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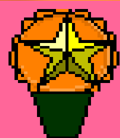

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Review

Spontaneously fermented kenyan milk products: A review of the current state and future perspectives

John Masani Nduko*, Joseph W. Matofari, Zacchaeus Okoth Nandi and Moses Barasa Sichangi

Department of Dairy and Food Science and Technology, Egerton University, P. O. Box 536-20115, Egerton, Kenya.

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Many spontaneously fermented milk products are produced in Kenya, where they are integral to human diet and play a central role in enhancing food security and income generation. Some of these products have demonstrated therapeutic and probiotic effects although recent reports have linked some to death, biotoxin infections, and esophageal cancer. These products are mostly processed from poor quality raw materials under unhygienic conditions resulting to inconsistent product quality and limited shelf-lives. Though very popular, research on their processing technologies is low. This review provides a comprehensive summary of the most common spontaneously fermented milk products from Kenya including *Mursik*, *Kule naoto*, *Amabere amaruranu* and *Suusa*. Their production challenges and future perspectives are highlighted; emphasizing the need for application of high throughput biotechnologies in their study. Available literature on their microbiology, biochemistry, and chemical composition is summarized. Moreover, knowledge on the value of clean starting raw material, fermentation parameters definition, and employment of standard equipment are discussed.

Key words: Starter culture, probiotics, lactic acid bacteria, fermented milk, high throughput biotechnology, spontaneous fermentation, *Kule naoto*, *Mursik*, *Amabere amaruranu*

INTRODUCTION

Fermentation of food is one of the oldest methods of food processing and preservation that is entrenched in traditional cultures and village life (Pederson, 1971; Campbell-Platt, 1994). It has evolved into a method of preserving foods during times of scarcity, imparting appropriate aroma and flavors to foods, decreasing product toxicity,and generating product diversity in diets including staple foodstuffs such as milk, tubers, cereals, and fish (Belton and Taylor, 2004; Chelule, Mokoena and Gqaleni, 2010). Additionally, fermentation reduces the bulk of material to be transported, it improves the nutritive value and appearance of food, and it reduces the energy required for cooking (Holzapfel, 2002). Traditionally, fermented products have a special part in social

*Corresponding author. E-mail: jnduko@egerton.ac.ke.

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functions such as circumcision,marriage,victory, naming, and rain making ceremonies (Hounhouigan, 1994; Muigei et al., 2013). Currently, fermented foods contribute 20 - 40% of the global food supply and approximately one third of the food consumed by man is fermented food. This renders fermented foods and beverages a substantial component of the global diet (Campbell-Platt, 1994; Chilton et al., 2015).

Several traditional fermented products have been documented in different African countries including non-alcoholic beverages, alcoholic beverages, breads, pancakes, porridges, cheeses, and milks. Some of these products including fermented cereals, tubers, and roots are dietary staples in Africa hence; they are important for food security (Marshall and Mejia, 2012). In Kenya, fermented foods and beverages are produced at household/ village level using traditional fermentation technology from various raw materials such as cereals, milk, honey, sugarcane, bananas, and coconut sap, among others (Nout, 1981; Mathara et al., 2004; Kunyanga et al., 2009; Nyambane et al., 2014). Milk fermentation is a common method for preserving milk and many communities ferment milk to produce desired products. In some communities, fermented milk plays a crucial role in the nutrition of vulnerable groups of population such as infants, pregnant women, young children, and the elderly (Chilton et al., 2015). Examples of fermented milk in Kenya include the *Mursik* from the Kalenjin community (Muigei et al., 2013), *Amabere amaruranu* from the Kisii community (Nyambane et al., 2014), *Kule naoto* from the Maasai community (Mathara, 1999), and *Suusa* from the North Eastern pastoralist communities (Lore et al., 2005). The fermentation processes for the production of these fermented products are typically uncontrolled and are dependent on microorganisms from the environment.

These household fermentation technologies have not been upgraded into industrial scale in order to meet the growing demand for traditional fermented products by the urban and immigrant population. The household production of these products is laborious and time-consuming for the urban/ immigrant population and the raw materials could be absent; hence the need for production of these products efficiently with assured safety, quality, packaged for extended shelf life, broader acceptance, and in a ready-to-use/ easy-to cook form from high qulity raw materials (Nout and Sarkar, 1999). This can be realized only if the traditional fermentation technologies are characterized to identify starter cultures that could be used to give products with consistent and improved quality and assured safety together with fermentations taking place in reactors under controlled conditions, and the need for high quality raw materials emphasized (Marshall and Mejia, 2012). Fermented foods and the microorganisms that contribute to the fermentation process have also been associated with many beneficial effects on human health and food preservation, however they are underexploited. Although there have been studies characterizing the microorganisms in some of the Kenyan spontaneously fermented products (Lore et al., 2005; Mathara et al., 2004; Nieminen et al., 2013; Nyambane et al., 2014), there has been no technology in Kenya that has been developed to modernize the production of the traditional fermented products using starter cultures under controlled conditions or exploiting their probiotic and antimicrobial potential (FAO, 2010; Reid et al., 2014). This is partly because most studies intended to characterize traditional fermentations employs the classical microbiological culturing techniques, which are inadequate in identifying and characterizing microbial consortia. The more powerful high throughput biotechnologies have not been employed in identifying starter cultures/probiotics and other fermentation aspects have not been characterized; thus there is limited progress in upgrading the traditional fermentation technologies. Consequently, modern socio-economic changes may cause some traditional technologies for the production of fermented foods to be lost together with the associated microorganisms (Akabanda et al., 2013; van Hijum et al., 2013; Reid et al., 2014).

This review aims to list and recap the production processes of common Kenyan traditional spontaneously fermented milk products and to highlight, where available, some of the microbiological and biochemical properties of the fermented milk products, challenges, and suggest areas of research/ innovation for the advancement of these traditional technologies for industrialization, culture preservation, and for satisfaction of the growing demand for the products.

FERMENTED MILK PRODUCTS IN KENYA

Traditional spontaneously fermented milks are produced in Kenya and they have been consumed for many years due to the belief that they promote good health (Mathara et al., 2008). These products are mostly produced in rural areas and their preparation methods have some variations e.g. smoking/ nonsmoking, boiling/ not boiling milk, and use of back slopping/ non usage. These variations results to textural and flavor differences and microbial compositions. The most common fermented milk products in Kenya are discussed below.

Mursik

Mursik is the spontaneously fermented bovine milk product which is mainly produced by the Kalenjin community in Kenya, whom milk is a staple diet. The milk is prepared in gourds (*sotet*), where the milk is left to

ferment for 3–5 days or more dependent on sensory preferences. The Kalenjins have preferred this milk and use it to mark special occasions such as symbolizing success in negotiating marriages and weddings and success in athletics. *Mursik* is also consumed by breast feeding mothers and initiates. It is believed that one is strengthened and the immune system against common diseases is boosted by consuming *Mursik* (Muigei et al., 2013; Mathara, 1999).

Mursik production

The production of *Mursik* starts with the preparation of the gourd. First, the top of the gourd is cut and the seeds are removed. It is then cleaned using a cured wood stick (*Sosiot*), obtained from branches of a palm tree. The gourd is then left outside for a few hours to dry. After drying, the preserving tree (*Senna didymobotrya*) sticks are burnt and the burning embers are added into the gourd but taking care not to burn the gourd. The gourd is considered ready when the inside is evenly covered with fine dust. The purpose of using the burnt stick is to improve the flavor of *Mursik*, pasteurize the gourd, and the coloring of *Mursik* (Mathara et al., 1995). After milking, the milk is boiled and cooled down. The cooled milk is poured into the prepared *sotet* then covered tightly with a lid. The milk is stored in a cool and dry place for about one week to allow it to ferment. Upon fermentation, the gourd is shaken to ensure that the *Mursik* has a smooth uniform consistency. It is commonly consumed after meals or sometimes served along with other staple foods such as hot *Ugali* (Mathara et al., 1995).

Microbiology of Mursik

Studies on *Mursik* have shown that the fermentation is carried out by lactic acid bacteria (LAB) (Mathara et al., 1995). Nieminen et al. (2013) analyzed *Mursik* samples that were fermented using small batches of *Mursik* collected from Kenya. The total microorganisms at 48 and 72 h of fermentation were 10.42±0.21 and 9.54±0.43 log_{10} cfu/mL, respectively. The pH of the fermented milks was 3.46±0.04 at 14 days. The dominant bacteria (determined by 16S rDNA sequencing) were *Lactobacilli* (8 species), which were present in 6 out of 8 samples that were analyzed. Eight *Lactobacillus* species were identified and the *Lactobacillus kefiri* was more frequent in the samples (6 out of 8 samples) with a mean of 37% of the colonies. In 5 of the samples, *L. kefiri* was found in combination with *Candida krusei*. The other common *Lactobacillus* species were *L. casei*, *L. paracasei* and *L. rhamnosus*. Other species were less prevalent (mean 27%, range <1–93%, of total colonies). Among these, *Bacillus* spp. were most prevalent (present in 5/8 samples). Yeasts were present in all the samples of *Mursik* and their proportion in the samples varied between 7 and 90%. Three *Candida* species (*C. krusei, C. sphaerica* and *C. kefyr*) and one *Saccharomyces* species (*S. fermentati*) were detected in the samples, with *C. krusei* being the most common (identified in 5/8 samples). *Candida kefyr* and *C. sphaerica* were present in 3/8 samples whereas, *S. fermentati* was found in only one sample (1/8) (Nieminen et al., 2013).

Muigei et al. (2013) attempted to identify exopolysaccharide-producing LAB in *Mursik* obtained from Nakuru County, Kenya. Among the LAB, the *Lactococcus* spp. was more dominant than the *Lactobacillus* and *Leuconostoc* spp. The most prevalent *Lactococci* was *Lactococcus lactis* subspp. *lactis*. In another study (Digo, 2015), it was found that the proportion of LAB in *Mursik* samples collected from Bomet County, Kenya was 82% of the total microorganisms. The coliform counts were 6.98% and the yeasts and molds count were 11.02% prevalent. The dominant LAB were *Lactobacillus* (56.10%) while the other LAB (*Lactococcus*, *Enterococcus* and *Leuconostoc*) consisted 25.9% of the total microorganisms. The *Lactobacillus* species identified were *L. plantarum*, *L. fermentum*, *L. brevis* and *L. casei* (Digo, 2015).

From these reports, it is clear that *Mursik* has not been clearly characterized. Whereas Nieminen et al. (2013) recorded *L. kefiri* as the dominant LAB; Digo (2015) did not record it. The differences could be ascribed to the broad variation of *Mursik* due to household differences in preparation of *Mursik* and/or due to geographical differences. Moreover, Nieminen et al. (2013) used a back-sloping method to produce *Mursik* using small batches of *Mursik* that had stayed at room temperature for 4 weeks. These differences could also explain why Digo (2015) detected coliforms that were not identified by Nieminen et al. (2013). If *Mursik* is left at room temperature, the LAB could produce excess lactic acid that will inhibit coliforms and other microorganisms; however this needs to be confirmed.

Although *Mursik* is a popular product that can be found in urban areas, its consumption has been linked to the occurrence of esophageal cancer and deaths (Patel et al., 2013; Daily Nation, 2015). The consumption of *Mursik* as a risk factor to the occurrence of esophageal cancer has been attributed to the polycyclic aromatic hydrocarbons (PAHs), which are carcinogens that originate from charcoal powder added to the *Mursik*. *Mursik* has also been shown to contain high levels of acetaldehyde that may contribute to esophageal carcinogenesis (Patel et al., 2013; Nieminen et al., 2013). Acetaldehyde is a mutagenic carcinogen and was detected in *Mursik* at levels (>1800 µg/L) that are 4 times times higher those in commercial yogurt.Ethanol levels>100 mmol/Lwerefound in *Mursik* (Niemeinen et al., 2013).

The microbes in *Mursik*, particularly *C. kefyl* have been

demonstrated to produce high acetaldehyde in fermented milk when added together with LAB starter cultures (Gadaga et al., 2001). The high acetaldehyde levels were found to correlate positively with ethanol levels. Many microbes such as *Candida* and *Streptococcus* are capable of producing acetaldehyde from ethanol. In *Mursik*, *L. kefiri* was in combination with *C. krusei* and this combination had high acetaldehyde and ethanol levels. Although *C. krusei* is a poor producer of acetaldehyde from ethanol in pure cultures, its existence with other microorganisms in *Mursik* can enhance its production potential (Nieminen et al., 2013).

It is therefore pertinent that standardization of the *Mursik* fermentation process using starter cultures that will reduce contamination and acetaldehyde and ethanol production will be a great milestone towards assuring safety and quality of *Mursik*. To achieve that, there needs to be molecular characterization of the microorganisms in *Mursik*, trials to select the best combination of microorganisms (starter cultures/probiotics), a protocol giving a safe and acceptable product, sensory evaluation for acceptability of developed products needs to be done, and safety and quality standards developed.

Amabere amaruranu

Amabere amaruranu is the product of spontaneous fermentation of bovine milk produced by the Kisii community in Kenya. Mostly, the milk to be fermented is boiled, cooled and added to the gourd (*ekerandi*) for fermentation. The fermentation is spontaneous, however normally back-sloping is used where a small batch of previous successfully fermented milk (*enduranerio*) is added to fresh milk to initiate the fermentation (Nyambane et al., 2014). The milk is valued for provision of nutrition to children and the elderly. It is normally consumed along with *ugali* prepared from maize or millet flour. Sometimes, the milk is mixed with blood to prepare a fermented product called *omokoora*. In the preparation of *omokoora*, blood is first boiled and once it thickens, it is cooled, broken into small pieces then added to the gourd. It ferments for 2–3 days, then it can be consumed similar to *Amabere amaruranu*. Addition of blood can affect microbial metabolism since blood is rich in iron; a cofactor for numerous cellular processes (Nieminen et al., 2013). Both products can be consumed for extended periods whereby after consumption, backslopping starter culture (*enduranerio*) is left in the gourd, then fresh milk is added and the cycle can be conducted several times. This ensures that the milk does not sour excessively and the backslopping maintains the quality of the milk. In recent times, many people use different containers including plastics for fermenting the milk.

There has been little research on the microbiological and biochemical attributes of the *Amabere amaruranu*. However, in a pioneering study of *Amabere amaruranu*, Nyambane, Thari, Wangoh & Njage (2014) analyzed the microbiological composition of the product. The total viable counts were high ($\log_{10}$ 8.06 ± 0.59 – 8.24 ±0.40 cfu/ml). Coliforms, yeasts and molds were analyzed in addition to the LAB, which carries out the fermentation. The LAB were identified to be members of the genera *Lactobacillus* (45%), *Streptococcus* (25%), and *Leuconostoc* (20%). Of the LAB, the most dominant was *Streptococcus thermophiles,* which comprised of 25% of all LAB isolated and was in all the analyzed samples. *Lactobacillus bulgaricus* subsp. *bulgaricus* made up 15% of the total number of LAB isolates. This organism, together with *Streptococcus thermophilus* are normally used as starter cultures for yoghurt production. *Leuconostoc mesenteroides* subsp. *mesenteroides* consisted of 20% of all the LAB isolates. Another highly prevalent *Lactobacillus* in *Amabere amaruranu* was *L. plantarum,* which comprised 20% of the isolates. A 5% of the isolates were identified as *Lactobacillus helveticus.*

Yeasts were isolated from *Amabere amaruranu* but no mold was detected. The yeast species that were identified belonged to the genera *Saccharomyces* (25%), *Candida* (20%), and *Trichosporon* (15%). However, the Analytical Profile Index (API) system used was unable to identify 40% of the yeast isolates. Yeasts have been detected in many spontaneously fermented types of milk. Therefore, their role in the fermentation needs to be studied.

The study by Nyambane et al. (2014) identified most of the microorganisms in *Amabere amaruranu* particularly the bacteria. The identified microorganisms need to be studied further for their properties and their interactions in the design of starter cultures for controlled production of *Amabere amaruranu*. The bacteria in *Amabere amaruranu* have been demonstrated to exhibit probiotic potential. For example, Boyiri (2014) used 16S rRNA gene sequencing to identify a *Lactobacillus rhamnosus* strain in *Amabere amaruranu* that was bile stable, nonmucinolytic and had antibacterial activity. The strain also stimulated increase in MUC4 and MUC3 expression in colon cells. Kotala and Onyango (2015) have also demonstrated that the cell extract of *L. rhamnosus* from *Amabere amaruranu* downregulates the expression of numerous adipogenic-related transcription factors. At high dose levels, the cell extracts were found to down regulate peroxisome proliferator-activated receptor-ϒ, sterol regulatory element-binding protein 1, and adipose triglyceride lipase. These results demonstrated that the cell extracts from *L. rhamnosus* from *Amabere amaruranu* could be employed for anti-obesity management regimes; hence the probiotic potential of *L. rhamnosus*. Moreover, Mokua (2004) demonstrated the antibacterial effect of *Amabere amaruranu* on *E. coli*. This clearly indicates that the microorganisms in *Amabere amaruranu* have probiotic potential that is yet to be

exploited in product development. The role of yeasts in the fermentation also needs to be characterized. The API system that was employed failed to identify some yeast hence; combination with advanced tools such as molecular diagnostic tools can achieve complete profiling of the microbial diversity of *Amabere amaruranu* for starter culture and probiotic design. The physical fermentation aspects such as temperature, aeration, agitation rates and chemical composition needs to be defined so as to produce a product that can be scaled-up for industrial production.

Kule naoto

Kule naoto is the traditional fermented bovine milk product of the Maasai pastoralist community in Kenya. In its production, raw milk is filled into treated gourds made from the hollowed out dried fruit of the plant *Lagenaria siceraria*, then spontaneously fermented. The milk and the gourd treatment involves the addition of fresh cow's blood and rubbing the gourd's interior with a burnt stick from the tree; *Olea africana,* locally known as *Enkidogoe* (Onyango et al., 2014). The milk-blood mixture is then spontaneously fermented at ambient temperature for up to 5 days (Mathara, 1999). After fermentation, the product is gently shaken before consumption and averagely; an individual drinks 2–3 liters of *kule naoto* per day. *Kule naoto* is preferred by the Maasai community because of its natural taste and aroma. Moreover, the Maasai's believe that it has therapeutic value for treatment/ prevention of ills such as diarrhea and constipation (Mathara, 1999).

Although the milk might contain some probiotic microflora that confers such benefits, studies on the microbiology and biochemical aspects of *kule naoto* are limited and currently the product is prepared in the traditional way by spontaneous fermentation. This gives products of inconsistent quality and safety is not assured. However, some efforts have been made to study the microbiology of *kule naoto*. Mathara et al. (2004) identified microorganisms in the product, whose pH was found to range at 4.17–5.19. The microbial levels were in the level of 6.1 to 9.2 $\log_{10}$ cfu/ml of which the LAB dominated. The *Lactobacilli* and the *Lactococci* species were the dominant LAB (10^7–10^9 cfu/ml), whereas the *Enterococci* were less dominant (3.3–9.9 cfu/ml). However, at this concentration, the *Enterococci* may play a crucial role in fermentation of the milk. Other bacteria detected were *Enterobacteriaceae* group as well as the yeasts and moulds. In samples where *Enterobacteriaceae* were detected, yeasts were absent, which suggests a possible interaction between bacteria and yeasts in the production of kule naoto (Mathara et al., 2004). Overall, more than 500 strains were detected in *kule naoto* and all the LAB isolates were Gram-positive of which 55% belonged to the genus *Lactobacillus*, 25% to *Enterococcus*, 14% to *Lactococcus*, and 6% were *Leuconostoc*. Approximately 60% of the Lactobacilli strains were *Lactobacillus plantarum*. Other *Lactobacillus* strains identified were; *L. fermentum*, *L. casei*, *L. rhamnosus*, and *L. acidophilus*. For the *Enterococci*, *Enterococcus faecium* was the dominant species. *Lactococcus lactis* were also detected and the *Leuconostoc* strains were identified as *Leuconostoc mesenteroides* subsp. *dextranicum*. In the studies, most of the *kule naoto* samples had pH below 4.5. However, samples that had pH higher than 4.5 harbored the *Enterobacteriaceae* and yeasts, suggesting a potential health risk.

The main strain in *Kule naoto*, *L. plantarum* could be responsible for the characteristics of the product. However, the other *Lactobacilli* that were present such as *L. rhamnosus*, *L. fermentum*, and *L. acidophilus* and other present microorganisms such as Leuconostoc and yeasts could also contribute to the quality/ probiotic potential of the product. For instance, Mathara et al. (2008) demonstrated the probiotic potential of the *Lactobacillus* spp. in *Kule naoto*. The *L. acidophilus* had resistance to gastric juice and bile, while some other strains exhibited bile salt hydrolase activity, assimilated cholesterol *in vitro*, and had up to 70% adherence to HT29 MTX cells. The *L. fermentum* had almost 100% survival under simulated stomach acidic conditions and physiological salt concentrations of bile salts and had over 80% hydrophobicity values. Most strains of the *L. casei* and *L. acidophilus* had aggregation abilities above 50% (Mathara et al., 2008). These studies demonstrated that most *Lactobacillus* species found in *kule naoto* are probiotic and thus; needs full characterization for their application in the food industry. The technological features of some of the LAB from *kule naoto* have also been studied (Patrignani et al., 2006). These include growth kinetics and survival at 4°C. From the study, optimum conditions were detected, which could enhance sensory properties of the fermented product.

In efforts to upgrade the production of this fermented milk product using starter cultures, the interactions between the bacteria and yeasts should be elucidated and process parameters fully defined so as to develop a product as similar as the traditionally produced *kule naoto* under controlled conditions. Pasteurization of the milk should be practiced to minimize contaminations/ zoonoses. Further characterizations of the micro-organisms using advanced molecular techniques are necessary since culturing techniques have been demonstrated to be insufficient in mapping a microbial consortium (van Hijum et al., 2013). Moreover, the claims made regarding the health benefits of consuming *kule naoto* needs to be substantiated and studied using animal models for the product to benefit the masses.

Suusa (_Suusac_)

Suusa is the spontaneously fermented raw camel milk product that is common among the North Eastern pastoralists of Kenya. Camel milk is generally opaque white, and normally has a sweet, sharp taste though sometimes it tastes salty (Yagil, 1982). *Suusa* is white in color, has low viscosity, a distinct smoky flavor, and astringent taste (Lore et al., 2005). The composition of camel milk is relatively similar to milk of other domestic animals. However, camel milk is rich in vitamin C and the enzyme lysozyme (Wilson, 1984; Yagil, 1982). It therefore forms an important source of vitamin C in the diets of North Eastern Kenya pastoralists, who inhabit arid and semi-arid regions where fruits and vegetables are scarce. The lysozyme is good in preservation of the milk by inhibiting Gram-positive organisms.

There have been some studies documenting the traditional fermented camel milk (*Suusa*) in Kenya (Njage and Wangoh, 2008). The fermented camel milk has been given different names all over the world. It is called *Kefir* in the Middle East; *Lehben* in Egypt, Israel and Syria; *Yoghurt* in Bulgaria; and *Chal* or *Shubat* in Russia (Mohamed, 1993; Yagil, 1982). *Suusa* in Kenya is prepared by leaving fresh camel milk at room temperature (25–30°C) for 1–2 days for souring spontaneously. Mathara (1999) has documented the traditional production of *suusa* in Isiolo District, Kenya; the camels are milked directly into a gourd that has been cleaned, smoothed and treated with smoke. The smoking of the gourd is done using smouldering twigs of the acacia tree (*Acacia seyal*) after rinsing the gourd with water. The hot smoking chips are put into the gourd then the gourd is closed for a few minutes. Once the gourd cools, the charcoal chips are removed. The smoking is believed to improve the keeping quality of *suusa* and gives it its characteristic flavor and aroma, and improves the color. After the milking, there are no any heat treatments and the milk is bulked into larger containers, which are closed and left for two to three days for the milk to ferment spontaneously. After the fermentation, the top fatty layer is removed and the product is ready for consumption for up to a week at room temperature (26–29°C).

The microorganisms that participate in the spontaneous fermentation of traditionally produced fermented milk are mixed, undefined or partially defined. Some earlier studies had demonstrated the presence of various LAB, yeasts and molds (Oberman, 1985). Lore et al. (2005) studied the microbial counts and pH in traditional and laboratory-produced *suusa*. The total microbial counts and coliforms were similar. However, there were higher LAB, yeasts, and molds numbers in laboratory-produced than traditional *suusa*. The pH of *suusa* ranged between 3.6–4.4 and the lactic acid ranged from 0.52–0.71% (Njage and Wangoh, 2008). Among the dominant bacteria in *suusa*, *L. mesenteroides* subsp. *mesenteroides* consist of 24% and *L. plantarum* (16%). Other bacteria that have been reported in *suusa* are *Lactobacillus curvatus, L. salivarious, L. raffinolactis,* and *Streptococcus infantarious* subspp. *infantarious,* a pathogenic microbe. About 30 yeasts were isolated from *suusa. Candida crusei* (50%) were the dominant and others were *Geotrichum penicillatum* and *Rhodotorula mucilaginosa. C. krusei* has been recruited as an adjunct starter culture along with dairy starter cultures to maintain the activity of LAB and, as such increasing their longevity (Frazier and Westhoff, 2001). Jay (1992) also observed that *C. krusei* plays a crucial role in flavor development during fermentation of cacao beans, owing to its proteolytic activity. *Candida krusei* has been identified in other spontaneously fermented products such as *busaa* and *Amabere amaruranu* and *Mursik* (Nout, 1981; Nyambane et al., 2014). It could be possible that it plays a role in flavor development in *suusa* and the other traditional spontaneously fermented products, hence having a synergistic association with LAB. The presence of *G. penicillatum* and *R. mucilaginosa* could be linked to flavor and aroma development.

The limitation of *suusa* production similar to other spontaneously fermented milks is that products with wide variations in quality are produced due to the uncontrolled nature of spontaneous fermentation. Moreover, little is known about the nature and the interactions of the microorganisms contributing to the fermentation of *suusa* (FAO, 1990). As mentioned, *C. crusei* and *Saccharomyces cerevisiae* are detected in most spontaneously fermented foods. Their interactions with LAB needs to be studied as it will be vital in starter culture design. The presence of *S. infantarious* subspp. *infantarious,* a pathogenic microbe in *suusa* renders it unsuitable for consumption as it could be a vehicle for other pathogenic agents. Moreover, the pathogenic bacteria, *Salmonella enterica* has been found in raw camel milk (Matofari et al., 2007). Therefore, boiling/pasteurization are necessary before fermenting the milk to produce *suusa*. The boiling of milk will require the use of starter cultures in subsequent fermentation; hence detailed microbial studies are necessary to design starter cultures for *suusa* production.

SUMMARY OF THE PRODUCTION AND MICROBIOLOGY OF KENYAN FERMENTED MILK PRODUCTS

The fermentation process for the production of all milk products in Kenya occurs spontaneously in gourds. In the production of *Amabere amaruranu*, back-sloping is often used while for the other products,fermentation occurs without back-sloping, which could affect the microbial dynamics in the products. In modern production techniques, milk is fermented using starter cultures,which

produces consistent products that are safe and have extended shelf-lives in comparison to the spontaneously fermented milk products. Whereas *Mursik*, *Amabere amaruranu* are boiled before fermentation, *kule naoto*, and *suusa* are fermented raw, which could compromise their safety. Other practices such as smoking of the fermentation containers are carried for *suusa*, *Mursik*, and *kule naoto.* Addition of blood is also another practice especially to *Amabere amaruranu* and *kule naoto*. The fermentation period of the products ranges from 2 to 7 days.

In the production of these fermented milk products, Lactic acid bacteria (LAB) are the predominant fermenting microflora. In the production of *Mursik*, *Amabere amaruranu* and *kule naoto*, *Lactobacilli* are the predominant microorganisms. For *suusa*, *Leuconostoc* are the predominant but the *Lactobacillus* are also present. The LAB are known to be safe in their fermentation processes hence they have been employed for food preservation and flavor development (Khalid, 2011). As a result of their safety, there is need for their application in food biopreservation. In all the milk products, yeasts are found to be present. In particular the *C. crusei* and some *Saccharomyces* species are present in the milk along with LAB. Their role in the fermentation need to be characterized so that they could be employed as starter cultures.

CHALLENGES OF CURRENT METHODS (STARTER CULTURE DEVELOPMENT, PROCESSING AND CONSUMPTION) AND FUTURE PERSPECTIVES

In Kenya, as already discussed, all the traditional fermented foods and beverages produced at the household and village level using spontaneous fermentation are a staple diet for many communities and a cultural heritage (Franz et al., 2014; Mokoena et al., 2016). The fermentations draw substrates from a diversity of locally available raw materials including cereals, milk, coconut, honey, fruits, and vegetables. The technologies for their production have vast potential for stimulating development in the food industry owing to their low cost, tractability, little energy and infrastructural requirements, and the broad acceptance of the traditional fermented foods in Kenya (Nout and Motarjemi, 1997; Tamang and Samuel, 2010). The rising awareness of the health benefits of fermented foods, urbanization, and migrations of people has seen the rise in demand of these foods (Franz et al., 2014). It is apparent that with the increased demand, there is need for improvement in quality and safety of the products.

The rising demand for the traditional fermented milk products requires modernization of the technologies to assure safety, offer products with consistent quality and broadly accepted by the community. The spontaneous fermentation has limitations including inefficiency, low product yields, and variable product quality (Marshall and Mejia, 2012; Chilton et al., 2015). Moreover, there are safety concerns relating to pathogenic bacteria or chemical intoxicants produced by contaminating microorganisms as demonstrated by the deaths and risks of oesophageal cancer reported by the consumption of *Mursik* in Kenya (Patel et al., 2013; Wakhisi et al., 2005; Nieminen et al., 2013; Daily Nation, 2015). Although back-sloping process that makes use of samples of a previous successful batch of a fermented product as inoculants has been practiced in the production of African fermented food products such as the *Amabere amaruranu* giving products of relative consistency, safety of the products is not guaranteed.

With regard to the fermentation technologies developed in more advanced countries, it is evident that the identification and full description of microorganisms and the insights on their interactions within the fermentation ecosystems of *Mursik*, *Amabere amaruranu*, *kule naoto*, and *suusa* is required for the design of starter cultures to facilitate production under controlled conditions and tap into their unexploited probiotic potential (Nout, 2009; Franz et al., 2014; Holzapfel, 2002). There are numerous research reports showing the microorganisms that are associated with some of these Kenyan traditional fermented milk products (Nyambane et al., 2014; Digo, 2015; Nieminen et al., 2013; Patrignani et al., 2006; Mathara, 1999; Lore et al., 2005). Most of these studies have applied the classical microbiological techniques involving culturing (culture-dependent techniques) to identify microorganisms. However, cultivability of the microbes in the laboratory using synthetic media is most often inaccurately presumed and the absence of prior knowledge of their presence, selectivity of culture media and microbial interdependence, can result to utterly lopsided conclusions and misleading information (van-Hijum et al., 2013). Hardly any of the studies has corroborated culture-dependent techniques with the modern high throughput biotechnologies (culture-independent techniques) in studying the microbial consortia for designing starter cultures or isolating probiotic microorganisms, and none of these products uses a defined culture or is being produced at industrial level (Reid et al., 2014; Marshall and Mejia, 2012). The design and consequential improvement of starter cultures and exploitation of the probiotic potential could be the impetus for transforming the traditional fermentation technologies into a science, which can further spur innovation in equipment design for controlled processing of these products.

The application of high throughput biotechnologies for the characterization of microorganisms in fermented food products started when Polymerase Chain Reaction (PCR) was employed to conduct community profiling of microorganisms in traditional fermented food products

using the sequencing of the 16S rRNA genes and Denaturing Gradient Gel Electrophoresis (DGGE) (Tamang et al., 2016; van-Hijum et al., 2013). The application of 16S rRNA sequencing has demonstrated its superiority over culturing techniques with regard to microbial abundance and detection of specific microbes (van-Hijum et al., 2013; Tamang, 2014; Jianzhonga et al., 2009). Sequencing approaches for the portions of 16S or 18S rRNA genes are mainly useful to discern different genera or at best species within the fermentation. The 16S rRNA gene is highly conserved such that primers can be developed that can be used for almost all bacteria (Mayo et al., 2014). However, the gene also contains hypervariable regions whose variability is species-specific. Therefore, an amplification of the of the variable 16S rRNA gene from all microorganisms whose DNA is extracted *in situ* followed by sequencing can allow production of a fingerprint, which corresponds to microbial identity in the food. After identification, comparisons can be done to publicly available data bases hence; help in the isolation of starter cultures and probiotic microflora (Ercolini, 2013). For example, the Polymerase Chain Reaction- Denaturing Gradient Gel Electrophoresis (PCR-DGGE) approach has been used to identify microbial species occurring in natural whey cultures used as starter for water buffalo Mozarella cheese (Ercolini et al., 2001). In this study, both thermophilic and mesophilic LAB were identified by sequencing the V3 region of the 16S rRNA gene from the DGGE fragments on natural whey culture profiles. Other fermented products that have been characterized by sequencing techniques include *pulque*, a traditional Mexican alcoholic beverage from Maguey (Escalante et al., 2008), *kimchi,* a naturally fermented vegetable product of Korea (Jung et al., 2011; Park et al., 2012), *narezushi*, a fermented salted fish and cooked rice of Japan (Kiyohara et al., 2012), *doenjang*, soy bean paste (Nam et al., 2012[a]), *kochujang*, a traditional Korean fermented food that is made with red pepper, glutinous rice, salt, and soybean (Nam et al., 2012[b]), seafood, and rice bran among others. Some of the other molecular diagnostic/ typing techniques that have been applied in the study of microorganisms in food and could be useful in the study of *Mursik*, *Amabere amaruranu*, *kule naoto* and *suusa* are listed in Table 1. The sequencing studies including the Next Generation Sequencing (NGS) can enrich our biodiversity knowledge in the traditionally fermented foods, which can be important also in selecting probiotic microorganisms besides the design of starter cultures. RAPD, Random amplification of polymorphic DNA; rep-PCR, repetitive extragenic palindromic sequence-based PCR; AFLP, Amplified fragment length polymorphism; DGGE of 16S rRNA, denaturing gradient gel electrophoresis of 16S rRNA; ARDRA, Amplified ribosomal DNA restriction analysis; MLSA, Multilocus sequence analysis.

Already in developed countries, starter culture design and bioreactor technology improvement for controlled fermentation processes have led to the development of high value-added products such as enzymes, microbial cultures, and functional food ingredients, which in most cases are imported to Kenya and other developing countries (Marshall and Mejia, 2012). Moreover, the starter cultures used for the synthesis of products such as yogurt, cheeses, and alcoholic beverages are imported from developed countries. There is a growing consumer interest in attaining wellness through diet, necessitating the need for incorporation of the probiotic strains into diets and the African traditional fermented products offer a vast source of those microorganisms, yet these opportunities have not been explored (Franz et al., 2014; Reid et al., 2014). As demonstrated, *Mursik*, *Amabere amaruranu*, *kule naoto*, and *suusa* are dominated by LAB. Most LAB have the 'generally regarded as safe' (GRAS) status and some are probiotics such as the *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Lactococcus*, *Streptococcus*, and *Leuconostoc* (Masood et al., 2011). It is therefore important to note that *Mursik*, *Amabere amaruranu*, *kule naoto*, and *suusa* have probiotic strains and efforts to purify, characterize, and incorporating them into foods could contribute to the wellbeing of Kenyans, particularly the vulnerable groups.

The application of high throughput biotechnologies for starter culture design can allow the tailoring of starter cultures to yield products with specific flavors and/or textures. For example, in Thailand, Random amplified polymorphic DNA (RAPD) techniques have been recruited in the development of defined starter cultures for flavor development for the production of fermented pork sausage, *nham*, which is now produced commercially (Valyasevi and Rolle, 2002). The successful application of starter cultures and improved bioreactor technology is highlighted by the fermented soy sauce production. The production of soy sauce has transited from a craft to a technology-based production system in Thailand (Valyasevi and Rolle, 2002). The process takes a shorter time and uses the *koji* culture *Aspergillus oryzae* for proteolysis of the soy proteins in the initial phase and *Saccharomyces rouxii* in the second phase (Moromi fermentation) (Beuchat, 1995; Valyasevi and Rolle, 2002). All the processes are controlled and the physical parameters (temperature and humidity) are controlled in the fermenters. All these developments have enhanced product safety and consistency and have ultimately led to economic gain for the soy sauce industry and greater value added to the product in terms of quality and safety. The production of *Som Fug*, a traditional fermented fish paste has also employed starter cultures.

In recent times, the genetic characterization of micro-organisms has advanced at a rapid pace with exponential growth in the collection of genome sequence information, high-throughput analysis of expressed products, that is,

Table 1. Some of the techniques appropriate for studying indigenous fermentations.

Name	Description	Reference
RAPD	A typing method based on the genomic DNA fragment profiles amplified randomly by PCR, and is commonly used for disintegration of LAB strains from fermented foods.	Coppola et al. (2006); Chao et al. (2008).
rep-PCR	A technique permits typing at subspecies level and reveals significant genotypic differences among strains of the same bacterial species from fermented food samples.	Tamang et al. (2008)
AFLP	A technique based on the selective amplification and Separation of genomic restriction fragments.	Tanigawa and Watanabe (2011)
Illumina	Sequencing technique generating millions of short reads from a single lane up to 100 bp.	http://www.illumina.com
Roche 454	Sequencing technique generating approximately one million longer reads (450–700 bp) from a sequencing plate.	http://www.my454.com
DGGE of 16S rRNA	Allows ‘‘community fingerprinting’’ by PCR amplified DNA of 16S or 26S rRNA from mixed microbial communities to visualize variations in microbial diversity and give an estimate of richness/abundance of predominant microbial members.	Alegría et al. (2011)
ARDRA	An adaptation of the restriction fragment length polymorphism that creates ‘fingerprints’ from the 16S rRNA gene that can be analyzed on agarose gel.	Jeyaram et al. (2010)
MLSA	Uses housekeeping genes as molecular markers alternative to the 16S rRNA genes for LAB species identification	Tanigawa and Watanabe (2011)
Metagenomics	Application of sequencing method(s) to DNA obtained directly from a given environmental sample. The sequencing reads can either be used or assembled into contigs for determining prevalence of open reading frames specifying molecular functions.	Bigot et al. (2015)

transcripts and proteins and the application of bioinformatics which allows high throughput comparative genomic approaches that provide insights for further functional studies (Ercolini, 2013; Alkema et al., 2015). Genome sequence information, coupled with the support of highly advanced molecular techniques, have allowed scientists to establish mechanisms of various host-defensive pathogen counter-defensive strategies and have provided industry with tools for developing strategies to design healthy and safe food by optimizing the effect of probiotic bacteria, the design of starter culture bacteria and functional properties for use in food processing. Characterization of the genomes of lactic acid probiotics has, for example, shed light on the interaction of pathogens with LAB (de Vos, 2001).

There has been a slow adoption of microbial starter cultures and application of high throughput biotechnologies in Kenya (Reid et al., 2014; Franz et al., 2014). This can be due to high poverty levels, whereby the price of the food is more relevant than the quality and safety. Studies aimed at upgrading the traditional fermentation technologies are few and the funded studies so far aim at identifying microorganisms and improving hygienic conditions of the fermentation processes and not process development.

However, with the demand of the products rising due to improved living standards and potential for export, exploitation of the vast microbial diversity in *Mursik*, *Amabere amaruranu*, *kule naoto* and *suusa* to optimize the current products or to create new ones, use of hygienic conditions, and development of fermentation processes and shelf-life extension of the products is essential. Moreover, co-creation processes where product end users and stakeholders are engaged to discuss the challenges and share the goal of valorization of the spontaneously fermented products will be key tothe broadening the acceptance of the developed products. This is because the stakeholders’ experiences, ideals and senses of value,which will allow the generation of products based on a common ground, will enable rapid adoption of newly developed products.

To achieve this, there needs to be an enabling environment. There needs to be collaborative research efforts between Kenyan research institutions, their counterparts in the developed countries and stakeholders in Kenya including farmers, consumers and regulatory bodies. The research institutions also need to link with the industry in the application of the developed technologies. The Kenyan government needs to come up with policy regarding traditional fermented foods in order to protect consumers and organize the stakeholders to promote fermented foods. The government should invest in manpower and research funds that can develop bioprocesses for the production of traditional fermented foods/ beverages. Moreover, food biotechnological information sharing is necessary in guiding research agenda. Programs should be set to scale research into business ventures. Since Kenya has laws protecting

intellectual property, development of traditional fermentation technologies can be protected. The information from scientists and industry therefore need to be transparent to gain consumer confidence. As the fermented foods have potential to contribute to food security, scientists in Kenya need to rise to the occasion and give direction in this arena. First, they need to apply the recent high throughput biotechnologies in characterizing the products for up-gradation. By doing so, they will contribute to process development, knowledge creation, industrialization, and curation of indigenous knowledge and it remains a challenge.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Nutritional composition of *Meristotheca senegalense* (Rhodophyta): A new nutrient source

Bodian Moussa Yagame[1], Ayessou Nicolas Cyrille Mensah[2,4]*, Cissé Mady[2,3], Ndiaye Cheikh[3] and Kandioura Noba[1]

[1]Département de Biologie végétale- Laboratoire de Botanique et de Biodiversité. Faculté des Sciences et techniques, Université Cheikh Anta Diop de Dakar, Sénégal.
[2]Laboratoire de Electrochimie et des Procedes, École Supérieure Polytechnique, Université Cheikh Anta Diop, B.P 5085 Dakar Fann, Sénégal.
[3]Laboratoire des Biotoxines et Contrôle des Aliments, Institut de Technologie Alimentaire (ITA) Route des Pères Maristes, B.P 2765 Dakar, Sénégal.
[4]Centre d'Etudes sur la Sécurité des Aliments et le développement des Molécules fonctionnelles (CESAM-RESCIF) - Université Cheikh Anta Diop de Dakar, Sénégal.

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Macroalgae are used in diverse global regions. *Meristotheca senegalense* J. Feldmann, a macroalga species found in a Senegalese bay, was the subject of this study focusing on the chemical and mineral composition. The present study was done in order to evaluate the potential of this macroalgal resource for biomass development and contribution to the economy in Senegal. The results of this study showed that *M. senegalense* J. Feldmann was relatively a good source of nutrients including fiber (6.67 ± 0.7%) and protein (6.37 ± 0.8%). Mineral analysis also showed significant concentrations of magnesium (216.87 ±12.9 mg/100 g), calcium (81.6 ± 17.5 mg/100 g), iodine (31.16 ± 1.5 mg/kg) and iron (28.13 ± 2.15 mg/100 g). However, zinc and copper 3.31 ± 0.26 and 2.43 ± 0.13 mg/100 g respectively were found at relatively low concentrations. Interestingly, the vitamin B12 content was significant with a content of 20 ± 1.0 mg/kg, potentially allowing for the use of the alga as a supplemental. The nutrient concentrations reported for *M. senegalense* J. Feldmann suggests that its cultivation and harvest can be a source of diversification in the activities of fishermen. In terms of food consumption, the results also showed that the red alga used in this study can be added to human diets as supplementation and might also be F

Key words: *Meristotheca senegalense*, nutrient composition, macroalgae, supplementation, food diversification.

INTRODUCTION

There are 218270 known macro and microalgae varieties of which 36238 have been identified at the species level (John, 1994). However only about 665 of the known species of marine macroalgae are used for food or

*Corresponding author. E-mail: mybodian@live.fr. Tel: 00221-764771312.

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Figure 1. Map of Cap-vert peninsula (Dakar Region) showing Ngor bay position (Modified from Google Earth 04/2015).

animal feed, and extracted for individual chemical components (Lobban and Harrison, 1994; Pereira, 2016). Components from industrial extraction of macroalgae include: hydrocolloids, nutraceuticals, minerals and food color. According to Mouritsen (2013) and Pereira (2016), the primary mineral components in seaweeds were iodine, calcium, phosphorus, magnesium, iron, sodium, potassium, and chlorine with trace elements such as zinc, copper, manganese, selenium, molybdenum and chromium. The mineral composition varies significantly from one seaweed species to another depending on oceanic residence time, seasonal variations, environmental and physiological conditions, and the processing method of mineralization. *Meristotheca senegalense* has been harvested for approximately three decades (Pérez, 1997; McHugh, 2002; John et al., 2004). It was originally isolated in Dakar between Ngor and Ouakam bays and initially characterized by Faye et al. (2004) and named *M. dakarensis* and was subsequently renamed *M. senegalense*. Previous studies on *M. senegalense* showed the presence of the phycocolloid iota carrageenan (Fostier et al., 1992) containing a potentially promising pharmacological glycolipid biomolecule with anti HIV properties previously extracted from *Cyanobacterium* (Diop and Samb, 2004).

This species of algae was previously exported as raw material to Japan (Pérez, 1997; McHugh, 2002) where *M. senegalense* (McHugh, 2002), *M. papulosa* (Faye et al., 2005; Rao et al., 2007; Pereira, 2016) and *M. procumbens* (Rao et al., 2007; Pereira, 2016) were used for human consumption (Pérez, 1997; Rao et al., 2007; Mouritsen, 2013; Pereira, 2016). Unfortunately the mineral composition of *M. senegalense* was unknown at this time. The exploitation of this marine resource was recently restricted for exportation as a harvested biomass by the Senegalese government. However due to malnutrition faced by some segments of the population of Senegal and elsewhere, diversification of sources of essential micronutrients appears as one of the solutions next policy strategies and enhancement of certain fruit and vegetables neglected (Ayessou et al., 2009, 2011, 2014; Gueye et al., 2014). Thus, strengthening nutritional balance requires the identification of new food resources. Accordingly, the purpose of the present study was to determine the nutritional composition of *M. senegalense* in order to increase the value of this macroalgal resource for economic development through the local use of the biomass which is still underexploited in Senegal.

MATERIALS AND METHODS

Plant materials

M. senegalense samples were collected in Dakar peninsula Ngor bay located at N 14° 44'715; W017° 30' 857 (Figure 1). Samples were collected early March (the beginning of the cold season) until late June (beginning of the warm season) from crops grown on the mariculture station nets.(Figure 2) This time interval corresponded to the period during which the natural fields of the species were harvested for biomass traded by local coastal populations. For biochemical analysis, three batches of *M. senegalense* samples were targeted. To insure preservation, samples were first naturally dried in shade with adequate ventilation. Prior to analysis samples

were washed in distilled water and dried at 70°C in an oven according to Afnor's
method NF V 03-707 (Afnor, 1982) for 48 h before grinding with a mortar pestle.

Major macronutrient analysis

Analyses of lipid and protein content were carried out according to the procedure described according to AFNOR standards (Afnor, 1982). Samples were dried in an oven at 105°C for two hours, cooled and then weighed to determine moisture content. Lipid extraction was performed using a Soxhlet extractor with diethyl ether as the solvent (NFV 03-905 standard). Nitrogen determination was performed using Kjeldhal method (NF 03-050 standard) and the protein content was calculated by using a coefficient factor of 5.7. Fiber content was determined in sample through AFNOR standards V76-101. Samples were hydrolysed with acid solution and then with basic solutions. Then it's mineralizing during 3 h incineration at 550°C.

Ascorbic acid

Ascorbic acid content of the samples was determined according to the method of Dhuique et al. (2007). Ten grams of *M. senegalense* was homogenized in ice-cold metaphosphoric acid solution (4% in distilled water) to extract the ascorbic acid. The mixture was centrifuged at 1000 X g for 15 min with a second extraction repeated after removing supernatant. Supernatants were pooled before determining the total ascorbic acid content. The analysis was performed using a Thermo Scientific HPLC 1000 SCM (Thermo Fisher Scientific France, Illkirch) with an RP 18 Licrospher 100 column (4.6 × 250 mm; 5 m, Merck, Darmstadt, Germany). The separation used a mobile phase consisting of a 0.01% isocratic sulfuric acid solution (Sigma-Aldrich, Saint-Quentin Fallavier, France) with an injection volume of 10 µl. Quantification was performed using a UV 3000 Spectra (254 nm). Quantification and identification of the peaks were done using an external calibration curve using a concentration range from 10 to 200 mg/l of L-Ascorbic acid solution (A5960 BioXtra, ≥99.0%, crystalline (Sigma) standards (200 to 10 $mg.L^{-1}$) with a 0.01 % limit of detection.

Vitamin A

To determine Vitamin A content samples were extracted with a mixture of ethanol/hexane (4:3 v/v), with $CaCO_3$ and 0.1% butylhydroxytoluene (added as antioxidant). *M. senegalense* (0.5 g) samples were mixed with 20 ml of the extraction buffer followed by centrifugation at 15000 X g for 15 min at 4°C. A second extraction was repeated using the same conditions after collecting the supernatant. All supernatants were pooled and evaporated to dryness under nitrogen. After evaporation to dryness, samples were solubilized in 1 mL of a dichloromethane/Tert-Butyl methyl ether/methanol mixture. Separations were carried out by gradient elution using an Agilent HPLC 1100 with water (solution A)/methanol (solution B)/Tert-Butyl methyl ether (solution C) as follows: Initial conditions 40 %A/60 % B; 0-5 min, 20% A/80% B; 5-10 min, 4%A/81% B/15% C; 10-60 min, 4% A/11% B/85% C; 60-71 min, 100% B 71-72 min, and back to the initial conditions for reequilibration. Samples were quantified at 350, 400, 450 and 470 nm wavelengths. Comparisons were carried out using authentic standards as reported by Dhuique et al. (2007) with a limit of detection (LOD) of 0.035 mg/100 g and a limit of quantification (LOQ) of 0.119 mg/100 g per sample.

Mineral determination

Samples were mineralized by incineration at 500°C and desiccated by adding fluorhydric acid at 40% (Sigma-Aldrich, Saint-Quentin Fallavier, France) and then evaporated to dryness. Sodium, potassium, calcium, magnesium, phosphorus, copper, zinc and iron were quantified using an ICP-AES (inductively coupled plasma atomic emission spectrometry) Varian Vista spectrophotometer containing a charge coupled device for detection (Agilent France, Massy). Mineral standards used in this assay (Ca, K, Mg, Fe, Zn, Cu) were obtained from Fisons Scientific Equipment (Loughborough, England).

Vitamin B12

Extraction of vitamin B12 was carried out in acetate buffer (pH 4.0) containing pepsine [50 $mg.ml^{-1}$ (4500 $U.ml^{-1}$)]. Partial purification was achieved using Amberlite XA D-2 followed by elution with 80% (v/v) methanol. Activated charcoal facilitated removal of impurities in the extract and in the further purification of vitamin B12. The purified fraction containing methyl cobalamin was analyzed by HPLC using a silica C18 column with water/methanol 50/50 (pH 4.5) as a mobile phase, and a UV detector.

Iodine

Iodine was assayed by "Aquanal" (Bordeaux, France) using the standard method EN 15111/ICP-MS with a VARIAN Spectrometer ICP-VISTA coupled to a mass spectrometer.

Quality control and statistical analysis

HPLC limits of detection (LOD) of vitamin C, β-carotene, and vitamin B12 were determined using a 5 point external calibration systematically performed before each series of analyses. The spectrophotometer limits of detection (LOD) used for mineral determination was a 5 point calibration and analysis of control samples whose mineral contents were known. All analyses were carried out in duplicate and the data were analyzed using SAS software (version 8.1 2000; SAS Institute, Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

The results from Table 1 show that the algae *M. senegalense* is very low on lipid content (0.08 mg /100 g) and rich in carbohydrates especially with a dry weight fiber content of 6.67%. The protein content was 6.38% and the minerals were relatively abundant most notably magnesium (216.87 mg/100 g), calcium (81.6 mg/100 g) and iron (28.13 mg/100 g). Moreover, *M. senegalense* also contained a significant concentration of iodine (31.16 mg/kg) zinc (3.31 mg/100g) and copper (2.43 mg/100g). Vitamin B12, an essential hydrosoluble vitamin was found at a concentration of 20 mg/kg. However, the vitamin A and vitamin C content were relatively low 0.7 mg/kg of sample and < 10 mg/kg respectively.

In this study, sample averages were obtained during a complete season and showed large variances for calcium (17.5 mg/kg) and magnesium (12.9 mg/kg) due to the minima and maxima values (64 and 99 for Ca; 203 and 228.5 for Mg). These variations could be related to

Table 1. Average chemical composition of *Meristotheca senegalense*

Components	Average (n=3)	Components	Average (n=3)
Lipid (mg/100 g)	0.08 ± 0.1	Zn (mg/100g)	3.31 ± 0.26
Protein (%)	6.38 ± 0.8	Cu (mg/100g)	2.43 ± 0.13
Fibers (%)	6.67 ± 0.7	Iodine (mg/kg)	31.16 ± 1.5
Ca (mg/100 g)	81.6 ± 17.5	Vitamin B12 (mg/kg)	20 ± 1.0
K (mg/100 g)	9.25 ± 2.58	Vitamin C (mg/kg)	< 10
Mg (mg/100 g)	216.87 ±12.9	Vitamin A (mg/kg)	0.7± 0.06
Fe (mg/100 g)	28.13 ± 2.15		

Table 2. Comparative study between *M. senegalense* and other macroalgae species.

Species*	Protein (%)	Lipid (%)	Fibers (%)	Ca (mg/100 g)	Fe (mg/100 g)	Zn (mg/100 g)	Cu (mg/100 ng)	Vit. B12 (mg/kg)
M. senegalense[1]	5.6 -7.2	2-8.10^{-5}	6 -7.5	64-99	25.7- 29.8	3.1- 3.6	2.3-2.5	20
Ulva lactuca[2]	10-25	0.6-1.6	29-55	840	66	-	-	600
Laminaria digitata[2,3]	8-15	1	36-37	1005	3.2-9	1.77	< 0.5	0.05
Undaria pinnatifida[2]	12-23	1.0-4.5	16-51	680-1380	1.54-30	0.94	0.18	0.36
Chondrus crispus[2]	11-21	1-3	10-34	420-1120	1.54-30	7.14	< 0.5	60- 400
Palmaria palmata[2]	8-35	0.7-3	29-46	560-1200	50	2.86	0.37	0.9
Porphyra tenera[2]	28-47	0.7-1.3	12-35	390	10-11	2-3	< 0.63	-
P. umbilicalis[2]	29-39	0.3	29-35	330	23	-	-	2.9
***P. yezoensi*[2;3]**	**31-44**	**2.1**	**30-59**	**440**	**13**	**10**	**1.47**	**0.52**

*Results expressed according to the dry wet; [1]this article; [2]Pereira (2011). [3]Pèrez (1997).

seasonal aspects of the samples which are linked to the physiological state of *M. senegalense* during Sample collection was in April, May (cold season) and June (beginning of the warm season).

M. senegalense was compared to other edible seaweeds (Table 2). The results show that *M. senegalense* contains less fiber and calcium compared to other species; whereas, the vitamin B12 contents of different edible seaweeds were highly variable. When considering seafood as an important source of iodine both red and brown algae are generally richer in iodine than green algae (Fleurence and Guéant, 1999). According to Teas et al. (2004) *Rhodophyceae* contained 10 to 100 mg/kg of iodine similar to the content of *Palmaria palmata*, while species like *Porphyra umbilicalis* and *Undaria pinnatifida* had iodine contents of 17.3 and 22 to 30 mg/kg respectively. The results of this study show that *M. senegalense* is a good source of iodine with an higher average concentration (31.16 mg/kg) than the species mentioned above. In the past years important actions were taken by world organizations, such as World Health Organization, to fight against iodine deficiency. The recommended concentration of iodine in iodized salt was fixed at 30 mg/kg. Therefore, *M. senegalense* could be a valuable dietary source of iodine. The specie used in this study could be used to fill the gap of iodine concentration in many foods which WHO and other organizations have suggested as an alternative source of iodine. In this context, *M. senegalense* should be promoted as a new local food. In fact edible seaweeds present many advantages such us the quality of their fatty acids content (Khotimchenko and Levchenko, 1997), traces metals such us Fe, Zn, Cu (Robledo and Pelegrin, 1997) or iodine content (Teas et al., 2004; Zava and Zava, 2011). In addition, some reports have shown quality protein content in some species including *Chlorophyta* and *Rhodophyta* (Galland-Irmouli et al., 1999; Fleurence and Guéant, 1999).

Although *M. senegalense* is considered as a rich source of iodine, the FDA and other world dietary guidance programs warn that consumption of excess iodine rich seaweeds could be dangerous for health (Brownlee et al., 2012; Leung and Braverman, 2014). An example of the potential health risk was reported by Brownlee et al. (2012), when seaweed was used in a study composed of lactating mothers in Japan and Korea. They found that the high concentrations of iodine in seaweed were transmissible from mother to infant through breast milk which could result in neonatal iodine toxicity and subsequent hypothyroidism or hyper-thyroidism. However, *M. senegalense* could be very valuable in new foods which are rich in iodione.

Figure 2. Plant of *Meristotheca senegalense* collected during May 2010 at Ngor Bay. Scale bar = 5 cm.

Currently new types of foods are been developed using *M. senegalense as a source of some important nutrients* such calcium, magnesium and iron as well as iodine.
The increase of the local consumption of this endemic seaweed in spite of exportation will also induce development of local markets.

Conclusion

Like other seaweed, *M. senegalense* could be an important source of nutrients, iodine, minerals and vitamin B12. These results also show that these red algae can be added to the human diet and diversify world food sources however, further studies are needed in order to boost the added value of *M. senegalense*. Farming of *M. senegalense* could promote diversification of fishing activities reducing pressure on other marine resources and contributing to sustainable food production.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Sensory quality of orange-fleshed sweetpotato cultivars as affected by curing and household-level storage methods

Richard A. Atuna[1], Francis K. Amagloh[2]*, Edward E. Carey[3] and Jan W. Low[4]

[1]Department of Biotechnology, University for Development Studies, Tamale, Ghana.
[2]Department of Food Science and Technology, University for Development Studies, Tamale, Ghana.
[3]International Potato Center, Kumasi, Ghana.
[4]International Potato Center, Nairobi, Kenya.

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A 2×2×3 factorial design was used to investigate the effect of two orange-fleshed sweetpotato cultivars, three curing treatments and two homestead storage methods on the general appearance, finger-feel firmness, sweetness and overall acceptability of boiled roots. The cultivars were Apomuden and Nane, and the two homestead storage methods were the sand box and the heap storage. In-ground curing (dehaulming) and field-piled curing, for seven days and then uncured treatment were the curing options investigated. A hedonic scale ranging from 1 = extremely dislike to 5 = like extremely was used. For cultivars, the sensory scores ranged from 3.20 to 3.84 (farming season I) and 3.32 to 3.93 (farming season II), indicating good consumer preference. Curing type significantly ($p<0.05$) influenced the sensory properties of roots in the second farming season. Storage type showed no significant difference ($p > 0.05$) in all sensory attributes in both farming seasons except for sweetness and the heap storage had significantly higher (3.84 *vs.* 3.47, $p<0.0001$; respectively) score relative to sand box in the first year. Apart from general appearance (3.64 *vs.* 3.32, $p = 0.002$) and finger-feel firmness (3.51 *vs.* 3.25; $p = 0.006$) in which females had a significantly higher score than males in the first farming season, all the other sensory attributes were similarly ranked by males and females for both years. In-ground and field-piled curing methods, there is increased consumer acceptability and it should be encouraged.

Key words: Curing, Field-piled, Gender, Sensory, in-ground, orange-fleshed sweetpotato.

INTRODUCTION

Sweetpotato (*Ipomoea batatas* (L) Lam.) is an important food security crop in sub-Saharan Africa (van Oirschot et al., 2003). In Ghana, the traditional sweetpotato cultivars are either white or cream-fleshed devoid of β-carotene, a vitamin A precursor. However, the orange-fleshed sweetpotato (OFSP) have been reported to contain

*Corresponding author. E-mail: fkamagloh@uds.edu.gh.

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significant amount of β-carotene; one variety in Ghana has concentration ranging from 2100 to 5500 µg/100 g on fresh weight basis (Tumwegamire et al., 2014). OFSP is gradually gaining attention in developing countries because of its potentials in ameliorating vitamin A deficiency (VAD) (Agbemafle et al., 2014; Laurie and Van Heerden, 2012). VAD is a public health concern in low income countries including Ghana (World Health Organization, 2009) and requires a multi-sectorial approach in addressing it. Food-based approaches through biofortification of indigenous crops have been shown to be a promising and sustainable means to address VAD (Birol et al., 2015; Low et al., 2007). Therefore, sweetpotato, particularly OFSP, is a very good candidate because; it is high in β-carotene and able to do well on marginal soils characterized by soils in low income countries.

Curing, a pre-or post-harvest treatment of sweetpotato could be achieved by either field-piled (Ravi et al., 1996) or/and dehaulming (Tomlins et al., 2002). Field-piled is a form of curing where roots are heaped on the field and covered with fresh vines for seven days before storage. Dehaulming which is a preharvest curing method, entails pruning the vines of sweetpotato plants seven days before harvest (Tomlins et al., 2002). This has been reported to improve the postharvest qualities of the roots (Tomlins et al., 2002). However, some changes may occur during these pre and post-harvest processes and could significantly influence consumer acceptability.

In Ghana, efforts are on-going in the release of more OFSP varieties. However, successful introduction of new varieties, not only depends on production characteristics but the sensory and utilization characteristics as have been found elsewhere (Tomlins et al., 2004, 2007). Therefore, a study that focuses on the changes in sensory quality with postharvest practices such as curing and storage is worth investigating.

During curing and storage, certain biochemical changes occur and may either be desirable or undesirable (van Oirschot et al., 2003). For instance, the sensory attributes of roots could be affected by location and sweetpotato management practices (Tomlins et al., 2007b). Moreover, curing seem to promote the synthesis of α and β amylase-important enzymes in the hydrolysis of starch during processing and the formation of monosaccharides that acts as precursors for vital flavour components (Wang et al., 1998).

Storage effect on sensory quality of sweetpotato roots has been contradictory. George and Kamara (1988) reported that storage of sweetpotato roots in baskets or on earthen floor had a little effect on the sensory qualities of boiled or fried roots of some cultivars. Furthermore, the sensory property of sweetpotato cultivars was not greatly affected by storage under tropical conditions (van Oirschot et al., 2003), but Mpagalile et al. (2007) reported that storage in traditional pit affected the sensory qualities of sweetpotato roots.

In this study, storage in sand and sprouting, denoted as the triple-S system that was designed for production of planting material (Namanda et al., 2013), was modified and investigated as a homestead storage method of roots for consumption. The objective was to assess the effect of curing and household-level storage methods on the sensory qualities of two orange-fleshed sweetpotato cultivars: Apomuden (19% dry matter content) and Nane (27% dry matter content).

MATERIALS AND METHODS

Experimental design

The experimental design used was a 2×2×3 factorial design. The treatment factors were two cultivars of orange-fleshed sweetpotato (Apomuden and Nane), two household-level storage methods (heap and sand box) and three curing treatments (field-piled, dehaulming and uncured).

Cultivars

Apomuden and Nane were planted in two successive farming seasons (August 2014 during the first season and July 2015 in the second season) at Bontanga in the Kumbungu district, Tamale, Ghana. All good agronomic practices were adhered until they were harvested at optimum maturity (Apomuden- 3.5 months and Nane- 4 months). Apomuden is an officially released variety by the Crops Research Institute of the Council for Scientific and Industrial Research, Ghana. Nane is a cultivar being evaluated for release as a variety in Ghana.

Curing and storage

In-ground curing (dehaulming) was done by removing part of the canopies and leaving about 30 cm of the vines from the base seven days prior to harvest. In the field-piled curing treatment, roots were carefully harvested, sorted and heaped on the field and then covered with fresh sweetpotato vines for seven days. During the seven-day curing period, no rains were recorded. Freshly harvested roots, the uncured treatment, together with roots from the two curing treatments stated above were stored in either sand box or under a moistened straw heap for nine and eight weeks, respectively in two successive farming seasons.

Sample preparation and sensory analysis

Wholesome roots (about 1 kg) of both Apomuden and Nane from each curing option in the two homestead storage methods were selected into labelled net bags. The roots were then washed, and wet cooked for (20 min) to become soft. The peels of the cooked roots were removed using a knife and sliced to thumb sizes for the consumer preference test. Three figure-coded disposable plates were used to serve the samples for scoring by the panelist. The consumer acceptability test took place at a dining room of Alimento catering service, University for Development Studies, Tamale. The boiled roots were evaluated by 121 untrained panelist (female = 76, male = 45) for farming season I and 91 untrained panelist (Male = 14, Female = 77) for farming season II. The ages of the recruited untrained panelist ranged from 17 to 37 years for both farming seasons. A five point hedonic scale: 1 = extremely dislike; 2 = dislike; 3 = neither like nor dislike; 4 = like; and 5 = like extremely

Table 1. Sensory scores of boiled OFSP roots after being cured/uncured and stored using two household-level storage methods (heap *vs.* sand box).

Cultivar*	Season I (*n*=121; Male=45, Female=76)				Season II (*n*=91; Male=14, Female=77)			
	Sensory attributes				Sensory Attributes			
	Gen. appearance	Finger-feel firmness	Sweetness	Overall acceptability	Gen. appearance	Finger-feel firmness	Sweetness	Overall acceptability
Apomuden	3.49±1.26[a]	3.26±1.16[a]	3.30±1.16[a]	3.44±1.15[a]	3.53±1.07[a]	3.35±1.11[a]	3.54±1.11[a]	3.63±1.15[a]
Nane	3.39±1.13[a]	3.39±1.15[a]	3.83±1.03[b]	3.76±0.98[b]	3.66±1.18[b]	3.63±1.12[b]	3.66±1.09[a]	3.75±1.13[a]
P-value	0.149	0.116	<0.0001	0.000	0.094	0.003	0.210	0.224
Curing#								
Field-piled	271.48[a]	304.94[a]	288.61[a]	288.62[a]	266.10[b]	298.93[b]	296.29[b]	288.99[b]
In-ground	329.58[b]	309.40[a]	312.42[a]	317.07[a]	278.98[b]	243.80[a]	238.49[a]	255.57[ab]
Uncured	307.97[ab]	297.98[a]	306.54[a]	304.49[a]	233.69[a]	233.69[a]	241.03[a]	229.59[a]
P-value	0.004	0.779	0.335	0.261	0.000	<0.0001	0.000	0.001
Storage type*								
Heap	3.40±1.14[a]	3.32±1.13[a]	3.84±1.04[a]	3.69±1.01[a]	3.63±1.16[a]	3.51±1.12[a]	3.54±1.13[a]	3.63±1.15[a]
Sand box	3.45±1.21[a]	3.35±1.17[a]	3.47±1.13[b]	3.60±1.10[a]	3.58±1.12[a]	3.49±1.13[a]	3.70±1.06[a]	3.75±1.13[a]
P-value	0.491	0.569	<0.0001	0.397	0.539	0.903	0.157	0.224

Means in the same category in a column with the same letter are not significantly different (P > 0.05); *Values are means ± standard deviation; #values are mean of ranks.

was used to assess the sensory qualities of boiled roots. The sensory attributes evaluated were: General appearance, finger-feel firmness, sweetness and overall acceptability. The attribute sweetness was explained to panelist to mean desired taste as described by other researchers (Kapinga et al., 2003). Consumers rinsed their mouth with water before and in-between samples' tasting.

Statistical analysis

The statistical analysis was performed using Microsoft® Excel 2010/XLSTAT©-Pro (Version 2016.02, Addinsoft, Inc., Brooklyn, NY, USA. The Mann-Whitney test was used to analyze treatments cultivar and storage type factors. Kruskal-Wallis non-parametric test procedure was employed to analyze the curing treatment. Multiple pairwise comparisons was done using the Steel-Dwass-Critchlow-Fligner procedure/Two-tailed test when p<0.05. Comparison of scores on the basis of gender was done using the two sample t-test procedure. Minitab.v16.2.4.4™ (Minitab Inc., State College, PA, USA) software was used for this data analysis.

RESULTS AND DISCUSSION

All the sensory attributes had a sensory score ranging between 3.20 to 3.84 and 3.32 to 3.93 during the first and second farming seasons, respectively, indicating good consumer preference for both cultivars. However, in the first farming season, Apomuden had significantly lower score for sweetness (3.30 vs. 3.83, p < 0.0001) and overall acceptability (3.44 vs. 3.76, p = 0.000) when compared with Nane (Table 1). Recently, Owusu-Mensah et al. (2016) reported on the variability in the sweetness of cooked sweetpotato cultivars. The differences in the cultivars' overall acceptability could be attributed to desired taste (sweetness) as it was among other factors that largely influenced overall acceptability of sweetpotato cultivars (Kwach et al., 2010). In the second farming season, cultivars did not differ (p > 0.05) in all sensory attributes except for finger-feel firmness and Nane had a significantly higher score (3.63 vs. 3.35, p = 0.003). This could be attributed to the high dry matter content, averagely, 27% for Nane. The finding support the works of Kapinga et al. (2003) that showed firmness is an indicator of high dry matter content, a preferred sweetpotato root quality.

The OFSP cultivars have often been rated poorly regarding finger-feel firmness (Leksrisompong et al., 2012) probably due to their generally, low dry matter (20 to 24%) contents (Tomlins et al., 2012; Vimala et al., 2013).

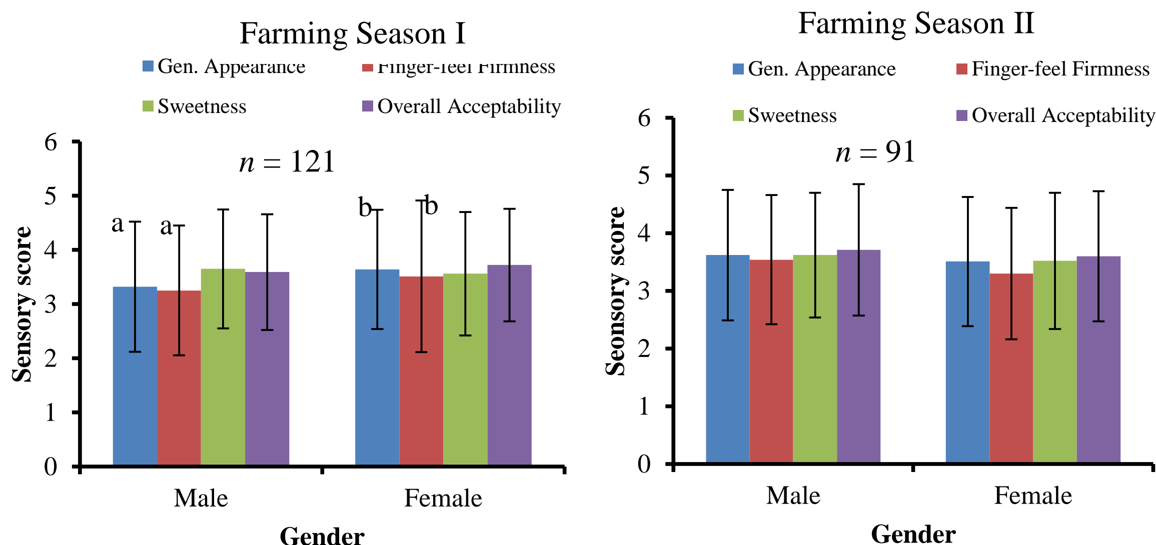

Figure 1. Sensory attributes of boiled OFSP roots as affected by respondent's gender. Bars represent means; and error bars are standard deviation. Comparison is between males and females with respect to the sensory attributes; bars with different alphabets are significantly different ($p < 0.05$).

However, Nane is relatively high in dry matter (27%) and it is reported that, African consumers prefer high dry matter cultivars (Tomlins et al., 2004).Thus, Nane, the cultivar under evaluation for release in Ghana could have high consumer acceptability. In the two successive farming seasons, in-ground curing consistently had higher mean rank for general appearance ranging from 278.98 to 329.58 ($p < 0.05$) when compared with field-piled (266.10 - 271.48) and uncured (233.69 - 307.97) as shown in Table 1. This implies that root that were cured in-ground and stored in sand box or under moistened straw heap were more preferred by consumers. Kuttappan and co-workers (2012) opined that the visual appearance among other factors is a very important criterion for assessing the quality of processed food product. Field-piled curing had significantly higher ($p < 0.05$) mean rank for all sensory attributes assessed for the second farming season except for general appearance. The curing method could have promoted the synthesis of α- and β-amylase enzymes that hydrolyses starch during cooking, leading to the formation of monosaccharides, precursors for vital flavour components as reported elsewhere (Wang et al., 1998). Both curing methods generally resulted in roots with better sensory quality than uncured treatment because the increased enzyme activity and sugars concentration make the boiled-cured roots become sweet and moist (Walter, 1987). Taste (sweetness) among other factors has been reported to be the main driver of overall acceptability of sweetpotato cultivars (Kwach et al., 2010).

Storage type showed no significant difference ($p > 0.05$) in all sensory attributes in both farming seasons. This is an indication that cultivars stored either in sand box or under moistened straw heap for a maximum of nine weeks will be equally accepted by Ghanaian consumers. The findings agree with van Oirschot and co-workers (2003) who reported that apart from fibrousness, storage had no significant effect on all the sensory qualities of sweetpotato roots. Mpagalile et al. (2007) also reported that improved open pit, improved house pit and raised woven structure had no significant influence on the acceptability of sweetpotato except for the traditional pit storage.

Gender is a major factor that determines the success and sustainability of any intervention including OFSP dissemination. In both farming seasons, males and females similarly ranked all the sensory attributes ($p > 0.05$) with the exception of general appearance and finger-feel firmness for farming season I (Figure 1). This is an indication that both cultivars could be equally accepted by both male and female. Because the men preferred the OFSP cultivars, and they are usually household heads, these β-carotene-rich food crops are likely to be prepared and consumed at the household-level. However, the finding in this study contradicts earlier studies by Tomlins et al. (2004) who reported that female consumers preferred some sweetpotato cultivars more than their male consumers.

The females scored the boiled roots higher when compared with ranking by the male participants for general appearance (3.64 *vs.* 3.32; $p = 0.002$) and finger-feel firmness (3.51 *vs.* 3.25; $p = 0.006$) in the first year. This probably could be attributed to the fact that females are more particular about the appearance of the food they consume, relative to their male counterparts. The preference of women is very important in determining

children preference of any food product. Reports have shown a significant but moderate direct relationship between preference of mothers and children (Skinner et al., 2002) since foods not preferred by mothers are not normally offered to children.

Generally, the sensory data from the two farming seasons suggest high consumer (males and females) preference after two months of storage. Therefore, in Ghana, and particularly in the rural communities where VAD prevalence is usually high, OFSP has the potential to be a dietary source of vitamin A for at least two months in a year.

Conclusion

The high sensory score for Nane is an indication that it possesses desirable sensory qualities that could be acceptable to Ghanaian consumers. Therefore, its evaluation process should be intensified for its release. In-ground and field-piled curing methods increased consumer acceptability and should be encouraged. The cceptance of the boiled OFSP cultivars by both males and females further suggest both cultivars could easily be accepted as part of the Ghanaian diet. Therefore, it is recommended that nutritional campaign should be intensified to increase its consumption as it could help in efforts target at reducing VAD.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Nutrient composition of cat's whiskers (*Cleome gynandra* L.) from different agro ecological zones in Malawi

Harris Jinazali[1]*, Beatrice Mtimuni[1] and Eric Chilembwe[2]

[1]Department of Human Nutrition and Health, Lilongwe University of Agriculture and Natural Resources, Malawi.
[2]Department of Horticulture, Lilongwe University of Agriculture and Natural Resources, Malawi.

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The aim of the present study was to determine the nutrient composition of *Cleome gynandra* L. Nutrient content determination of *C. gynandra* was limited in the previous studies done in Malawi. *C. gynandra* is a readily available vegetable used for relish and medicine. The study was done from February to March 2014. Nutrient analysis was done on *C. gynandra.* L. leaf samples collected from different agro-ecological zones in Malawi. Nutrient analyses included moisture, protein, crude fibre, ash, iron, calcium and zinc. All the analysis of the nutrients was done on dry weight basis. Results obtained indicated significant differences ($p<0.05$) in ash, iron and calcium contents ranging from 5.2 to 6.88, 22.93 mg/100 g – 44.7 and 1667.0 – 2497.5 mg/100 g, respectively. However *C. gynandra* from different agro ecological zones did not differ significantly ($p > 0.05$) in moisture content ranging from 79.28– 83.58 g/100 g, protein content ranging from 3.85– 5.80 g/100 g, crude fibre content ranging from 1.76– 2.06 g/100 g, vitamin C ranging from 214.31– 319.12 mg/100 g and zinc ranging from 2.28 – 2.9 mg/100 g. This study revealed that *C. gynandra* contains macro and micro nutrients which are essential for the growth and maintenance of the human body. Hence, it can be promoted for consumption to contribute some nutrients to the diet. The values generated by this study can contribute to Malawi Food Composition database.

Key words: Agro ecological zone, *Cleome gynandra*, Malawi, nutrients.

INTRODUCTION

Spider plant/cat's whiskers (*Cleome gynandra* L.) is one of the indigenous vegetables found in Malawi. Kwapata and Maliro (1997) reported that *C. gynandra* was fourth in the top ten of indigenous vegetables consumed around Karonga Agricultural Development Division in Malawi. Furthermore, in the National Nutrition Education and Communication Strategy for preventing child stunting in Malawi (2011 to 2016), *C. gynandra* was amongst vitamin A rich vegetables being promoted to be consumed for prevention and control of vitamin A deficiency

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

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(Government of Malawi, 2012). This vegetable is readily available since it grows as a weed in crop fields and also in road sides and in open grass lands including around households (Mishra et al., 2011). Usually, *C. gynanrda* does not require any formal cultivation (Nnamani et al., 2009). Hence, it can be a readily available source of relish, providing nutrients to the household. Narendhirakannan (2005) reported that *C. gynandra* is a rich source of nutrients such as vitamins A, C and minerals (calcium and Iron). In addition, Omale and Ugwu (2011) also reported that vegetables contribute to the mineral, vitamin and fibre contents of diets.

In terms of botany, *C. gynandra* is an erect herbaceous annual with branched stems in the family Cleomaceae. Leaves are alternate, digitately palmate and petiolate. Inflorescences are showy, many flowered, terminal racemes. The fruits are long-stalked, dry, dehiscent siliques. The plants inhabit wasteland and arable land (Mishra et al., 2011). Its nutritional value may vary with soil fertility, environment, variety, plant age and the production techniques used (Chweya, 1997). This vegetable grows as a weed in most tropical countries, but is a semi cultivated tropical leafy vegetable in many parts of sub-Saharan Africa, especially in eastern and southern Africa. The leaves and shoots are gathered from the wild or are cultivated.

Nutrient content of *C. gynandra* is affected by environment. Greenfield and Southgate (2003) reported geographical location, season, physiological state and maturity and cultivar/breed as major sources of nutrient variability. Geographical location causes variation in terms of soils and climatic conditions while physiological state and maturity affects the concentration of sugars, organic acids and vitamins in many plants. In terms of season, plant foods are especially prone to variation, particularly in their water, carbohydrate and vitamin content. In relation to this, Van Der Walt et al. (2009) reported differences in nutrients of *C. gynandra* from different districts in South Africa due to geographic and climatic conditions.

In a related study, Tidemann-Andersen et al. (2011) reported differences in iron and zinc of vegetable leaves including *C. gynandra* from two districts. These differences were attributed to soil differences and soil contamination.

The available literature on *C. gynandra* especially in Malawi, suggest limited research work on nutrient composition of *C. gynandra* from different agro-ecological zones in Malawi.

The current study was done to find the nutrient composition of *C. gynandra* from three different agro-ecological zones in Malawi. The hypothesis was that nutrient composition is different in *C. gynandra* from different agro-ecological in Malawi. The information generated by the current study can highlight the nutrients found in the *C. gynandra* from different agro ecological zones and can contribute to the production of Malawian Food Composition database.

MATERIALS AND METHODS

Study area

Samples of *C. gynandra* leaves were collected from Dedza (14.3817°S, 34.7741°E), Lilongwe (13.9626°S, 33.7741°E) and Salima (13.7796°S, 34.4586°E) districts representing high plateau and hilly area, medium altitude and lakeshore and low shading agro ecological zones in Malawi respectively. High plateau and hilly area zone has altitude of >1500, annual rainfall of >1200 mm and mean temperature range of 10 – 26°C. Medium altitude zone has altitude of 1000 – 1500, annual rainfall range of 800 – 1200 mm and mean temperature of 16 – 26°C. Lake shore and low shading area zone has altitude of 400 – 1000, annual rainfall of 600 – 800 mm and mean temperature of 20 – 29°C (Ministry of Agriculture and Food Security, 2003).

Sample collection

Collection of the samples was done from February to March 2014. The samples were collected around households and in the wild by the researcher. The samples collected were growing wild as weeds (not cultivated). The young leaves (those growing on the tips) of *C. gynandra* were the ones that were sampled and collected across all the sampled districts. This was done to minimise variation. After collection, *C. gynandra* samples were put in Ziploc™ bags to prevent moisture loss and any outside interference causing moisture loss. In each sampled district, 1500 g of fresh samples was collected for chemical analysis. Chemical analysis was done on the C. *gynandra* leaves collected across the districts.

Sample preparation

The collected samples were kept in Ziploc™ bags and put in a cooler box to maintain the fleshiness. The samples were transported using private transport from the sampled areas to the laboratory. Moisture content determination of the fresh samples was done soon after arrival at the laboratory. After moisture content determination the remaining *C. gynandra* samples were oven dried for 1 day at 60°C (Odhav et al., 2007).

Chemical analyses

Moisture content

Moisture content was determined using the oven drying method as indicated in AOAC (1984). A 2 gram sample for each of the sampled leaves was dried at 105°C for 5 h to constant weight and was placed in a dessicator to cool to room temperature. Three replications were done for each samples and moisture content was calculated as the loss in weight expressed as a percentage of the initial weight of the sample.

Protein content

Protein was determined by the method of the Association of Official Analytical Chemists (AOAC, 1990). Two grams of the sample was weighed into a digestion flask and 0.5 g of selenium catalyst was added. 25 ml of concentrated H_2SO_4 was added and the flask was shaken to mix the contents. The flask was then placed on a

digestion burner for 8 h and heated until the solution turned green and clear. The sample solution was then transferred into a 100 ml volumetric flask and made up to the mark with distilled water. Twenty five millilitres of 2% boric acid was pipetted into a 250 ml conical flask and two drops of mixed indicator (20 ml of bromocresol green and 4 ml of methyl red) solution was added; and into the decomposition chamber of the distillation apparatus was added 15 ml of 40% NaOH solution. Ten millilitres of the digested sample solution was then introduced into a Kjedahl flask. The condenser tip of the distillation apparatus was then dipped into the boric acid contained in the conical flask. The ammonia in the sample solution was then distilled into the boric acid until it changed completely to bluish green. The distillate was then titrated with 0.1 N HCl solution until it became colourless. The percent total nitrogen and crude protein were calculated using a conversion factor of 6.25.

Crude fibre content

Crude fibre was determined by the method of the Association of Official Analytical Chemists (AOAC, 1990). The defatted sample was transferred into a 750 ml Erlenmeyer flask and 0.5 g of asbestos was added. Two hundred millilitres of boiling 1.25 % H_2SO_4 was added and the flask was immediately set on a hot plate and condenser connected to it. The content was brought to boil within 1 min and the sample was digested for 30 min.

At the end of the 30 min, the flask was removed and the content was filtered through a linen cloth in a funnel and subsequently washed with boiling water until the washings were no longer acidic. The sample was washed back into the flask with 200 ml boiling 1.25% NaOH solution. The condenser was again connected to the flask and the content of the flask was boiled for 30 min. It was then filtered through the linen cloth and thoroughly washed with boiling water until the washings were no longer alkaline. The residue was transferred to a clean crucible with a spatula and the remaining particles washed off with 15 ml ethanol into the crucible.

The crucible with its content was then dried in an oven overnight and cooled in a desiccator and weighed. The crucible with its content was then ignited in a furnace at 600°C for 30 min, cooled and reweighed. The loss in weight gave the crude fibre content and was expressed as a percentage of the initial weight of the sample.

Ash content

Ash was determined by the method of Association of Official Analytical Chemists (AOAC, 1990). A 2.0 g sample was weighed into a previously dried and weighed porcelain crucible. The crucible with its content was placed in a furnace preheated to 600°C for 2 h. The sample was allowed to cool in the furnace to 250°C. The crucible and the ash were then transferred into an oven at 100°C for 30 min cooling. After this period, the crucible with its content was cooled in a desiccator. The crucible with its content was weighed. The weight of the ash was expressed as a percentage of the initial weight of the sample.

Vitamin C content

Vitamin C was determined using spectro photometric method (Azra et al., 2012). Potassium iodide (2.0 g) and iodine (1.3 g) were dissolved in 100 ml distilled water to make iodine solution (0.005 mol/l). This solution was diluted ten times. The concentration of prepared iodine solution was determined by titration with a standard solution of ascorbic acid. Soluble starch (0.25 g) was added to 50 ml of near boiling distilled water to make starch indicator solution (0.5 %). Stock solution of ascorbic acid containing 0.1 mol/l of ascorbic acid was prepared by dissolving appropriate amount of ascorbic acid in distilled water and stored in a glass stopped bottle at 4°C in the dark. Solutions of variable concentrations were prepared by diluting the stock standard solution in water before use. Methylene blue solution (0.4 mmol/l) was prepared by dissolving 0.0128 g of methylene blue in 100 ml distilled water.

For ascorbic acid determination, 2.5 g *C. gynandra* leaf samples were coarsely ground and glacial acetic acid (2 ml) was added. The mixture was stirred for about 20 min and rapidly filtrated using a suction pump and Buchner funnel. After that, the volume of the sample was made up to 100 ml with distilled water. Analysis with spectrophotometric method used T 90 UV/VIS Spectrometer (England serial number 20-1901-01-0351) to determine the amounts of AA in the samples. Fifty microliters of a sample solution was mixed with 125 µL of MB (c=0.4 mmol/l) solution and diluted up to 10 ml with distilled water. Decrease of absorption was measured at λ max = 665 nm. All analysis was carried out in triplicates. Results were expressed in mg of ascorbic acid per 100 g of dry sample.

Minerals determination

Calcium, iron and zinc

Calcium, iron and zinc were determined by atomic absorption spectrophotometry (AAS-6200 model) at wave length of 213.9 nm and range of 0.5-5 with flame rich air C_2H_2 (Norhaizan and Ain, 2009). One gram of the sample was dry ashed in a muffle furnace at 550°C for 5 h until a white residue of constant weight was obtained. The minerals were extracted from the ash by adding 20.0 ml of 2.5% HCl, heated to reduce the volume to 7.0 ml, and this was transferred quantitatively to a 50 ml volumetric flask. It was diluted to the mark (50 ml) with distilled water, stored in clean polyethylene bottles and calcium, iron and zinc content determined using atomic absorption spectrophotometer. All the chemical analysis were carried out on dry weight basis and expressed per 100 g edible portion.

Statistical analysis

Data on nutrient and mineral composition was entered in Microsoft excel and analysed for analysis of variance (ANOVA) in Genistat software version 15 where means and standard deviations were generated at 95% confidence interval level. Means were compared using Fisher's Protected Least Significant Difference and Fisher's unprotected Least significant difference at 5% level of significance.

RESULTS AND DISCUSSION

Nutrient content of raw cats whiskers (per 100 g)

Significant differences ($p < 0.05$) were found in ash content (total measure of mineral content) among *C. gynandra* from the three districts representing agro-ecological zones in Malawi (Table 1). Ash content increased with a decrease in altitude across the agro-ecological zones. The differences in the ash contents might be attributed to the differences in amount of the minerals present in the *C. gynandra*. The differences in minerals might also be attributed to the differences in soil types from the different districts, which affects minerals present for example Salima district contains calcimorphic alluvial soils and medium textured sandy clay loam soils,

Table 1. Nutrient content of raw *C. gynandra* (g/100 g) collected from Dedza, Lilongwe and Salima districts in Malawi.

Districts nutrients	Dedza	Lilongwe	Salima	Mean for all districts
Moisture content	83.2 ± 1.45	83.6 ± 3.42	79.3 ± 3.38	82.0
Protein content	4.8 ± 1.67	3.9 ± 0.97	5.8 ± 1.37	4.8
Crude fibre content	2.1 ± 0.39	1.8 ± 0.01	1.9 ± 0.25	1.9
Ash content	5.2 ± 0.07^{a}	6.3 ± 0.11^{b}	6.9 ± 0.27^{c}	6.1
Vitamin C (mg/100 g)	319.1 ± 107.82	262.1 ± 77.87	214.3 ± 37.97	265.2
Calcium (mg/100 g)	2497.5 ± 316.43^{b}	1667.0 ± 518.66^{a}	2465 ± 749.76^{b}	2209.8
Iron (mg/100 g)	22.9 ± 2.04^{a}	44.7± 11.46^{b}	39.4 ± 13.0^{ab}	35.7
Zinc (mg/100 g)	2.7 ± 1.1	2.3 ± 0.25	2.9 ± 0.45	2.6

Values are triplicate mean value ± standard deviation. Values on the same row with different superscript are significantly different ($p < 0.05$).

Lilongwe district contains ferruginous latosol soil, clay loam soil while Dedza district contain leached latosols (Saka et al., 2003). High ash content in samples from Salima district, might also be attributed to warmer conditions which generally accelerates plant growth (Whiting, 2014). The samples from Salima might have accumulated higher biomass than the samples collected in Dedza and Lilongwe which have cooler conditions. However, the ash content of *C. gynandra* reported in the present study is lower than 11.2 g/100 g reported by Mibei et al. (2011).

Significant differences were also observed in calcium and iron contents of *C. gynandra* from the different agro-ecological zones. This might also be attributed to the differences in the soil types from different districts. Joy et al. (2015) in their study in Malawi reported differences in concentrations of Ca, Cu, Fe and Se in leafy vegetables grown on calcareous soil than non-calcareous soil. In addition to that, mineral and trace element content of plant leaves is a function of the environment and in leafy vegetables which is strongly influenced by the chemical composition of the soil and the climate (Modi, 2007). Iron content found in this study are higher than 14.4 mg/100 g and 6.3 mg/100 g iron content of *C. gynandra* reported by Maroyi (2013) and Hilger (2005), respectively. However, the calcium values found in the present study are higher than 288 mg/100 g calcium content of *C. gynandra* reported by Mbugua et al. (2008). Differences in minerals calcium and iron from the 3 districts reported in the study agree with van der Walt et al. (2009), who reported significant differences in iron and zinc from Rustenburg and Capricorn districts. Minerals are important for vital body functions such as acid-base and water balance (Omale and Ugwu, 2011).

No significant differences ($p > 0.05$) were found in protein content among *C. gynandra* from the three districts representing the three agro-ecological zones (Table 1). In addition, no significant differences were found in crude fibre content, moisture content, vitamin C and zinc of *C. gynandra* samples from the three districts. The finding of no significant differences in moisture, protein, crude fibre, vitamin C and zinc was not expected by the current study. However, the values of nutrients found by this study are similar to other previously reported studies. Moisture contents of *C. gynandra* for Dedza and Salima found in this study are within the range of 81.8 to 89.6% reported by Chweya and Mnzava (1997). Moisture content determines the freshness of food. In the present study, all the leaf samples had high percentage of moisture content, this is an indication that they possess large number of cell saps. Water is clearly the most important nutrient and the most abundant substance in the human body (Adeniyi et al., 2012).

Protein content of *C. gynandra* found by this study are similar to those by Oldhav et al. (2007) and Mbugua et al. (2008) who reported 5 and 4.8 g/100 g protein contents, respectively. Protein values of vegetables might be of low quality and low bioavailability because of phenolic compounds which might be found within the vegetable (Chweya and Mnzava, 1997). The non-significance in protein contents of *C. gynandra* among districts found by this study disagrees with the findings of Modi (2007) who indicated that cool environmental conditions are associated with high total protein in leafy vegetables while hot temperatures had a significant decrease in leaf protein content. Protein is necessary for building the structural components of human body, such as muscles and organs (Omale and Ugwu, 2011).

Crude fibre contents found in this study were lower than the results found by Hassan et al. (2007) who indicated 6.0 to 6.3% of crude fibre in *C. gynandra*. However, crude fibre values found by this study are higher than 0.8 g/100 g reported by Mibei et al. (2011). The differences of fibre contents found in this study and those reported in literature are expected. This is because the total dietary fibre content of African leafy vegetables may vary due to differences in stages of plant maturity, seasonal variation, fertilizers or chemicals used, variety of plant, geographical location and the method used for analysis (Punna and Parachuri, 2004). The fibre content ranging from 1.7 to 2.1 g/100 g found in the current study are very low as compared to the adequate intake (AI) of total fibre for a normal adult which is 38 and 25 g/day for males and females, respectively (Food and Nutrition Board, Institute of Medicine, 2003). Hence, *C. gynandra* is a poor source of fibre to human beings.

Vitamin C values of *C. gynandra* found in this study are higher than 107 and 13 mg/100 g found by Mibei et al. (2011) and Maroyi (2013), respectively. These differences were expected because the amount of vitamin C in plants varies greatly due to variety, environment grown weather and level of maturity (age) of the plants (Mibei et al., 2011). In agreement with that, Ayua et al. (2016) reported vitamin C concentration in the three *C. gynandra* varieties to be significantly higher in the mature leaves than in immature ones. This was because young leaves have more demand for vitamin C and cannot accumulate enough vitamin C to meet their physiological processes. Vitamin C is important to humans because it is an anti-oxidant and has health promoting properties.

The study findings of zinc contents of *C. gynandra* are lower than 8.4 mg/100 g reported by van der Walt et al. (2009). The zinc values found by the study is also lower than 6 and 6.3 mg/100 g, reported by Tidemann-Andersen et al. (2011). The lower zinc concentrations obtained by this study are supported by Gibson (1994), who reported that vegetables and fruits have much lower concentrations of zinc due to their high water content. In terms of bioavailability, zinc from animal sources has higher bioavailability as compared to plant sources due to phytates and fibres that inhibit zinc uptake by intestines (FAO/WHO, 2002). The differences in the nutrient composition of *C. gynandra* reported in this study and those reported in literature might be linked to species, climate, growing conditions, nature of soil, application of natural or artificial manure and period of analysis (Adeniyi, 2012).

Conclusions

In this study, there are significant differences in ash, iron and calcium contents of *C. gynandra* leaves collected from different agro ecological zones (high plateau and hilly area, medium altitude and lakeshore and low shading agro ecological zones) in Malawi. In addition, it can also be concluded that *C. gynandra* from different agro ecological zones do not have significant differences in moisture content, protein content, crude fibre content, vitamin C and zinc. Hence, promotion of this vegetable to achieve dietary diversity and food security in these different agro ecological zones will not have discrepancies in terms of some of the nutrients. It can also be concluded that *C. gynandra* contains some macro and micro nutrients which are essential for the human body. Therefore, *C. gynandra* can be a readily available cheap source of relish and contribute some of these nutrients to the human diet.

Conflicts of Interests

The authors have not declared any conflict of interests.

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