxin. The determination of this rate was used to correct the final results.

Table 2. Quantity of AFB1in sorghum malt.

Sample	Positive 1	Positive 2	Positive 3	Positive 4	Positive 5
Concentration after correction (ppb)	66.51	64.18	56.26	46.33	254.73

Purification of aflatoxin B1 from sorghum beer

Ten 10 ml of the neutralized solution were placed in a flask to which 10 ml of ultrapure water were added and then vigorously vortexed. The solution was then ultrafitrated. Purification was done by passing 10 ml of the ultrafiltrate on immunoaffinity (IA) column Aflatest® through the purification vessel at a rate of approximately one drop per second; washing of the column was done with 10 ml of ultrapure water and the IA column eluting was done with 1 ml methanol (HPLC grade). The eluate was diluted with 1 ml of ultrapure water and transferred to a vial, ready to be injected in the HPLC system. The vials were placed on the auto-sampler along with the vials containing the mobile phase (600 ml of ultrapure water, 200 ml of acetonitrile, 300 ml of methanol and 132 mg of KBr + 385 .mu.l of HNO_3)

Purification of ochratoxin A from sorghum beer

10 ml of each neutralized solution sample were placed in a conical vial and 10 ml of a buffer solution (phosphate buffer) were added. After that the mixture was vortexed and ultrafiltrated. The next step was the purification of the mixture and for that, 10 ml were passed through an IA Ochratest® through a purification tank connected to a device with a vacuum pump in a flow of about one drop per second. Washing the immunoaffinity columns was done with 5 ml of PBS solution at a flow rate of two drops per second and 5 ml of ultrapure water at the same rate as PBS; the immunoaffinity column eluting was performed with 2 ml methanol (HPLC grade) and the eluate was collected in haemolysis vials. This phase ended with the evaporation of the eluates through an evaporator under nitrogen and the recovery was made in vials with 250 µl of mobile phase (water / acetonitrile / acetic acid, 990: 990: 20, v / v / v). After purification, the injection of the samples was performed.

Purification of aflatoxin B1 from sorghum malt

Extraction and purification were conducted following the procedure described in “Aflatest® Instruction Manual. Massachusetts: VICAM.” After spraying, a test sample of 25 g of malt was conducted and 5 g of NaCl were added to each sample. For extraction the samples were placed into vials and 100 ml of the extraction phase (methanol / water, 70:30, v / v) were added therein, then the whole was placed in stir for 30 min. This was followed by filtration by using a filter paper. 20 ml of filtrate were placed in a conical vial where 40 ml of ultrapure water were added. The mixture was vortexed. The extraction was completed by an ultrafiltration. The purification steps, injection and results reading followed the same procedure as for aflatoxin B1 in sorghum beer.

Purification of ochratoxin A from sorghum malt

Malt samples were sprayed in a mill (ROMER®), and then 5 g of NaCl was added to a test sample of 25 g malt. Sodium chloride was added to prevent the formation of foam during the addition of the extraction solution. The test samples were placed in vials and 100 ml of the extraction phase (methanol / water, 80: 20, v / v) were added to each sample and then subjected to a rotary shaker (HEIDOLPH UNIMAX 2010) for 30 min. The mixture was filtered (paper filter porosity of 10 µm), 10 ml of the filtrate were placed in a conical vial and 40 ml of PBS was added. The solution was vortexed, then ultrafiltrated by using micro-filters (0.41 microns porosity).
The steps in the purification of the injection and the reading of the results followed the same procedure as used for Ochratoxin A in sorghum beer.

Data analysis

The different parameters were compared using the comparison test of proportions and the comparison test of averages at 5% level.

RESULTS

Fifty sorghum beer samples and twenty sorghum malt samples were collected. Sorghums used by the producers in the two cases were all from the red sorghum variety and harvested in the year 2011. Producers in this study were all interviewed, and they belong to different ethnic groups: Mossé (45 samples), Gourounsi (one sample), Bissa (two samples), Bwaba (one sample), Dagara (one sample). Only 14.3% of the producers reported they knew about the risks associated with sorghum beer consumption; including misbehaviour related to drinking. The majority of the producers believe that the consumption of sorghum beer was free from risk. The analysis of sorghum malt samples did not yield any positive result for OTA. A quality control was performed for both Aflatoxin B1 and Ochratoxin A in malt and sorghum beer.

Sorghum malt

The recovery rate was 86.6% for Ochratoxin A and 76.9% for Aflatoxin B1.

Sorghum beer

The recovery rate was 70.0% for Ochratoxin A and 105% for Aflatoxin B1. Analysis of malt samples showed that 25% of samples were contaminated with AFB1. Contaminated malts were from three districts. Concentrations of AFB1 were corrected for the recovery rate (ppb) and are reported in Table 2. The average was 97.6 ± 88.2 ppb for malts samples. The value of the standard deviation showed a high variation of Aflatoxin B1 contents of malts. Analysis of the 50 sorghum beer samples

showed no detectable contents of Ochratoxin A and Aflatoxin B1.

DISCUSSION

The absence of detectable mycotoxins (aflatoxin B1 and Ochratoxin A) in sorghum beer samples raised a certain number of questions related to other results and especially the presence of aflatoxin B1 in malts. Although, it also revealed the limitations of the study because malt residues had not been quantified. Therefore, it was difficult to envisage the hypothesis that the absence of quantifiable Aflatoxin B1 and Ochratoxin A in sorghum beer was due to the fact that malts originally used for the production of sorghum beer were free of mycotoxins, despite its possibility. Indeed, this hypothesis was plausible because: 1) our study was carried out during the dry season, when water activity and the temperature would not favour the development of toxigenic moulds; and 2) sorghum used in the malting process was harvested in 2011 with a short period of storage. Similarly, other studies on sorghum beer have not found mycotoxins in sorghum beer, while they found that the malt samples were contaminated (for example, Nkwe et al. (2005) in Botswana and Trinder (1988) in South Africa). Other studies such as the one by Matumba et al. (2010) in Malawi reported that sorghum beer samples were contaminated with Aflatoxin (22.32 ppb). Thus, the absence of mycotoxins in sorghum beer could be to the heating during the preparation of sorghum beer. In the United States of America, Chu et al. (1975) showed that when brewing beer, the temperature had little effect on Aflatoxin B1 and OTA; he found about 14 to 18% of Aflatoxin B1 and 27 to 28% of OTA in the produced beer. These results were much greater in other studies; Oluwafemi et al. (2004) showed that 99.5% Aflatoxin B1 was destroyed in contaminated corn in 30 min at 250°C, compared to only 20% at 100°C. Uma Reddy et al. (2012) showed that the addition of moisture at different temperature gradients was able to improve the destruction of Aflatoxin B1 in extrusion of corn flour. They reported a destruction ranging between 30 and 90%, with 90% destruction obtained with a thermal treatment at 100°C combined with 30% humidity.

Furthermore, addition of salt added to the effect of temperature and moisture. Boudra et al. (1995) reported in their study that no change occurs when dry contaminated wheat with OTA was heated to 100°C for 40 to 160 min. However, 50% reduction of OTA resulted from humid heating at the same temperature. According to Kabak (2009), destruction of mycotoxins by temperature involves many factors among which the initial level of contamination, the type and concentration of the mycotoxin, the heating temperature, the degree of heat penetration correlated with moisture, pH and ionic strength of the medium; all play a crucial role in the degradation of mycotoxins. In Germany, Raters and Matissek (2008) found that OTA was stable up to 180°C, while AFB1 was completely destroyed at 160°C. According to the same authors, the degradation of mycotoxins is improved by the existence of certain substrates in the matrix, such as soy protein, carbohydrates and polyphenols. The above studies support the difficulty to eliminate entirely mycotoxins by the cooking temperature.

According to Kabak (2009), ochratoxin A' disappearance does not necessarily mean an absence or decreased risk of toxicity because the decomposition product can be just as dangerous as the parent molecule itself. A second possibility that might explain the absence of mycotoxins in sorghum beer is their microbiological denaturation. Many microorganisms capable of degrading OTA are reported in the literature. Commonly, the yeast used for fermentation in sorghum beer production is not pure. Usually, it contains many other germs other than lactic acid bacteria. However, as it is the case for the thermal degradation hypothesis, biodegradation also raises many shadows. Despite the absence of mycotoxins (Ochratoxin A and Aflatoxin B1) in beer in this study, we would recommend a degree of caution among consumers. The absence of mycotoxins does not mean a zero risk, especially with the finding showing that 25% of malt samples were contaminated with AFB1. Further studies are needed to shed some light on the difference in the microbiological quality between malt and beer; these studies should consider:

1. A controlled longitudinal study with contaminated malt. This study will remove the ambiguity on the alleged distortion of mycotoxins or rather their mode of elimination (search for AFB1 in the malt residues).
2. Microbiological studies to identify the microorganisms that are responsible about the elimination of mycotoxins during the preparation of sorghum beer.
3. Develop methods that minimize contamination of sorghum malts. This will certainly require sensitization of producing malt and sorghum beer. The collaboration of producers associations may be requested as well as public health authorities.

Conflict of interests

The authors did not declare any conflict of interest.

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Donkey cheese made through pure camel chymosin

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Cheese from donkey milk was never produced by traditional way. Research suggests that asinine milk by bovine chymosin form a very weak gel compared to the gel formed from bovine milk and with out curd formation. Encyclopedia of Dairy Sciences 2nd edition reported that no cheese is made from donkey milk, due to its particular caseinic composition that makes inefficient the traditional rennet coagulation. However, the author has recently discovered that pure camel chymosin is able to clot effectively the casein micelles of donkey milk. This investigation is aimed at experimental manufacturing of donkey cheese through pure camel chymosin. A total of five experiments were included in the present investigation. Before making cheese, the raw donkey milk samples were analyzed for their physico-chemical composition in order to better evaluate the successive properties of fresh donkey cheese. Cheese making using pure camel chymosin gave a yield of about 3.32% of fresh donkey cheese, however relative to the content of protein and fat of donkey milk. The fresh cheese after 12 h in molds, had a good content in total solids (35.65%). The low pH in cheese, after 30 min from the production (pH_{at} 30 min 5.34), shows the efficient activity of starter cultures in asses' milk, and allows a good protection against pathogens agents. This application represents an informative step for further trials and could be useful for industrial scale cheese processing of donkey milk. More research is needed to study the mechanism of enzymatic coagulation in equid species and if donkey cheese can be addressed, such donkey milk, as alternative food for people suffering from food allergies.

Key words: Donkey milk, camel chymosin, donkey cheese, milk clotting enzyme.

INTRODUCTION

The use of asinine milk by humans for alimentary and cosmetic purposes has been popular since ancient times, but it was not until the Renaissance that the first real scientific consideration was given to this exceptional milk, more recently it has been used successfully as a substitute for human milk in Western Europe (Vincenzetti et al., 2008), and as an alternative food for infants with food allergies (Salimei and Fantuz, 2012; Polidori and Vincenzetti, 2013) and to upregulate the immune response of healthy elderly humans (Salimei and Fantuz, 2012). Moreover, donkey milk whey proteins showed *in vitro* antiproliferative and anti-tumor activity (Mao et al., 2009). These properties have allowed its diffusion as fresh milk, milk powder and even in cosmetic products.

However cheese from donkey milk was never produced by traditional way and, in scientific literature, alternative

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production methods are not reported.

Cheese production and applications of enzymes

Cheese production represents one of the earliest bio-technological applications of enzymes (Szecsi, 1992). The active ingredients in this process were identified as the proteolytic enzymes pepsin and chymosin, previously referred to as 'rennet' (Foltmann, 1966).

The dairy industry characterizes rennet enzymes using two properties. The first is the milk-clotting activity (C) expressed in International Milk-Clotting Units (IMCU). It is determined by a standard method (International Dairy Federation, 2007) that describes the ability to aggregate milk by cleaving the Phe_{105}-Met_{106} bond or nearby bonds of κ-casein. The second property is the general proteolytic activity (P), which is the ability to cleave any bond in casein (Kappeler et al., 2006). The ratio between the two properties, the C/P ratio, captures the essential quality of a milk-clotting enzyme. The higher the value of this ratio the better the rennet, and in this regard chymosin is superior to all other known rennet enzymes (Foltmann, 1992). The C/P ratio of bovine chymosin towards bovine milk is higher than those of the chymosins from lamb, pig, cat and seal (Foltmann, 1970).

In bovine milk with rennet, chymosin hydrolyses the Phe_{105}-Met_{106} bond of κ-casein. As a result, the micelles lose steric stabilization and become susceptible to aggregation, particularly in the presence of Ca^{2+} (Walstra, 1990; Walstra et al., 2006).

Equine milk with calf chymosin is susceptible to hydrolysis at the Phe_{97}-Ile_{98} bond of equine κ - casein (Egito et al., 2001), but its hydrolysis is considerably slower than that of bovine κ -casein (Kotts *et* Jenness, 1976) and with out gel formation (Uniacke-Lowe *and* Fox, 2011).

Preliminary research, suggests that asinine milk under the action of bovine chymosin, forming a very weak gel, G' of 10–15 Pa, after 60 min at 30°C, compared to 180–200 Pa for bovine milk coagulum under similar conditions (Uniacke-Lowe and Fox, 2011) and with out curd formation. This result is due by the particular caseinic composition of donkey milk, because in asses' milk the k-casein, the key protein in the clotting process, is present only in traces (Amadoro et al., 2011). Differently, k-casein represents about the 13% of total caseins in cow's milk (Malacarne et al., 2002); small quantities in horse (Malacarne et al.,2002); and 5% in camel (Jardali et Ramet, 1991).Therefore, it is not surprising that the majority of pastoral systems have produced at least one type of cheese, no traditional methods exist for making cheese from donkey milk; in Encyclopedia of Dairy Sciences 2nd (Fuquay and Fox, 2011) was reported that no cheese is made from donkey milk.

However, until today, only through lactic fermentation or thermo-acid coagulation could produce a dairy product from donkey milk (i.e. yogurt cheese, ricotta, cottage cheese without rennet), but these products have very different organoleptic, nutritional and rheological characteristics from a cheese obtained with the use of coagulating enzymes, from rennet.

A decisive step in the possibility to use donkey milk in the dairy sector, for cheese production, has been made recently thanks to the surprising finding of the italian food technologist, Iannella (2014), which has discovered that pure camel chymosin, enzyme found in camel rennet, is able to clot effectively donkey milk (Iannella,2014).
For this reason pure camel chymosin now used to curdle camel milk (Bruntse, 2011), can also be used for donkey cheese production (Chr Hansen Co. and Iannella, 2015).

Unfortunately, computational methods for proteins have not yet reached a state that enables the modelling of the interactions of an entire casein micelle with chymosin, in particular the pure camel chymosin with casein micelles of donkey milk. Without wishing to be bound to any theory, it is contemplated that curd is formed when raw donkey milk is treated with pure camel chymosin (Iannella, 2014) with a proper dairy technology, because Iannella found that donkey's milk subjected to a prior heat treatment, pasteurization or thermization, did not give a clot (Iannella, 2014).

Taking into account the complexity of the natural casein substrate, Iannella affirms that the improved milkclotting activity of camel chymosin can be attributed to variations on the surface charge, at the binding sites, that facilitate the association between camel chymosin and the casein micelles.

The promising industrial applications of camel chymosin were first reported by Kappeler et al. (2006), who have shown that camel chymosin synthetized by *Aspergillus niger* have different characteristics than bovine chymosin. Camel chymosin exhibits a 70% higher clotting activity for bovine milk and has only 20% of the unspecific protease activity for bovine chymosin. This results in a sevenfold higher ratio of clotting to general proteolytic activity. Kinetic analysis showed that half-saturation is achieved with less than 50% of the substrate required for bovine chymosin and turnover rates are lower (Kappeler et al. 2006). Camel chymosin, moreover, contains two additional positive patches that favor interactions with the substrate. The improved electrostatic interactions arising from the variations on the surface charges and the greater malleability both in domain movements and substrate binding of camel chymosin contributed to better milk-clotting activity in bovine milk (Langholm Jensen et al., 2013). In addition, an increase flexibility of the relative orientation of the two domains in the camel enzyme contributes to improved substrate binding by camel chymosin in casein micelle (Langholm Jensen et al. 2013).

While raw camel milk cannot be clotted with bovine chymosin (FAO, 2001; Ramet, 2001), a high clotting activity was found with camel chymosin (Kappeler et al.,

2006). However the ability of pure camel chymosin to clot donkey milk (Iannella, 2014) was a surprising found because as mentioned k-casein is present in trace amounts in casein micelles of asses' milk (Amadoro et al., 2011). Taking into account the complexity of the natural casein substrate, Iannella hypothesizes that the improved milk clotting activity of camel chymosin can be attributed to variations on the surface charge, at the binding sites, that facilitate the association between camel chymosin and the casein micelles. Iannella supposes that the key proteins in the efficient coagulation process, by pure camel chymosin, of donkey milk are especially β-caseins.

The best breeding Asinine management practices, which has improved the quantity and quality of milk produced, combined with the growing interest in donkey's milk due to its nutraceutical properties, might spawn the interest of producing cheese with donkey milk.

However, the processing of donkey milk into cheese is technically more difficult than milk from other domestic dairy animals.

This is mainly due to its low total solids and casein content. This investigation is aimed to develop cheese making technology from donkey milk through pure camel chymosin, and could be useful for industrial scale cheese processing of donkey milk.

MATERIALS AND METHODS

Sample collection and physicochemical properties of donkey milk

Fresh whole donkey milk was obtained from Montebaducco pharma© (1990), specialized in livestock and production of donkey milk; District in province of Reggio Emilia (Italy).

The animals were of indigenous breed (*Romagnola*). The milk samples were collected from 40 donkey between 2° to 4° month of lactation.

Individual milks samples, not refrigerated, were mixed in one batch to perform donkey cheese manufacturing in the laboratory. The tests were started within 2 h from collection occurred just after the morning milking**.**

Coagulant

Pure camel chymosin, named FAR-M® (2011) and product from Chr Hansen©, Denmark, was used; product in highly-stable powder. Dosage used in all tests was 4 g /50 L of donkey milk.

Coadjuvant

Commercial thermophilic starter culture for fresh cheese (Freeze-dried DVS of *L. delbrueckii ssp. bulgaricus and S. thermophilus*) was used for acidification of milk. Dosage used as indicated on the package.

Skimmed milk samples were analyzed for pH values by using pH meter (Hanna Instruments HI221 pH/mV/ORP), percentage of fat, total protein and lactose, was determined by automatic milk analyzer device (Lactoscan MCC which uses ultrasonic technology) calibrated for donkey milk.

Donkey cheese manufacturing

A total of five trials of cheese-making from donkey milk were conducted, 1 test per day for 5 consecutive days, to prepare fresh donkey cheese.

Fifteen liters of donkey milk, kept at room temperature, were taken in a stainless steel container and heated to 37°C in a water bath at 43°C. Then the milk was inoculated with thermophilic starter cultures *(L. delbrueckii ssp. bulgaricus* and *S. thermophilus*), after 1 h 30 min was added pure camel chymosin, FAR-M® (0.4 g /5 L of donkey milk).

The milk was incubated for 5 h, at room controlled temperature at 37°C. After the curd formation, most of the whey was removed, and the curd was cut and then the remaining whey was drained off.

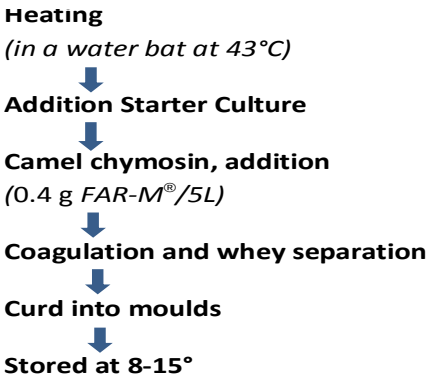

Figure 1. Flow diagram for the manufacture of fresh cheese from donkey milk. “Nativity equid donkey cheese making method , by Giuseppe Iannella©.

The curd was moulded and pressed at room temperature. Cheese was kept in the mould, weighed, sampled and stored at 15°C for further evaluation.

Physico-chemical evaluation of fresh donkey cheese

Cheese yield

Cheese yield (after 12 h in mould) was calculated using an analytical balance, and was expressed as a percentage. Cheese yield % = weight of cheese (g)/weight of the milk sample (g) x100.

pH

pH were measured using pH meter (Hanna Instruments HI221 pH/mV/ORP), after 1 h that cheese was in the modules.

The total solids

The total solids of fresh donkey cheese, after 12 h in modules, were analyzed following standard procedures (AOAC, 1995).

Statistical analysis

Test results are expressed as Mean ± Standard deviation (SD) of five test using Excel 2010 as a statistical program.

RESULTS AND DISCUSSION

The flow steps for the manufacturing of fresh donkey cheese are summarized in Figure 1.

Starter cultures are responsible for the production of lactic acid, which improved curd firmness and inhibited the growth of undesirable bacteria in the curd. In addition the pure camel chymosin in previous studies has shown an optimum pH at 5.1 (Kappeler et al., 2006). The longest time of coagulation (5 h) is due to different caseins in donkey milk, which may related to the poor rennet aptitude of donkey milk. The gross composition of donkey milk differs considerably from milk of the principal dairy species. In comparison with bovine milk, donkey milk contains less fat, protein, inorganic salts but more lactose, with a concentration close to that of human milk (Table 1).

Table 1. Average percentage composition (g/100g) found in donkey's milk, compared with other milk (Salimei et al. 2004; and Guo et al. 2007).

Parameter	Donkey	Cow	Uman
pH	7.0-7.2	6.6-6.8	7.0-7.5
Proteins	1.5-1.8	3.1-3.8	0.9-1.7
Fat	0.3-1.8	3.5-3.9	3.5-4.0
Lactose	5.8-7.4	4.4-4.9	6.3-7.0
Ashes	0.3-0.5	0.7-0.8	0.2-0.3
Solid content	8.8-11.7	12.5-13.0	11.7-12.9
Casein %	45	78	28

Table 2. Average chemical composition (Mean±SD) of donkey milk used for cheese preparation (five replications).

Parameter	Value in donkey milk
pH	7.06±0.06
Fat (%)	1.11±0.34
Protein (%)	1.61±0.12
Lactose (%)	6.08±0.28

Table 3. Physico-chemical parametres (Mean±SD) of Fresh donkey cheese.

Parameter	Value in donkey cheese
pH_{10min}	5.34±0.08
$Yield_{at\ 12\ h}$	3.32±0.31
Total solids (%) at 12 h	35.65 ± 4.35

Moreover, the fat content, according to Salimei et al. (2004) and Guo et al. (2007) shows marked variability indicating that it could be affected by breed, breeding area forage, milking technique, interval between milkings and mainly stage of lactation as also reported by Fox (2003), with a negative trend throughout lactation.

Mean composition of donkey milk used for manufacturing cheese samples is shown in Table 2. The protein contents was in the range of the normal values reported in the literature (Salimei et al., 2004; Guo et al., 2007) whereas fat content, which is a parameter more variable in ass milk, was higher than reported (Table 1).

Physicochemical evaluation of fresh cheese made from donkey milk

In all the samples, the coagulum obtained with donkey milk, after 5 h from the addition of pure camel chymosin (FAR-M®), was a precipitate in the form of clot, with elastic properties.The pure camel chymosin utilized in this investigation, FAR-M® is able to allow the production of donkey's cheese with doses of 0.4 g / 5 L. Table 3 shows some physico-chemical parameters of fresh donkey cheese, after 10 min in the mold for pH and after 12 h for yield and solids content.

The curd (3.32%) with a good content in solid substance (35.65%) was obtained when cheese was prepared following the previous procedure. In a previous work Iannella (2014) has observed that only raw donkey's milk, therefore not heat treated nor pasteurized, is able to clot; for this reason the donkey's milk cannot be heat treated in this process.

The negative result in donkey milk heat-treated is due to the change of proteins conformation in response to this process; this result in donkey milk is very important and this prevents the subsequent enzymatic reaction.

Conclusion and recommendation

Manufacture of fresh cheese is feasible. The average cheese yield obtained from donkey milk (3.32%) however, was lower than that reported for cow milks, depending to the contents of protein and fat of this milk (Tables 1 and 2). The result is promising and shows possibilities of improving the quality of such cheese in the future.

In the process, the use of starter cultures is necessary because they are responsible for the production of lactic acid, which improve curd firmness and suppresses the growth of undesirable bacteria in the curd, obtained from raw milk. In addition the pure camel chymosin have optimum pH at 5.1 (Kappeler et al., 2006), therefore also for this reason the pH of the milk must be lowered. These data indicate that cheese making without use of starter cultures should be discouraged.

However, more research is needed to study the mechanism of enzymatic coagulation of donkey milk, with pure camel chymosin and to improve cheese yield.

Further investigation is warranted to determine if there are differences in the coagulation properties of asinine and equine milk and if donkey cheese can be to people sensitive to milk protein allergies

Conflict of interests

The author did not declare any conflict of interest.

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