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African Journal of Food Science

Full Length Research Paper

Consumer acceptability of modified and traditionally produced *amala* from fermented orange-fleshed sweet potato

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Amala **is a food made out of sweet potato, yam and/or cassava flour product that is traditionally consumed in Nigeria. The aim of the study was to evaluate the acceptability of *amala* produced from orange-fleshed sweet potato using traditional and modified methods of fermentation. Consumer acceptability studies provided information on the sensory attributes of traditional and modified *amala* samples. It was found that the pH of the *amala* was the same as the pH of the fermented sweet potato at Day 3 for both the cold and hot traditional (4.4) and modified (3.5) processes. The mean appearance, odour and familiarity scores were in a narrow range (7.2 to 7.8), while taste and overall acceptability showed a wider range of mean scores (6.9 to 8.0). The major differences ($P < 0.05$) observed were in taste and overall acceptability of the two products. The study indicated a higher significant acceptability for modified *amala* than traditional *amala*. It further demonstrated the usefulness of consumer acceptability test in quantifying the sensory attributes of the two products. This study was the first of its type and can serve as a good opening for policy makers wishing to promote the use of orange-fleshed sweet potato to fight against vitamin deficiencies in developing countries, particularly Nigeria. The study has successfully created varieties as well as alternative to *amala* from yam and the traditional method.**

Key words: Orange-fleshed sweet potato, traditional, modified fermentation, *amala,* vitamin A and C, Nigeria.

INTRODUCTION

Amala is a stiff paste produced from fermented and sun-dried sweet potato, cassava or yam flour in Nigeria. It is a staple food that has become increasingly popular in Nigeria (Adewumi and Adebayo, 2008). The cooked paste *amala* is mostly consumed when hot and is rarely stored (Omemu et al., 2011). Fetuga et al. (2013)

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reported that a*mala* produced from yam and cassava is more widely used than *amala* made from sweet potato. They also found that *amala* made from yellow-fleshed sweet potato is more widely eaten than *amala* made from orange or white-fleshed varieties. Furthermore, the colour of the products produced from orange-fleshed sweet potato poses problems and challenges associated with acceptability and could put some consumers off this product (Akoroda and Egeonu, 2009). Therefore, effort should be made to improve acceptability of *amala* made from orange-fleshed sweet potato amongst consumers.

Sweet potato is one of the world's most important food crops that have the potential of bridging food gap due to diversified processing and utilization technologies that have been produced but not yet fully exploited (Woolfe, 1992). Developing countries produced more than 95% of the world's sweet potato over the period of 2004 to 2009 (Harvestplus, 2004-2009). Nigeria is the second producer of sweet potato in the world and the highest in Africa (FAOSTAT, 2005; Akoroda and Egeonu, 2009; Lebot, 2009), where production is mainly targeted at food security (Minde et al., 1999). Micronutrient deficiency is increasingly becoming a public health problem in developing countries, particularly affecting women and preschool children (UN SCN, 2004). There is potential for orange-fleshed sweet potato, which contains high levels of β-carotene and ascorbic acid, to contribute to overcoming this deficiency. Orange-fleshed sweet potato is regarded as a healthy crop that contains significant amounts of natural health promoting compounds (Bovell-Benjamin, 2007). Processing can result in the loss of these key nutrients and previous studies have shown that modifying the process can result in the retention of higher levels of vitamins (Yusuf et al., 2015).

The roots are normally processed into other food products before consumption and the traditional processing method commonly used is fermentation (Odebode et al., 2008). In Nigeria, the common fermentation methods involve cold water fermentation (soaking) and hot water fermentation (parboiling) (Fetuga et al., 2013) and are aimed at improving the shelf life, texture, taste, aroma and nutritional value of the end product (Kohajdova and Karovicova, 2007). It has been reported that the addition of fermentable sugar (glucose, fructose or sucrose) to fermentations compensate the loss of sugars as a result of the initial microbial activity and provide enough fermentable sugar during the important stage of lactic acid bacteria growth to produce sufficient lactic acid to decrease the pH level (Shao et al., 2004, 2005). It is considered that the addition of fermentable sugar will extend the growth and metabolism of lactic acid bacteria to produce lactic acid and sustained pH reduction, which will eventually result in a safe and nutritious food that would benefit the poorest rural people. A modified fermentation method for producing *amala* from orange-fleshed sweet potato that retained more vitamins was prepared and its acceptability to consumers was determined. If *amala* produced by the modified method were accepted by the consumers it could increase the use of orange-fleshed sweet potato. The acceptance of an orange-fleshed sweet potato product (*amala*) produced from a modified method by the targeted consumers is important (Oyunga-Ogubi et al., 2011). The present study was aimed at comparing the acceptability of *amala* produced from traditional and modified fermented orange-fleshed sweet potato.

MATERIALS AND METHODS

Sample preparation

Orange-fleshed sweet potatoes (CRI-Apomuden variety (CIP440254)) were purchased from a retail super-market in Medway and washed using tap water in the Natural Resources Institute Food Processing Laboratory, University of Greenwich, United Kingdom. Sweet potato roots were peeled, cut into slices (2.0 cm long and 15 mm thick) and dried in an oven (Gallenkamp) at 60°C for 18 h.

In the traditional method, sweet potato slices (2000 g) were weighed into a 5000 ml sterile beaker and 2000 ml deionised sterile water was added. For the modified fermentation, 100 g (5% w/v) food grade fructose (Tate and Lyle Fruit Sugar, Tesco, United Kingdom) was dissolved in 2000 ml of sterile deionised water and 2000 g of sweet potato slices were added. The top of the beaker was covered with aluminium foil. The beaker was then incubated at 30°C (to represent ambient temperature in West Africa) for 3 days for the cold water fermentation. For the hot water fermentation, the beaker was placed in a water bath set at 65°C (based on the range of 63 to 67°C observed in the field survey) (Fetuga et al., 2013). Once the water in the beaker had reached 65°C, the temperature was taken down to 30°C. The samples were kept at this temperature in the water bath for 3 days.

The changes in pH of the fermenting sweet potato medium at Days 0 and 3 and *amala* for both the traditional and modified fermentation methods was measured and recorded in triplicate at each sampling time using automatic pH meter (3510, Jenway).

Preparation of cooked *amala* samples

Amala was prepared by taking slices of the fermented sweet potato (after 3 days of fermentation) and drying them in an oven at 60°C for 18 h. The dried samples were pounded into flour using a pestle and mortar. The flour was sieved using a domestic sieve and mixed with boiling water at a ratio of 1:4 and then stirred continuously to prevent lumps from forming. The paste was cooked for 10 min over a low heat. The cooked paste was wrapped in aluminium foil with the shiny side on the inside and kept at 63°C in a calibrated oven (to hot-hold *amala*) before serving.

Consumer acceptability test

Approval to conduct the study was sought from the University of Greenwich Research Ethics Committee and was given on 22nd November, 2013. The consumer acceptability test was carried out in the Food Processing Laboratory at the Natural Resources Institute, University of Greenwich, United Kingdom on 17th December 2013.

Table 1. The mean acceptability test scores of *amala* produced from traditional and modified fermented orange-fleshed sweet potato.

Fermentation method	Appearance	Odour	Taste	Overall acceptability	How much you like this product compared to the *amala* that you are familiar with
Traditional cold	7.4 ± 0.13	7.3 ± 0.13	6.9 ± 0.13	6.9 ± 0.13	7.2 ± 0.13
Modified cold	7.6 ± 0.13	7.6 ± 0.13	7.9 ± 0.13	7.9 ± 0.13	7.8 ± 0.13
Traditional hot	7.3 ± 0.13	7.3 ± 0.13	7.1 ± 0.13	7.0 ± 0.13	7.3 ± 0.13
Modified hot	7.5 ± 0.13	7.5 ± 0.13	8.0 ± 0.13	8.0 ± 0.13	7.8 ± 0.13

A group of people was recruited to assess the acceptability of *amala*. *Amala* is a traditional product commonly consumed in Nigeria. An invitation to participate in the acceptability group was e-mailed to staff and students of the University of Greenwich. Familiarity with *amala* traditional fermented food product from West Africa was one of the selection criteria for participating. Participants with any health issues or an allergy to sweet potato or fermented food products were excluded from the study. Responses were received and participants were selected based on the above mentioned criteria. Sixty two healthy adults were selected to take part in the study and were asked to sign a consent form. *Amala* was cooked on the day of tasting and cut into approximately 30 g pieces and served on a white plastic tray coded with a three-digit random numbers (care was taken to ensure that each consumer received samples in a different order). Participants were seated at a tasting booth presented with four *amala* samples (traditional cold, traditional hot, modified cold and modified hot) randomly placed on the plate. Each sample was accompanied with a score sheet and participants were asked to score the sensory attributes of *amala* using a 9-point hedonic scale which ranged from "like extremely" to "dislike extremely" (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely). The scores of the attributes were recorded and the mean test scores were calculated and tabulated (Table 1). Bottled water was provided for participants to rinse their mouth between samples. Good hygienic practice was followed throughout the process.

Statistical analysis

The consumer acceptability data recorded for the four *amala* samples were subjected to a two-way analysis of variance (ANOVA) using the R-package. The significant differences between the mean scores for the four *amala* samples (appearance, odour, taste, overall acceptability and how much you like the product as compared to the *amala* that you are familiar with) were tested. A p-value of <0.05 was considered to be statistically significant.

RESULTS

Amala was produced from traditional and modified fermented orange-fleshed sweet potato for both cold and hot processes. The pH value of the traditional and modified fermented orange-fleshed sweet potato and that of *amala* were measured and recorded (Figure 1). Figure 2 shows the changes in the mean scores of *amala* for the two fermentation methods over sensory attributes. The following interpretation of the 9-point hedonic scale was used for the discussion of the results: 7 to 9 was considered to be positive and indicated that the participant liked the product, 4 to 6 was considered the neutral part of the scale (neither liked nor disliked the product), while 1 to 3 was considered to be negative (the participant disliked the product).

Analysis of the differences in mean test scores recorded between *amala* produced from traditional and modified fermented orange-fleshed sweet potato was shown to be statistically significant (p≥0.05).

DISCUSSION

Amala is a popular food that is eaten every day in Nigeria. By modifying the method of producing *amala* using orange-fleshed sweet potato, more vitamins could be introduced into the diet. However, promoting the new method of production and acceptability of the new product by the target consumers will determine its success. The present study was the first to assess consumer acceptability of *amala* produced from traditional and modified fermented orange-fleshed sweet potato using a 9-point hedonic scale.

The pH of the fermenting sweet potato at Days 0 and 3 and *amala* for both the traditional and modified methods was measured. It was found that the pH level dropped to 4.4 and 3.5 on Day 3 in the traditional and modified fermentation methods, respectively (Figure 1). The pH of the final *amala* was the same as the pH of the fermented sweet potato at Day 3 for all the processes. The minimum pH levels (4.4 and 3.5) recorded would classify the products as a high acid food in which growth of known foodborne pathogens would be inhibited and outgrowth of bacterial spores would not take place. The cause of the decrease in pH could be due to the production of organic acids such as lactic and acetic acids in the first three days of the fermentation as a result of microbial activity. The results suggest that in the traditional fermentation method, key nutrients needed for microbial growth and concomitant acid production are limited. It can be considered that the addition of 5% w/v fructose in the modified fermentation method enhanced acid production and resulted in a concomitant pH reduction (Shao et al., 2004, 2005).

The sensory attribute score means (n=62) for the four

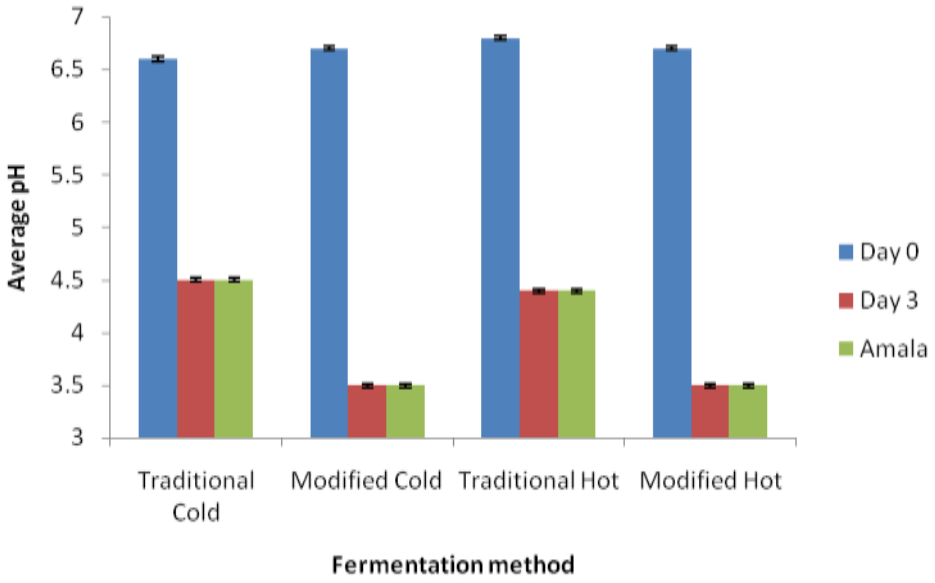

Figure 1. Comparison of changes in mean pH ± S.E between traditional and modified fermentation methods.

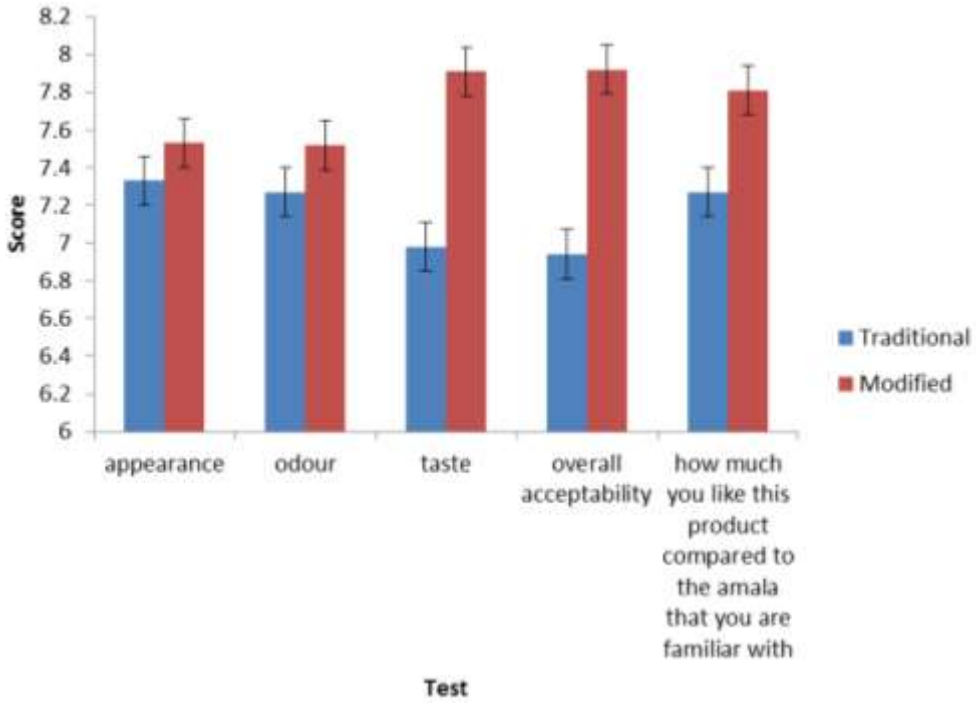

Figure 2. Comparison of changes in mean test score ± S.E of *amala* produced from traditional and modified fermented orange-fleshed sweet potato.

different *amala* samples are shown in Table 1 and Figure 2. The mean appearance and odour scores were in a narrow range of 7.3 to 7.6, with modified *amala* having slightly higher scores than the traditional *amala* for both cold and hot methods. This indicated little or no difference in the appearance and odour of traditional and

modified *amala* for both cold and hot processes. A wider range of mean scores from 6.9 to 8.0 was obtained for taste and overall acceptability, with modified *amala* recording the highest mean scores. This showed a clear difference in the taste and overall acceptability between traditional and modified *amala* for both the cold and hot processes. When examining consumer acceptability of the modified product as compared to the *amala* that they were more familiar with, the mean scores ranged from 7.2 to 7.8, with the mean score for modified *amala* being slightly higher than that of traditional *amala*. There was very little or no difference between *amala* that consumers are used to eating and the *amala* (traditional and modified) produced for this study. Overall participant's acceptability of *amala* produced from the modified fermentation was significantly higher ($p \geq 0.001$) than that of *amala* produced from traditional fermentation methods. This could be attributed to the addition of sugar (5% w/v fructose) to allow the fermentation to continue, as demonstrated by a lower pH in the modified process. It is possible that a sustained lower pH level produces *amala* which has a taste and flavour preferred by the participants.

The high ranking scores observed for modified *amala* indicated a higher acceptance among participants (Table 1). This suggests a readiness amongst participants to accept the modified *amala*. A survey report from Fetuga et al. (2013) has shown that if sweet potato flour were to be made readily available, then sweet potato *amala* could offer food security and improved nutritional status to the rural poor as compared to cassava and yam *amala*.

The acceptability of *amala* produced from the cold and hot fermentations for both the traditional and modified water fermentation were analysed. This study revealed a non-significant difference ($p \leq 0.05$) in the acceptability of *amala* produced from both types of fermentation. This was probably due to the fact that both types of fermentation were carried out for the same period of time.

CONCLUSION AND RECOMMENDATIONS

Evidence from the consumer acceptability test showed a greater acceptability of *amala* produced from the modified fermentation method than the traditional method. The major differences were in taste and overall acceptability of the two products. The study indicated a higher significant acceptability for modified *amala* than traditional *amala*. Hence, the modified method results in a safe and nutritious food that would benefit the poorest rural people. This is a useful conclusion for pre-school children and pregnant women at risk of becoming vitamin deficient in developing countries. Consideration would have to be given to whether the use of an additional substrate would be economically viable. Fructose is commercially available in retail super markets but the additional cost (250 g of fructose is required to ferment 5000 g of orange-fleshed sweet potato with a total cost of ₦271 (1.65 $ or 0.99 £) could be a constraint to this method being used by the rural poor. This is because 70.8% of the Nigerian population are living on less than ₦164.66 (less than 1 $ or 0.60 £) a day (Human Development Report, 2006).

In summary, therefore, it has been demonstrated that the modified fermentation could improve vitamin A and C levels in *amala* (Yusuf et al., 2015) and could help to make the method more widely used.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Nutritional and sensory qualities of commercially and laboratory prepared orange juice

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Physico-chemical analyses and sensory evaluation were carried out on commercially and freshly prepared orange juice (100%) in the laboratory and its shelf-life after the storage period of 90 days using various storage methods. At the end of the study, the result showed that the laboratory processed orange juice in terms of the nutritional composition when compared with the commercially processed orange juice had a better quality considering the parameters assessed. The results of the laboratory prepared orange juice showed that the chemically treated, pasteurized, concentrated and carbonated orange juice had pH 1.6 to 4.5, TTA 0.03 to 0.31%, maturity ratio 1.16 to 8.55, total soluble solids (TSS) ranging from 26 to 33°Brix. The vitamin C content for the untreated juice (fresh) and the commercially produced were 43 and 2.67 mg/100 ml, respectively. The experimental samples of chemically treated, pasteurized, concentrated and carbonated had vitamin C content ranging from 22-29, 27-32, 28-30 and 17-29 mg/100 ml, respectively. Sodium and phosphorus were found in small amounts ranging from 0.01-0.12 and 0.001-0.02%, respectively in the orange juice. Among the various processing methods concentrated orange juice ranked first followed by chemically treated, pasteurized and carbonated in terms of the sensory evaluation as assessed by the panelists.

Key words: Orange juice, physico-chemical properties, processing techniques, sensory evaluation.

INTRODUCTION

Fruit juices are well recognized for their nutritive value, mineral and vitamin contents (Wardlaw, 2004; Mannay and Shadaksharaswany, 2005). They are important source of bioactive compounds such as phenolics (such as flavanone glycosides, hydroxycinnamic acids), vitamin C and carotenoid which is an excellent source of bio-available antioxidant phytochemicals and it improves blood lipid profiles especially for people affected with hypercholesterolemia (Franke et al., 2005). Juices are the aqueous liquids expressed or otherwise extracted usually from one or more fruits or vegetables, purees of the edible portion of one or more fruits or vegetables, or any concentrates of such liquids or purees (Fraternale et al., 2011).

Orange fruits have been reported as a rich source of these phytochemicals such as flavonoids, especially flavanones, which have been shown to possess several physiological properties which can help inhibit cell

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proliferation and cell differentiation (Vanamala et al., 2006; Ndife and Abbo, 2009). However, due to the perishable nature of fruits and vegetables, high post harvest losses occur immediately after harvest, during distribution and marketing (Manny and Shadaksharaswany, 2005; Potter and Hotchkiss, 2006) resulting from lack of cold storage facilities on the farms, improper handling and inadequate processing facilities (Landon, 2007: Adubofuor et al., 2010). Reports have shown that the post harvest losses for oranges range between 31 and 50% (Alaka et al., 2003; Landon, 2007). One of the ways of preserving these fruits and vegetables from deterioration and subsequent loss is to process them into fruit juices (Vanamala et al., 2006). The processing of fruit juices such as orange juice presents the problem of storage, an unstable product for periods of 12 months or more. These juices are susceptible to three types of deterioration such as microbial spoilage, colour changes and off flavours due to enzymatic reactions (Obire et al., 2008; Lawlor et al., 2009: Sospedra et al., 2012*).* Juices are no exception, and there is, therefore, a strong tendency towards consumption of premium quality juices. These juices are directly obtained from fruits (not from concentrate), are distributed through the refrigerated chain and have a relatively short shelf life (Esteve et al., 2005). Traditionally, thermal pasteurization is used to inactivate micro-organisms to prolong shelf life on the one hand, and to inactivate heat-stable pectinmethylesterase (PME) for preventing cloud loss on the other hand (Chen and Wu, 1998). Typically, for shelf stable juices, processing times for thermal pasteurization are equivalent to 90°C for 1 min (Eagerman and Rouse, 1976). Thermal processing has a negative impact on the quality of orange juice such as loss of fresh flavor (Braddock, 1999), degradation of ascorbic acid (Chen et al., 1993), and discoloration (Arreola et al., 1991; Li et al., 1988; Tatum et al., 1975). To avoid quality degradation of orange juice by thermal processing, non-thermal preservation techniques such as high pressure and pulsed electric fields have been studied during the last decade.

Various methods which can be used for the processing and preservation of orange juice include; the traditional thermal processing method (pasteurization) also called high temperature short time (HTST), evaporation (concentrated juice), pressurized carbon dioxide process (carbon dioxide saturation), high pressure processing (HPP), pulse power submerged electrical arc technology method, sterile filtration and spray drying (Andres et al., 2001; Deliza et al., 2005). The pasteurization method kills bacteria and extend the shelf life of juices, but affects the taste of the product (Andres et al., 2000). Orange juice that has been extracted and pasteurized for few seconds to inactivate yeasts and moulds will have a shelf life of up to 21 days when stored at 4°C or less.

A shelf life of up to three months at chill temperatures (4.4 to 7.2°C) can be attained with orange juice treated with chemical preservatives. Preservatives used are typically sulphur dioxide at 100 ppm and a combination of sorbic and benzoic acid at up to 400 ppm (Mehmood et al., 2008).

Concentrated juices produced through the evaporation method are another method of storing juices, but this when reconstituted could not approach the quality of the original. The reason is because most of the compounds which give a fruit juice its flavour and aroma are volatile, and lost in the usual evaporation process (Moshonas and Shaw, 1998). The carbon dioxide saturation method has a high degree of stability in juices and it prevents the development of yeasts, probably by retarding the conversion of pyruvic acid into acetaldehyde and carbon dioxide. Moulds and bacteria are less affected and could cause spoilage pressurized carbon dioxide process, according to (Giovanna et,al; 2009) showed that the process is as effective as heat pasteurization but does not change the taste and preserves more of the vitamins found in fresh squeezed juices.

Today's society is characterized by an increasing health consciousness and growing interest in the role of food for maintaining and improving human well-being and consumer health. Orange juice is a heterogeneous, two-phase system consisting of the serum, a clear aqueous phase containing soluble compounds, and a water insoluble phase made up of particles ranging from 0.05 µm to a few hundred micrometers in size. These insoluble particles enhance the colour, flavour, aroma and body of the juice; as such they are highly desirable in commercial product (Rega et al., 2004). However, like most fruit juices, orange juice undergoes changes in chemical, nutritional and flavour characteristics during processing. Studies by Dauda and Adegoke (2014) showed that non-enzymatic browning during processing or storage of juices may affect the flavour, colour or other quality factors of the product. Kampen (1976) stored freeze-dried orange juice crystals and a synthetic mixture for 40 days at 50°C and monitored losses of total amino acids (75%), ascorbic acid (100%), citric acid (5.1%) and sucrose (4.4%). The orange juice crystals were discoloured from Maillard browning and several carbonyl compounds and furfural derivatives were identified as products of reactions. Several attempts have been made to examine the influence of different processing techniques on the quality and shelf life stability of orange juice. Nagy and Smoot (1977), Roig et al. (1999), Kaanane et al. (1988), and Kennedy et al. (1990) studied the kinetics of ascorbic acid degradation in pasteurized orange juice during storage as a possible marker for the end of shelf life.

During the processing of fruit juices, a large part of the quality characteristics of the fresh fruits undergo remarkable changes which could reduce the nutritional value of the products. Moreover, the fruit juices may be stored for several months in unfavourable conditions before consumption, thus leading to undesirable quality

changes due to the influence of temperature, time, oxygen content, light exposure and packaging material (Averbeck and Schieberle, 2010). Products obtained from orange juice are frozen concentrated orange juice (FCOJ) in which about 85% of the water content is removed, freshly- squeezed or not from concentrate (NFC) also known as single strength orange juice (SSOJ) and powdered juice (Anonymous, 2003). Processed products are expected to be essentially uniform in composition and sensory characteristics from one batch to another. Infact, compositional limits for many processed foods such as orange juice are set out in official regulations and purchasing specifications. To obtain the required degree of uniform quality in these products, processors often have one option that is, to use carefully selected raw materials (Board and Woods, 1983).

Sensory quality attributes and nutritive value of fruit play an important role in consumer satisfaction and they influence further consumption. Sensory ratings of fruit juice by products and physical measurements of fruit juice properties are useful methods in the evaluation of fruit juice quality (Colaric et al., 2005). Sensory quality is a difficult concept to define; it should be comprehended as interaction between the product and the consumer. It is necessary to establish a relationship between the physical and chemical composition of the product and its sensory attributes such as colour, texture, aroma (volatile compounds) and taste (sweet, sour, salty and bitter sensations) as well as between the sensory perceptions and the acceptability for the consumer (Escribano et al., 2010).

Several factors need to be considered when assessing for quality of fruit juice. The composition of a fruit juice depends on the variety, origin and growing conditions of the fruit, its quality and the processing and storage procedures (Ndife et al., 2013). Apart from nutritive value of the juice, it should have acceptable organoleptic and physicochemical characteristics as well as free from microbial and chemical contaminants. The juice organoleptic features of interest include colour, aroma, taste/flavour, texture, degree of spreadiblity and overall acceptability by the consumers (Iwe, 2010). The juice must have the characteristic colour, flavour and taste typical of the fruit from which it comes.

The physicochemical characteristics of juices considered in quality assessment include pH, titratable acidity (TA), total soluble solids (°Brix), dry matter contents, ash content, crude protein, ascorbic acid, total sugar, reducing sugar and °Brix (sugar)/acid ratio. The constituent of juice predominantly is water and also contains carbohydrate, sucrose, fructose, glucose, sorbitol and small amount of protein (Pao et al., 2001). Fruit juices have a low pH (2-5) because they are comparatively rich in organic acid (Tasnim et al., 2010). The total acidity of fruit juices is due to presence of a mixture of organic acids, whose composition varies depending on fruit nature and maturity. The total soluble solids (TSS) content is significantly influenced by the combined effect of stages of maturity and ripening conditions (Tasnim et al., 2010). Consumption of fruit juices is popular in Nigeria because of their health and invigorating benefits. Though some fruit juices are produced locally, most of the fruit juices and drinks found in Nigerian markets are imported (Okorie et al., 2009).

There are many studies on the effect of mild preservation techniques on micro-organisms, quality and shelf life of food products. Variance in initial quality of the food, caused by use of different species, maturity and storage conditions, and the use of different process conditions make it difficult to compare the results obtained. In view of the growing demand for processed products with the guarantees of better quality and quantity, researchers has to focus most of their efforts on studying ways of extending the shelf life of processed products so as to deliver specific benefits in terms of health, safety and environmental quality.

The objectives of our study were to examine the physico-chemical analyses and sensory evaluation of commercially and freshly prepared orange juice (100%) in the laboratory and its shelf-life after the storage period of 90 days using various storage methods.

MATERIALS AND METHODS

Sample collection

Matured, firm and ripe sweet intact oranges (*Citrus sinensis*), variety-*Valencia* and “lolly” commercially processed orange juice were purchased from the market in Zaria, Kaduna State, Nigeria.

Preparation of Juice and treatment

Six hundred fresh intact juicy sweet oranges were processed on a laboratory-scale into juice samples using a modified method of Akpapunam et al. (1993) with some modifications was adopted for the study and the flow chart is as shown in (Figure 1). The fruits were washed, manually peeled, cut into halves with sterile knife using hand gloves and their seeds removed. Sweet oranges were washed, peeled and sliced into halves with sterile knife using hand gloves during processing. The cut oranges (mesocarp) were pressed with a hand juicer squeezer to extract the juice. The juice and pulp obtained were homogenized (blended) in a sterile hand Monilex blender ®. The homogenate was clarified manually using a sterile muslin cloth to obtain a clear juice. The juice was subjected to different treatments: Four hundred millimeters (400 ml) each of the extracted juice was carbonated (carbondioxide Saturation) with1.5 kg/100 ml of juice at a pressure of about seven atmospheres at 15°C, concentrated by heating to boiling with distillation apparatus and treated with a preservative (sodium metabisulphite at the rate of 0.035 gm). As a guideline FDA recommends a minimum temperature-time equivalent for juice of 71.1°C for 3 s for products with a pH in the range of 3.6 to 4.0. However, this specific temperature–time is insufficient to inactivate spoilage organisms (Standard Organization of Nigeria (SON), 1976).

The pasteurized and concentrated samples were allowed to cool to 30 to 40°C. The freshly laboratory prepared and commercially

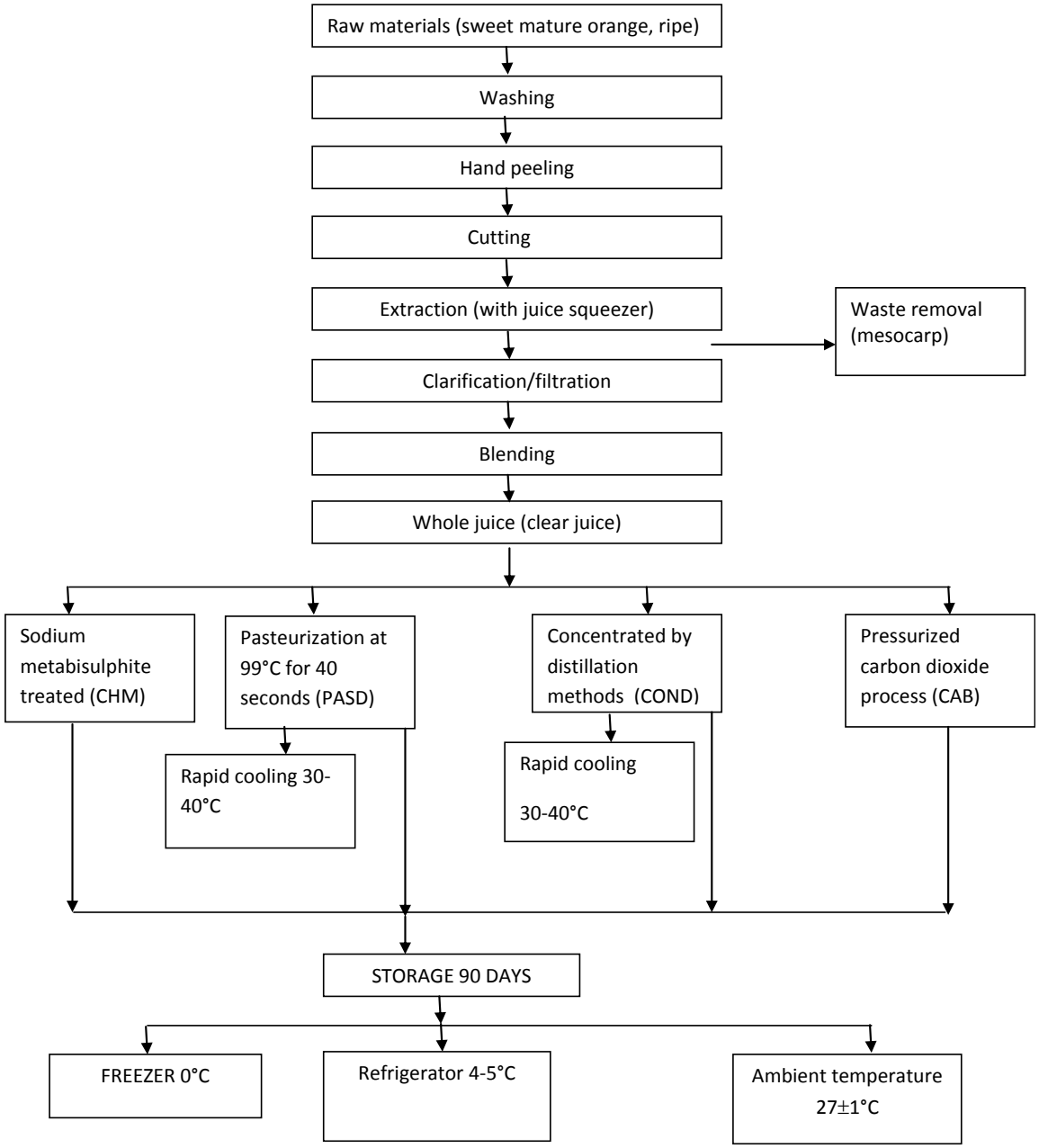

Figure 1. Flow chart for the production of orange juice.

processed samples served as the control. Individual aliquots were kept in the ambient temperature (27°C), the refrigerator (4-5°C) and the freezer (0°C), respectively as described in Table 1. Orange juice was bottled under hygienic conditions in 250 mL PET bottles and sealed with PE caps. The experimental setup were disposed in a factorial completely randomized design 4×3+2, where 4 traditional types (chemically, carbonated, concentrated and pasteurized), 3 storage mode (freezer, refrigerator and ambient temperature) with 2 controls. In all treatment XX replicates was used.

This was divided into five batches of 400 ml each in a plastic container and given the following different treatments:

1. Fresh orange juice was treated with sulphur dioxide, that is, 0.035 gm of sodium metabisulphite was added into each 100 ml of the fresh juice sample (for 400ml 0.14 gm was added).
2. Orange juice was pasteurized at 99°C for 40 s.
3. Orange juice was concentrated by heating to boiling and 10 ml of distillate was collected from every 100 ml of orange juice using distillation apparatus.
4. Pressurized carbon dioxide (carbon dioxide saturation) was introduced into the juice at the coca-cola bottling plant in Kaduna. The concentration of gas used was 1.5 kg/100 ml of orange juice with a pressure of about seven atmospheres at 15°C.
5. The freshly laboratory prepared orange juice without any treatment serving as control
6. A second control sample, that is a commercially prepared orange juice, a product of “lolly” was purchased from Zaria market Kaduna State, Nigeria and assessed along with the treated samples for their

Table 1. General characteristics of the fruit juices used for the assay.

S/N	Sample code	Type of treatments
1	*FRH	Fresh laboratory (lab) produced orange juice(control 1)
2	*COS	Commercially processed orange juice ("lolly",Pasteurized product of Prestige Ventures, Lagos) purchased from Zaria market(control 2)
3	CHM_1	Chemically treated lab produced orange juice stored in the freezer (0°C)
4	CHM_2	Chemically treated lab produced orange juice stored in the refrigerator (4-5°C)
5	CHM_3	Chemically treated lab produced orange juice stored in an ambient temperature (27°C)
6	CAB_1	Carbonated lab produced orange juice stored in the freezer (0°C)
7	CAB_2	Carbonated lab produced orange juice stored in the refrigerator (4-5°C)
8	CAB_3	Carbonated lab produced orange juice stored in an ambient temperature (27°C)
9	$COND_1$	Concentrated lab produced orange juice stored in the freezer (0°C)
10	$COND_2$	Concentrated lab produced orange juice stored in the refrigerator (4-5°C)
11	$COND_3$	Concentrated lab produced orange juice stored in an ambient temperature (27°C)
12	$PASD_1$	Pasteurized lab produced orange juice stored in the freezer (0°C)
13	$PASD_2$	Pasteurized lab produced orange juice stored in the refrigerator (4-5°C)
14	$PASD_3$	Pasteurized lab produced orange juice stored in an ambient temperature (27°C)

*±Control samples.

physico-chemical and sensory evaluation qualities. The pasteurized and the concentrated samples were allowed to cool to 30 to 40°C. All the samples with different processing techniques were stored for 90 days under freezer, refrigeration and room temperature, respectively.

Storage stability

This test is carried out in order to maintain quality in storage. Sensory tests for storage stability involve:

i. Difference tests to establish that stored lot is not different from the control. If no difference is found, product stability is good.
ii. Descriptive and scalar tests to describe the changes that may have occurred or to grade the product as acceptable or not acceptable.

A group of about 3 to 10 trained panelist and 1 to 18 experimental samples are used to determine if difference exist in the samples quality at storage with that of the control.

Sensory evaluation (Product rating method)

The organoleptic attributes evaluated for were colour/appearance, taste, aroma, and general acceptability using a five point hedonic scale, varying from "dislike extremely" (score 1) to "like extremely" (score 5) according to the method of Stone and Sidel (1992). Five highly trained panel members carried out the sensory evaluation. The juices were served in a coded and transparent white glass cups for proper assessment. Five samples were coded and presented to each of the panelists.

Physico-chemical analyses

The proximate analysis (quantitative) of the fresh samples of orange juice (FRH), concentrated (COND), carbonated (CAB), pasteurized (PASD), chemically treated (CHEM) and the commercially processed (COS) was carried out according to the methods of Association of Official Chemists (AOAC, 1995).

pH determination

The pH of orange juice samples was measured using a glass electrode connected to a standard pH-Meter (HB-652359,Hanna, USA) at 20°C. pH meter was standardized using a phosphate buffer solution of 4, 14 and 7. Aliquot of 5 ml each of the orange juice samples was used to determine the pH using pH meter (HB-652359, Hanna, USA) at 20°C.

Total titratable acidity

The test of maturity for oranges is based on the ratio of total solids to acid present in the juice. Since the major part of the total solids is sugar, this is often termed the sugar: acid ratio for oranges and the minimum ratio are 8:1. The ratio is determined by dividing the degrees Brix of a sample by the percentage of titratable acidity of a sample (AOAC, 1995). Aliquot of 5 ml of each sample was titrated with 0.1 M of NaOH using 1% phenolphthalein as the indicator. The percentage acidity was expressed as citric acid.

Maturity ratio (MR)

Citric acid of 0.8% was added to 5 ml of each sample, to obtain the degree brix which is determined by ratio. For oranges the minimum ratio is 8:1.

Total soluble solids

The total soluble solids content was determined using an Abbé 60 refractometer (HB-6959980, Hanna, USA.) at 20°C calibrated against sucrose (AOAC, 1995).

Total solids, moisture and ash content

Total solids, moisture and ash content of the samples were

Table 2. Sensory evaluation of the orange juices (commercially and laboratory processed)

Code	Colour	Viscosity	Odour	Flavour	Overall acceptability
Results of test method (x[± SD]) (A)					
PASD	3.87^{b}	3.13^{a}	3.67^{a}	2.67^{b}	3.27^{b}
CHM	4.07^{a}	3.13^{a}	3.47^{a}	2.13^{b}	2.73^{b}
CAB	4.14^{a}	3.36^{a}	3.86^{a}	1.93^{c}	2.71^{b}
COND	3.27^{b}	2.93^{b}	3.73^{a}	2.53^{b}	4.00^{a}
COS	3.20^{b}	2.20^{b}	2.80^{b}	3.40^{a}	3.80^{b}
FRH	3.60^{b}	3.60^{a}	2.20^{b}	3.00^{a}	4.00^{a}
Total	3.77	3.10	3.51	2.45	3.29
Result of test treatment methods (x [± SD]) (B)					
$PASD_1$	4.00^{b}	320^{a}	3.20^{b}	3.40^{a}	3.80^{a}
$PASD_2$	3.80^{b}	3.20^{a}	4.20^{a}	3.00^{a}	3.40^{a}
$PASD_3$	3.80^{b}	3.00^{a}	3.60^{b}	1.60^{c}	2.60^{b}
TOTAL	3.87	3.13	3.66	2.67	3.27
CHM_1	4.20^{a}	2.60^{b}	2.60^{c}	3.20^{a}	3.60^{a}
CHM_2	4.20^{a}	3.40^{a}	4.60^{b}	1.80^{b}	2.00^{b}
CHM_3	3.80^{b}	3.40^{a}	3.20^{b}	1.40^{b}	2.60^{b}
Total	4.07	3.13	3.47	2.13	2.73
CAB_1	4.40^{a}	3.40^{a}	3.00^{a}	3.00^{a}	4.00^{a}
CAB_2	3.83^{b}	3.67^{a}	4.17^{a}	1.33^{b}	1.83^{c}
CAB_3	4.33^{a}	2.67^{b}	4.67^{a}	1.33^{b}	2.33^{b}
Total	4.11	3.36	3.86	1.93	1.68
$COND_1$	3.20^{a}	3.00^{a}	3.60^{b}	2.80^{a}	3.80^{b}
$COND_2$	3.80^{a}	2.80^{b}	4.00^{a}	2.40^{a}	4.20^{a}
$COND_3$	2.80^{b}	3.00^{a}	3.60^{b}	2.40^{a}	4.00^{a}
Total	3.27	2.93	3.73	2.53	4.00

CV(95%; DMRTP≤ $0.05^{a,b}$ Means with the same alphabet are not significantly different at (P≥0.05) level of significant, while means with different superscripts within columns where significantly different at (P≤0.05);1= Samples stored in the freezer; 2 = refrigerator; 3 = Ambient temperature.

determined using the weight reduction method (AOAC, 1995).

Vitamin C (ascorbic acid) content

The ascorbic acid content (vitamin C) of the orange juice samples were determined by the method described by Nielsen (2003). Aliquot of 10 ml of each sample was added, 10 ml of 3% metaphosphoric acid solution and titrated with the standard dye solution of sodium 2.6-dichlorophenol dye to a faint pink end point. The ascorbic acid content was expressed as milligram per 100 ml (mg/100 ml).

Estimation of Amino Acids (free amino nitrogen)

Five milliliters (5 ml) of each sample and 10 ml of 40% formaldehyde solution with distilled water was titrated against 0.1 M NaOH according to the method of (AOAC, 1995).

Mineral assay

The orange juice samples were digested by the wet ashing method prior to mineral content determination using flame photometer (400, Corning Sherwood,United Kingdom.) for potassium (k) and sodium (Na) (Abulude, 2005). While the phosphorus content was determined colorimetrically with spectrophotometer (6100, Jenway Beckman, England.) using the method described by Nielson (2003).

Statistical analysis

The sensory evaluation data was analysed using analysis of variance (ANOVA) and the post Hoc test using multiple Duncan range procedure (DMRT) with significance level at P≤0.05 in order to determine the levels of significance between the different methods and the control (Ihekoronye and Ngoddy, 1985). A correlation test was used to analyse the different variables of the physico-chemical properties at significance level of P≤0.05 using

SAS computer software.

RESULTS AND DISCUSSION

Sensory analysis

The results for the sensory evaluation and overall acceptability of the various samples of orange juice before and after the period of storage (90 days) are shown in Table 2a and b. There were no significant differences (P≥0.05) between colour and viscosity, but there was significant difference (P≤0.05) between odour (aroma) and flavour when compared with their rating with the control samples for general acceptability (Table 2a). The differences obtained in flavour and aroma could be attributed to the different compounds formation based on the different methods of processing and storage of the tested samples of orange juices (Adubofuor et al., 2010). Moreover, orange juice can lose its flavour, colour or other quality factors due to non-enzymatic browning reactions during processing or storage of the juices (Dauda and Adegoke, 2014). The result of this study which showed off-flavour and aroma is also in support of the reports of Moshonas and Shaw (1998), they reported that orange juice can lose some of its fresh flavour during storage because of decreased levels of volatile flavour constituents, thus developing a stable flavour. They also reported that for juices packaged in plastic containers, a major contributor to this flavour lose is often caused by absorption of flavour constituents by the plastic containers.

At the end of storage for 90 days, the panelist had preference for concentrated juice stored under the refrigeration temperature and the room temperature; pasteurized and carbonated both were stored under the freezer and room temperature. Shelf life of the orange juice on sensory evaluation after the period of storage was determined. A mean value equal to 3 was chosen as the acceptance limit to determine the end of the shelf life of juices. The hedonic rating showed that samples of pasteurised (PASD), chemically tested (CHM), concentrated (COND), stored in the freezer and refrigerator with the values (3.80 and 3.40; 3.60 and 3.80 and 4.20), respectively was highly accepted by the consumers preference. These treated samples were judged superior organoleptically in quality than the commercially processed one, retaining more of its colour, viscosity and flavour.

Samples of PASD, CHM and carbonated (CAB) stored at ambient temperature was least generally accepted. The samples of CAB, PASD stored in the freezer with values (4.00 and 3.80) and COND stored in the refrigerator and ambient temperature with values of 4.20 and 4.00 had the best in overall acceptability. The result of this study is in agreement with the report of other researchers (Ploydera et al., 2003; Ndife et al., 2013). The deterioration of orange juice during storage comprises changes in organoleptic quality or sensory evaluation, nutritional value and appearance. The main parameters that influence the deterioration of orange juice is the temperature and the presence of oxygen (Dauda and Adegoke, 2014).

A shelf-life study was carried on mango, orange and pineapple juices by Obeta and Ugwuanyi (1997) and these juices were inoculated with ascospores of *Neosartorya* sp. The result showed that only fruit juices at 4 and 5°C storage and those containing sodium benzoate stored at room temperature were protected from spoilage by these fungi for 64 days.

Physico-chemical analyses

Table 3 shows the physico-chemical properties of the laboratory prepared orange juice fresh, pasteurized, pressurized carbon dioxide (Carbonated), chemically treated, concentrated and commercially processed orange juice. For all the samples the pH ranged from 1.6 to 4.5 and the pH values for the commercially processed sample showed the lowest value compared with the laboratory prepared orange juice with different treatments. The titratable acidity values ranged from 0.03 to 0.24, the Brix acid (maturity) ratio from 1.16 to 4.73, the total soluble solids (TSS) 26 to 33° Brix, the vitamin C content from 2.79 to 43.78% (mg/100 ml juice), total solids from 3.46 to 7.43%, moisture content 2.0 to 10.59%, estimation of amino acids from 0.08 to 0.25 molarity, ash content 0.29 to 1.29%, and minerals (macro-nutrients): Potassium from 0.51 to 1.73%, sodium 0.01 to 0.12% and phosphorus 0.01 to 0.02%.

The decline in pH was in good agreement with previous work (Akpapunam et al., 1993). The increase in hydrogen concentration could be explained by the dissociation of component acids by heat. The increase in the TTA of the processed orange juices as was observed in this present study. This could be as a result of increase in temperature, oxidation of aldehydes and alcohols to acid during processing and consequently contributing to an increase in acidity (Adubofuor et al., 2010). The maturity ratio (MR) increases as fruit ripens and is used for assessing the quality of the juice (Kareem and Adebowale, 2007).

The increase in soluble solids contents and the reduction in total solids show the effect of a decrease in acidity. The reduction in acidity during ripening plays an important role in the Brix: Acid ratio and consequently in influencing the taste and flavour of the juice (Braddock and Marcy, 1985; Akpapunam et al., 1993; Cinquanta et al., 2010; Ndife et al., 2013; Dauda and Adegoke, 2014). The increased soluble solids contents obtained compares with the result of Braddock and Marcy (1985). The increase in soluble solids is explained by the evaporation of water during pasteurization and concentration of the juice (Akpapunam et al., 1993).

A correlation analysis of the different variables of the

Table 3. Physico-chemical properties of various types of orange juices (table 4 shows the statistical values correlation test results).

S/N	Sample code	pH value	Titratable acidity (%)	Brix acid ratio (maturity ratio)	Total soluble solid (^{o}Brix)	Vitamin C content mg/100 ml	Total solid (%)	Estimation of amino acids (%)	Moisture content (%)	Ash content (%)	K (%)	Na (%)	P (%)
1	FRH	3.5	0.10	3.39	33.88	43.78	4.81	0.12	7.53	0.63	1.73	0.04	0.02
2	COS	1.6	0.03	8.55	26.67	2.79	3.46	0.08	10.97	0.29	0.51	0.02	0.001
3	CHM_1	3.4	0.20	1.50	30.05	22.89	5.61	0.13	10.59	0.55	1.67	0.04	0.01
4	CHM_3	3.3	0.22	1.54	33.88	29.65	5.09	0.22	4.46	0.45	1.47	0.12	0.02
5	CHM_3	3.5	0.07	3.81	26.67	25.97	4.86	0.23	4.38	0.48	1.44	0.11	0.01
6	CAB_1	3.4	0.20	1.59	31.70	17.01	5.33	0.08	10.79	0.45	1.83	0.01	0.01
7	CAB_2	3.2	0.31	0.92	28.37	29.45	5.39	0.23	4.79	0.62	1.65	0.12	0.01
8	CAB_3	3.0	0.06	4.45	26.67	29.75	5.34	0.17	5.12	0.62	1.62	0.01	0.01
9	$COND_1$	3.1	0.07	1.28	30.60	10.75	6.74	0.18	12.4	0.65	2.27	0.01	0.02
10	$COND_2$	4.5	0.12	2.78	33.34	21.99	3.25	0.12	2.81	0.72	2.30	0.02	0.02
11	$COND_3$	3.4	0.24	4.73	28.37	8.96	4.18	0.13	3.56	0.99	2.63	0.01	0.02
12	$PASD_1$	3.4	0.12	4.61	32.25	24.88	5.65	0.09	11.06	1.29	1.74	0.01	0.01
13	$PASD_2$	3.4	0.06	1.16	27.80	31.44	7.43	0.25	7.26	0.62	1.77	0.01	0.01
14	$PASD_3$	3.2	0.12	2.36	28.37	32.84	6.99	0.20	4.99	0.84	1.93	0.01	0.01

K, Potassium; Na,Sodium; P, Phosphorus.

Table 4. Correlation test results of the physico-chemical properties of the commercially and laboratory processes orange juices.

Correlation	pH	Acid	Brat	TSS	Vit.C	TOSOL	MC	EMACID	ASH	K	NA	P
Ph	1	0.2723	0.5818	0.5232	0.088	0.0184	0.4435	0.1382	0.3703	0.7161*	0.0681	0.6334*
ACID	0.2723	1	0.4499	0.2514	0.2237	0.0877	0.2651	0.0709	0.1265	0.3122	0.4445	0.2497
BRAT	0.5818	0.4499	1	0.3402	0.0378	0.5931	0.0871	0.5211	0.0193	0.4777	0.2486	0.4001
TSS	0.5232	0.2514	0.3402	1	0.0704	0.1444	0.0952	0.3178	0.1812	0.294	0.0626	0.642*
Vit.C	0.088	0.2237	0.0378	0.0704	1	0.1013	0.1914	0.2355	0.1854	0.4979	0.2724	0.208
TOSOL	0.0184	0.0877	0.5931	0.1444	0.1013	1	0.2657	0.5303	0.179	0.1852	0.1412	0.0805
MC	0.4435	0.2651	0.0871	0.0952	0.1914	0.2657	1	0.4729	0.0696	0.2763	0.3757	0.3345
EMACID	0.1382	0.0709	0.5211	0.3178	0.2355	0.5303	0.4729	1	0.157	0.055	0.5246	0.0973
ASH	0.3703	0.1265	0.0193	0.1812	0.1854	0.179	0.0696	0.157	1	0.5744	0.3217	0.2498
K	0.7161*	0.3122	0.4777	0.2940	0.4979	0.1852	0.2763	0.055	0.5744	1	0.3065	0.7233*
NA	0.0681	0.4445	0.2486	0.0626	0.2724	0.1412	0.3757	0.5246	0.3217	0.3065	1	0.0625
P	0.6334*	0.2497	0.4001	0.6420*	0.208	0.0805	0.3345	0.0973	0.2498	0.7233*	0.0625	1

No. of cases = 14; 1, Tailed; significant: 0.1**001;'''' '''' is printed if a coefficient cannot be computed; Key: pH- hydrrogen ion concentration; Acid, titratable acidity, Brat-brix, acid ratio; Tss, total solubkle solid; Vit.c, Vitamin c; Tosol, total solid; Mc, moisture content; Emacid, estimation of amino acid; K, potassium; Na, sodium; P, phosphorus.

physico-chemical properties of the laboratory prepared orange juice is shown in Table 4. The result showed that there was a significant correlation between pH and the percent Brix:acid ratio ($r = 0.58$, $P < 0.05$), total soluble solid ($r = 0.52$, $P < 0.05$), potassium ($r = 0.7$, $P < 0.05$), and phosphorus ($r = 0.63$, $P < 0.05$). There was a significant correlation between percent Brix:acid ratio and total solid ($r = 0.59$, $P < 0.05$), and level of amino acid ($r = 0.52$, $P < 0.05$). Also there was correlation between level of amino acid and sodium ($r = 0.05$, $P < 0.05$), percent ash and potassium ($r = 0.57$, $P < 0.05$), total soluble solid and potassium ($r = 0.64$, $P < 0.05$), potassium and phosphorus ($r = 0.72$, $P < 0.05$). There was no significant correlation between other variables.

Vitamin C (Ascorbic acid) content

The vitamin C of the orange juice studied under different processing conditions compared favourably with freshly prepared juice. More of the vitamin C content was conserved in the pasteurized, chemically treated, and carbonated. The results showed that the chemically treated and carbonated orange juice is as effective as pasteurized because it does not change the taste and it preserves more of its vitamins found in fresh-squeezed orange juice. Thus this result agrees previous studies (Murat, 2000; Polydera et al., 2003; Dauda and Adegoke, 2014). According to the Association of the Industry of Juices and Nectars from fruits and Vegetables of the European Union and the report by Kimball (1999), they stated that orange juice should contain at least 20 to 25 mg/100 ml of vitamin C (ascorbic acid) at the time of expiration of storage, which is about 50% of the initial amount. The loss in vitamin C which was observed drastically in the commercially processed sample as stated by Nagy and Smoot (1999) and Dauda and Adegoke (2014) could be attributed to the oxidation by residual air layer within the container during processing. According to Nagy and Smoot (1999) temperature and storage affects the presence of vitamin C content in orange juice and orange fruit. This also accounts for the low level of vitamin C observed in the juice stored at the freezer and ambient temperature for carbonated and concentrated juice.

Considering other parameters like, amino-acids, ash content and mineral elements, potassium was a major macro mineral in orange juice, followed closely by phosphorus, and the result compared favourably with the work of Parichart et al. (1999), and Nnam and Njoku (2005). The result of amino acid showed that the treatments did not show any significant difference in the nutrient compositions during the period of storage and it was consistent with the data obtained from Mears and Shenton (1973). The amino acids also affect sensorial quality attributes, including taste, aroma, and color (Ames, 1998; Kirimura et al., 1969).

The kinetics of reactions involving those amino acids in pineapple juice during heat treatments such as pasteurization, need to be assessed because of their role in the Maillard reaction, which can be of importance for human nutrition.

Statistically, a correlation test shown in Table 4 was applied to assess the nutritional components in order to determine possible significant relationships between the parameters analyzed. In the test, pH value was significantly related to total soluble solids, Brix:acid ratio, potassium and phosphorus ($P < 0.05$). No significant relationship was found between pH and the other parameters. Brix:acid ratio was negatively correlated with pH, total solids and estimation of amino acids ($P<0.05$).
Total soluble solids were significantly related to pH and phosphorus. There was no significant relationship between vitamin C, acidity, moisture and ash among the other selected parameter ($P >0.05$).

CONCLUSION AND RECOMMENDATION

Nigerian processors of fruit juices employ techniques similar to the ones that was adopted in this study. These techniques are pasteurization, carbonation, concentration and use of chemical preservatives and their products being stored under similar methods applied in this work; freezer, refrigeration and ambient temperature. The processes were designed among other things to prolong shelf-life of the products by controlling microbial contamination and growth, maintain nutritional value and the chemical composition. From this study, it was observed that the different methods employed, showed that orange juice can store in any of these methods when suitable conditions are being applied in terms of satisfactory cleaning/sanitary conditions of handling and processing methods based on the result obtained, it can be said that the product under study can still be acceptable and fit for human consumption since the result of the nutritional values that is the chemical composition when compared with the Standard Organization of Nigerian (SON) (1976) and the International Standards. Therefore, orange juice under proper quality control and application of good manufacturing practices will still maintain its nutritional values, organoleptic qualities, not exceeding its microbiological limits as provided by Nigerian Agency for Food and Drug Administration Committee (NAFDAC) still serves as a most invigorating fruit drink. The technologies for commercial production have been revolutionized with the fruit juices with the introduction of ultra-high pressurized packaging such as hydrostatic pressure (HHP), high pressure homogenization, pulsed electric field (PEF) and ultrasound (US) (Quek et al., 2012; Vasantha and Li, 2012). These emerging non-thermal techniques should be encouraged since they have the potential to provide “fresh-like” and safe fruit juices with prolonged shelf life.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Microbiological performance of Hazard Analysis Critical Control Point (HACCP)-based food safety management systems: A case of Nile perch processing company

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This study aimed at giving insight into microbiological safety output of a Hazard Analysis Critical Control Point (HACCP)-based Food Safety Management System (FSMS) of a Nile perch exporting company by using a combined assessment, FSMS-diagnosis and actual microbiological assessment. The FSMS diagnosis indicated FSMS activities at an average level operating in moderate-risk context level but with good system output. Likewise, microbiological assessment revealed a better system output with respect to pathogens (*Vibrio cholerae, Listeria monocytogenes* and *Salmonella* spp.) and faecal hygiene (*Escherichia coli*) as none of these were detected in any critical sampling location throughout the study. Although indicators of general process hygiene (that is, *Enterobacteriaceae* and TVC) exceeded regulatory limits and guidelines in raw materials and food contact materials, *Staphylococcus aureus* on operator's hands were beyond the general microbiological guidelines in the fish industry. Higher contamination levels of general process hygiene and personal hygiene indicators call for improvement on hygienic design, specific production and sanitation procedures, independent validation, process automation, and change in personnel recruitment criteria.

Key words: Fish export, food safety management system, food safety, microbiological performance.

INTRODUCTION

Globally fish production has significantly increased and contributes to more than 15% of animal source protein (FAO, 2012). In 2010, capture fisheries and aquaculture supplied the world with 148 million tons of fish valued at US$217.5 billion (FAO, 2012). The world average per capita consumption of fish has also increased to 18.6 kg

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making fish and fishery products among the most traded food commodities globally (FAO, 2012). Developing countries contribute to the bulk of world fish exports (FAO, 2012). Fish industry is among the largest food manufacturing and exporting sectors in Tanzania (Ruteri and Xu, 2009). The total annual production of fish is estimated at 365,023 tons earning the country about US$ 800,000 (United Republic of Tanzania, 2013). Tanzania exports 45,550 tons of ornamental fish and 41,291 tons of fisheries products, which worth US$ 159.1 million (United Republic of Tanzania, 2013). Moreover, fish industry provides substantial employment, income, and foreign exchange contributing to the economic development of the nation. It employs more than 4 million Tanzanians and contributes to about 1.4% national GDP (United Republic of Tanzania, 2010).

Fish importing countries like European Union (EU), United States of America (U.S.A) and Japan have set stringent requirements along the fish market chain (Onjong et al., 2014a). Consequently, exporting countries including Tanzania have taken various initiatives at various levels to translate the requirements into their production systems. At the company level, various quality assurance standards (ISO 22000, BRC, and ISO 9001) and guidelines (HACCP, GMP, and GHP) have been translated into their food safety management systems, FSMS (Kussaga et al., 2014; Onjong et al., 2014a). At the sectoral level, sector organisations like Tanzania Industrial Fish Processors Association (TIFPA) exercised the due diligence in fish safety and quality assurance systems to ensure the quality and safety of export products (www.tifpa.org). At the government level, various regulations were promulgated, the competent authority was designated, workers trained, inspection system improved and landing sites (that is, supplied with potable water, toilets, fenced and paved) were built (Kussaga et al., 2014).

However, despite such efforts, fish companies are still experiencing notifications and border rejections of their products (Rapid Alert System for Food and Feed, 2009b; Kussaga et al., 2014; Rapid Alert System for Food and Feed, 2014b). The major reasons behind such notifications and rejections are filthy, microbiological (like *Salmonella* spp. and *Vibrio cholerae*) and chemical contaminations (pesticides and illegal fishing by using chemical poisons/dynamite) (Rapid Alert System for Food and Feed, 2009b, 2014b). A recent study covering all Tanzanian fish exporting companies identified various inadequacies in the design (hygienic design of equipment and facilities, sampling design and measuring plan, sanitation programmes) and operation (procedures and capability of physical packaging equipment) of core FSMS control activities and set-up of core assurance activities like validation and record keeping system (Kussaga et al., 2014). However, typical microbiological assessment to identify the actual microbiological output of the system was not performed. Therefore, this study aims at getting deeper insight into the typical causes of insufficient microbiological performance of HACCP-based FSMS of Nile perch exporting company in order to propose intervention measures for improvement towards an effective system. This study involved a combined assessment applying two diagnostic tools; the FSMS-Diagnostic Instrument, FSMS-DI (Luning et al., 2008; Luning et al., 2009; Jacxsens et al., 2010; Luning et al., 2011b) and microbiological assessment scheme, MAS (Jacxsens et al., 2009) to provide a deeper insight in the actual microbiological system output and causes of inadequate performance (Luning et al., 2011a).

MATERIALS AND METHODS

Characteristics of the company analysed

The company analysed in this study processed fresh chilled and frozen Nile perch fillets for the export market. At the time of sampling, this company implemented pre-requisite programmes (PRPs), HACCP and ISO 22000; however, it was not ISO 22000 certified. It is a large-scale company with a total of 150 employees with a daily capacity of processing 120 metric tons (however, currently it processes less than 30 metric tons due to limited availability of Nile perch). It has also a big quality assurance department with 10 personnel and a QA manager. Eventually, this company is approved for export to the EU after being audited by the national competent authority (Department of Fisheries, Ministry of Livestock and Fisheries Development) to determine if the hygiene requirements are in compliance with the EU demands (that is, Commission Regulations (EU) 852/2004, EU 853/2004, and EU 2073/2005). This company was selected over other companies because it agreed to conduct both FSMS diagnosis and microbiological sampling as majority of the companies would not allow for actual microbiological to be conducted. The processing line for the frozen Nile perch fillets (Figure 1) was selected for assessment because at the time of sampling it was the only product being processed. It is also, the major processed product in this company accounting for more than 80%.

Diagnosis of food safety management systems performance

The FSMS-DI is a tool that enables systematic analysis and assessment of a company's specific FSMS (Luning et al., 2008, 2009, 2011b). The diagnostic tool involves a set of 58 indicators representing four crucial parts; part 1 describes set of indicators of context factors including product (3 indicators), process (3), organisational (7), and chain environment (4) characteristics that affect performance of FSMS. Context factors are structural elements of a system environment that can affect decision making activities in the FSMS and system output, and cannot (easily) be changed. The FSMS context is narrower than the overall environment of a company (Luning et al., 2015). For each context indicator a grid was designed including three situational descriptions, corresponding with a low (score 1), moderate (score 2), and high-risk situation (score 3) indicating levels of riskiness for decision-making in the FSMS activities (Luning et al., 2011b). The description for low, moderate, and high-risk situations for product and process characteristics pertains to low, potential, and high likelihood of contamination, growth and survival of pathogens. For organisational characteristics, low, moderate, and high-risk situations respectively represent supportive, constrained/restricted, and lack of administrative conditions to support appropriate

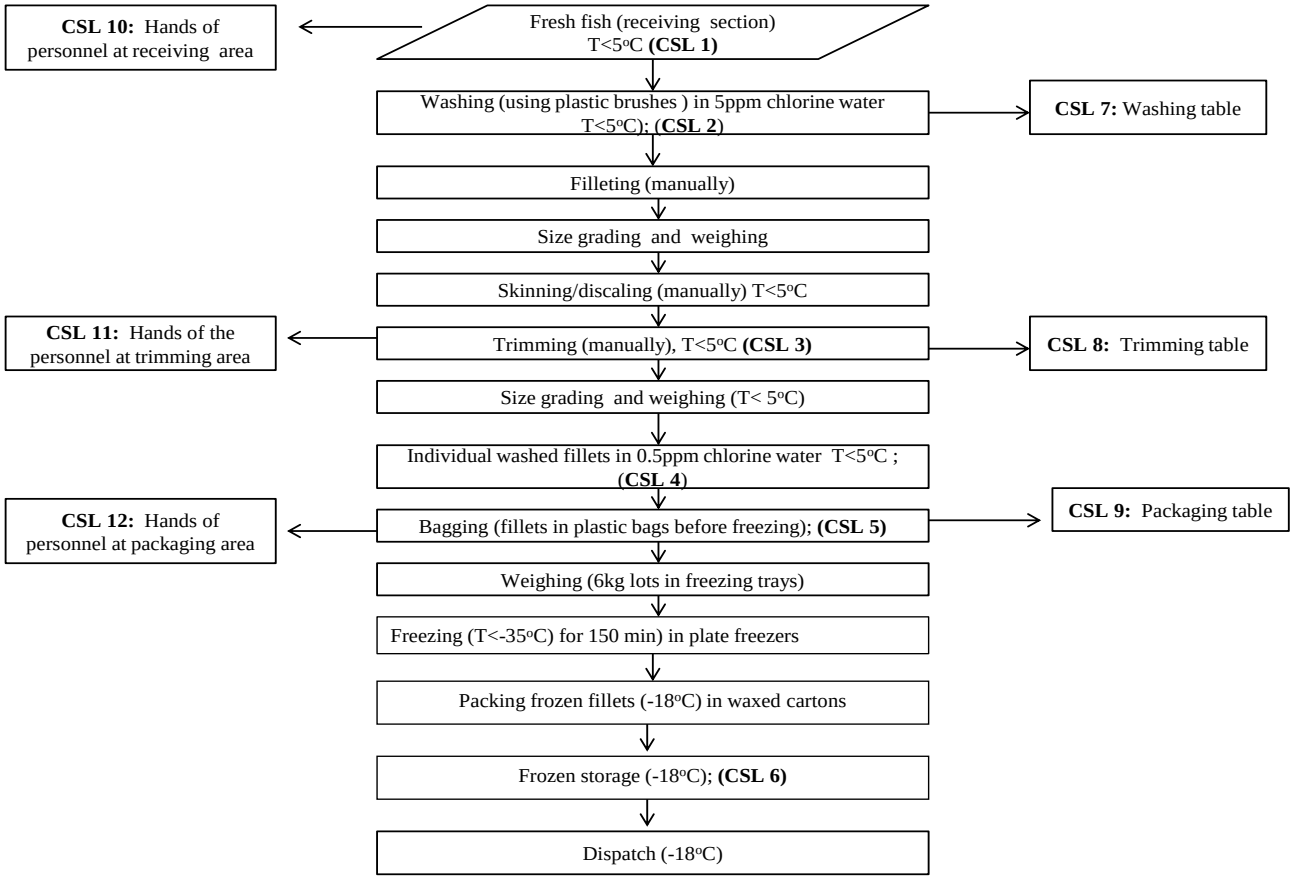

Figure 1. Process flow diagram of frozen Nile perch fillets indicating the critical sampling locations.

decision-making in the FSMS. Concerning chain environment characteristics; low, moderate, and high-risk situations correspond to low, restricted, and high dependability on other chain actors resulting in a more vulnerable decision-making situation, respectively (Luning et al., 2011b).

Part 2 includes sets of indicators that represent core control activities such as design of preventive measures (6), design of intervention processes (4), monitoring system design (8), and actual operation of control strategies (8) (Luning et al., 2008). Control activities are aimed at keeping products and processes within acceptable tolerances. For each control activity indicator a grid with description of four different performance levels, that is, low (score 0), basic (score 1), average (score 2), and advanced (score 3) was constructed (Luning et al., 2008, 2009). A low level represents that an activity is not possible in the given production circumstances (e.g. in freshly packed fish, commonly no physical interventions can be applied), just not applied, or when information is not known. The basic level for control activities is typified by use of own experience, general knowledge, ad-hoc analysis, incomplete, not standardised, unstable, and regularly problems. The average level for control activities is characterised by being based on expert (supplier) knowledge, use of sector/legislative guidelines, best practices, standardised, sometimes problems. The advanced level indicates that the control activity is characterised by use of specific information, scientific knowledge, critical analysis and procedural methods.

Part 3 pertains to set of indicators of core assurance activities including setting system requirements (2), validation (3), verification (2), documentation (1), and record keeping (1) (Luning et al., 2009). Assurance activities aim at providing evidence and confidence that control activities are effective and function well in actual practice. Likewise, for each assurance activity indicator a grid with description of four different performance levels, that is, low (score 0), basic (score 1), average (score 2), and advanced (score 3) was constructed (Luning et al., 2008, 2009). A low level represents that an activity is not applied, or when information is not known. The basic level is characterised by problem driven, only checking, scarcely reported, and no independent positions. The average level corresponds with active, additional analysis, regular reporting, and experts support. The advanced means that the assurance activity is characterised by use of specific information, scientific knowledge, procedural methods, systematic activities, and independent positions.

Part 4 involves assessment of external (4) and internal (3) system output indicators (Jacxsens et al., 2010). Moreover, for each system output indicator, four levels were described; level 0 (no indication of system output) refers to absent, not present or not conducted. Level 1 (poor system output) is characterised by aspects like ad-hoc sampling, minimal criteria used for FSMS evaluation, and having various food safety problems due to different problems in the FSMS. Level 2 (moderate system output) corresponds to regular sampling, several criteria used for FSMS evaluation, and having restricted food safety problems mainly due to one (restricted) type of problem in the FSMS. Level 3 (good system output) pertains to a systematic evaluation of the FSMS using specific criteria and having no safety problems (Jacxsens et al., 2010). The basic principle behind the FSMS-DI is that companies operating in a high-risk context require core control and

Table 1. Detailed microbial assessment scheme of a frozen Nile Perch fillets processing line.

Critical sampling location	Microbiological parameter	Sampling method
CSL1: Raw fish (in trucks at point of receipt)	Total viable counts (TVC), Enterobacteriaceae, *E. coli, V. cholerae, Salmonell*a spp., and *L. monocytogenes*	3 samples (1 sample/sampling day) by abrasive swabbing on 50 cm^2 of fish skin
CSL2: Raw fish after dipping in 5 ppm chlorine water	TVC, Enterobacteriaceae, *E. coli, V. cholerae, Salmonella* spp., and *L. monocytogenes*	3 samples (1 sample/sampling day) by abrasive swabbing on 50 cm^2 of fish skin after disinfection
CSL3: Trimmed fish fillet	TVC, Enterobacteriaceae, *E. coli, V. cholerae, Salmonella* spp., and *L. monocytogenes*	3 samples (1 sample/day) by abrasive swabbing on 50 cm^2 of fillet after trimming
CSL4: Trimmed fillet after dipping in 0.5ppm chlorine water	TVC, Enterobacteriaceae, *E. coli, V. cholerae, Salmonella* spp., and *L. monocytogenes*	3 samples (1sample/day) by swabbing on 50 cm^2 of disinfected fillet
CSL5: Bagged fresh fillet before freezing	TVC, Enterobacteriaceae, *E. coli, V. cholerae, Salmonella* spp., and *L. monocytogenes*	3 samples (1 sample/day) by abrasive swabbing on 50 cm^2 of bagged fresh fish fillet
CSL6: Final packaged fillet after freezing	TVC, Enterobacteriaceae, *E. coli, V. cholerae, Salmonella* spp., and *L. monocytogenes*	3 samples (1 sample/day) by abrasive swabbing on 50 cm^2 of frozen fillet
CSL7-9: Working tables (receiving, trimming and packaging)	TVC, Enterobacteriaceae, *E. coli, V. cholerae, Salmonella* spp., and *L. monocytogenes*	9 samples (3 samples × 3 times/day) by cotton swabs on 25 cm^2 of the table, ISO 18593:2004 (ISO, 2004)
CSL 10-12: Hands of operators (receiving, trimming and packaging)	*E. coli,* Enterobacteriaceae, and *S. aureus*	9 samples (3 samples × 3 times/day) by cotton swabs on 25 cm^2 of personnel hands/gloves, ISO 18593:2004 (ISO, 2004)

assurance activities at an advanced, fit-for-purpose level, whereas in a low-risk context, activities at a lower level could be sufficient to guarantee good system output (Luning et al., 2008, 2009, 2011b).

Microbiological food safety output diagnosis

The principles of the microbial assessment scheme (MAS) protocol developed by Jacxsens and co-authors (Jacxsens et al., 2009) were used to determine the actual microbiological output of an implemented FSMS. Microbiological analysis was conducted at an accredited NFQCL of the Department of Fisheries, Ministry of Livestock and Fisheries Development in Mwanza, Tanzania, which is the competent authority. The next sections clearly indicate the MAS procedure (Table 1).

Selection of critical sampling locations

In this study, 12 critical sampling locations (CSLs) were selected (Figure 1 and Table 1) including the raw materials, the whole fresh fish before offloading from the collection trucks (CSL1), washed whole fresh fish in 5 ppm chlorine water (CSL2), trimmed fresh fillets before washing with 0.5 ppm chlorine water (CSL3), and trimmed fresh fillets dipped in 0.5 ppm chlorine water (CSL4). Other CSLs were bagged fresh fillets before plate freezing (CSL 5) and final packaged plate-frozen fillets (CSL6), tables at receiving (CSL7), tables at trimming (CSL8), and tables at packaging (CSL9), operators' hands at receiving (CSL10) and trimming (CSL11), and operator's hand gloves at packaging (CSL12) areas. Sterile dry enviro-sponges (abrasive) made in USA, 3M St. Paul were used to sample 50 cm^2 on the products, whereas cotton swabs were used to sample 25 cm^2 of food contact materials (filtration tray and surface of filling machine) and hands of the personnel.

Selection of microbiological parameters

Seven microbiological parameters including indicators of food safety (*L. monocytogenes, V. cholerae* and *Salmonella* spp.), faecal hygiene (*E. coli*), personal hygiene (*S. aureus*), and general process hygiene (*Enterobacteriaceae* and TVC*)* were selected.

Sampling frequency

Samples were taken three times in three consecutive months (October 2010 to February 2011). Products were sampled once per sampling day, whereas food contact surfaces and hands of the personnel were sampled three times, that is, start, middle and end of production day (Table 1). A total of 214 samples [(4 samples × 6 (CSL 1-6) × 3 (1 sampling/month in 3 months) + 4 samples × 3 (CSL 7-9) × 3 times of sampling/day × 3 (1 sampling/month in 3 months)) + (1 sample × 3 (CSL 10-12) × 3 times of sampling/day × 3 (1 sampling/month in 3 months))] were taken over the three months period.

Selection of sampling and analytical methods

Sampling and laboratory analysis were conducted according to classical ISO and U.S. Food and Drug Administration-Bacteriological Analytical Manual (FDA-BAM) methods. In this study, non-destructive sampling technique was used for products, food contact surfaces and hands of the personnel. On each product, a sterile template was used to delineate 50 cm^2 and sterile

pre-moistened dry-sponge (3 M, St. Paul, Minnesota, USA) in the respective dilution medium (as each parameter uses a specific medium) was used to sample vertically, horizontally, and diagonally in the delineated area. Swabbing using abrasive sponges is regarded as the best alternative to destructive/excision sampling (Pearce and Bolton, 2005; Lindblad, 2007). The muscle of a healthy fish is considered sterile (Apun et al., 1999); as the micro-organisms on the surface of fish fillets are a result of cross contamination from personnel, processing water and equipment, and/ or food contact surfaces. Thus, swabbing on the surface of fish fillets by abrasive sponges would give an indication of the level of process hygiene and preventive measures of the company. In low contaminated products, abrasive sponge is superior (in recovering micro-organisms) to dry/wet swab and excision, and it is recommended when contamination levels are not known (Tenhagen et al., 2011). For the food contact surfaces and hands/gloves of the personnel, ISO 18593:2004 (horizontal methods for sampling techniques using cotton swabs on surfaces in food industry) was applied (ISO, 2004). Similarly, a sterile template was used to delineate 25 cm^2 on working tables whereas pre-moistened cotton swab with respective medium for the specific microbiological parameter was used to sample the delineated area. After sampling, enviro-sponges and cotton swabs were put back into their respective stomacher bags and tubes containing the media. Samples were stored and transported (at ≤4°C) in a cool box containing ice packs to the laboratory for microbial analysis. At the laboratory ISO 6887-3:2003 standard was used to prepare analytical samples. For detection (absence/presence) tests, 100 mL samples (abrasive sponges for the products) and 5 mL samples (cotton swab for food contact surfaces) were used for laboratory analysis. Enumeration of TVC*, Enterobacteriaceae, E. coli, S. aureus* and *L. monocytogenes* were respectively carried out by ISO 4833:2003, ISO 21528-2:2004, ISO 16649-2:2001, ISO 6888-1:1999/Amd.1:2003 and ISO 11290-2:1998 standards. Detection of *Salmonella* spp., *L. monocytogenes,* and *V. cholerae* performed according to ISO 6579:2002, ISO 11290-1:1996/Amd.1:2004 and BAM: 1995 standards, respectively.

Data analysis and interpretation

The actual microbiological assessment and FSMS-diagnosis data were analysed by using Microsoft Office Excel 2007. Microbiological results were interpreted according to the criteria described in European Union, Tanzanian and East African Community standards and the guidelines developed by Ghent University (Table 3). With regards to FSMS diagnosis data, the mean scores were calculated and transformed to assigned scores as indicated by Jacxsens et al. (2010) and Luning et al. (2011a). For the indicators of context factors if the mean risk-level is between 1 and 1.2, then score 1 is assigned. If the mean risk-level score is between 1.3 and 1.7, then score 1 to 2 is assigned. If the mean risk-level is between 1.8 and 2.2, then score 2 is assigned. If the mean risk-level is between 2.3 and 2.7, then score 2 to 3 is assigned. Lastly, if the mean risk-level is between 2.8 and 3.0, then score 3 is assigned (Luning et al., 2011a). For the indicators of core FSMS activities and system output, if the mean level is between 0 and 1.2, then an assigned score of 1 is defined. If the mean level is between 1.3 and 1.7, then an assigned score of 1 to 2 is attributed. If the mean level is between 1.8 and 2.2, then an assigned score of 2 is defined. If the mean level is between 2.3 and 2.7, then an assigned score of 2 to 3 is given. Finally, if the mean level is between 2.8 and 3.0, then an assigned score of 3 is attributed (Jacxsens et al., 2010; Luning et al., 2011a). Analysed companies with similar score for each indicator were counted (frequency counting) to get insight into the similarities in the level of design and operation of core FSMS (control and assurance) activities and risk-level of the context wherein the systems operate. The spider web diagrams were developed by using Microsoft Office Excel 2007 to indicate the risk level of the indicators of context factors and performance levels of the FSMS activities and system output. The medians were also calculated by using Microsoft excel. For comparison purposes, the means and medians of all fish companies (adopted from (Kussaga et al., 2014) are also indicated in Table 3.

RESULTS AND DISCUSSION

Diagnosis of food safety management systems

Figures 3 to 4 illustrate the results of FSMS diagnosis. More coloured spider webs indicate that the indicators of FSMS activities and system output are elaborated at high level or there is high-risk level of the context. This study revealed an average FSMS (median 3, mean 2.2) which operates in a medium-risk context (median 2, mean 1.9) with a subsequent better system output (median 3, mean 3). Likewise, a recent study covering all fish processing companies in Tanzania revealed an average FSMS (median 2.5, mean 2.2) operating in moderate-risk context (median 2, mean 1.9) but with relatively good system output, median 3, mean 2.7 (Kussaga et al., 2014). Although, the FSMS-diagnosis results indicated a better system output, the actual microbial assessment (score 2-3) revealed some inadequacies in the system with regards to indicators of general process hygiene (*Enterobacteriaceae* and TVC), personal hygiene (*S. aureus*) (Figures 4 to 5). However, the current FSMS is effective to pathogens including *L. monocytogenes, V. cholerae* and *Salmonella* spp., as none of the pathogens was detected throughout the study. Thus, with regards to pathogens, the current FSMS does not require any further improvement (Jacxsens et al., 2009).

Diagnosis of the risk level of context characteristics

In overall, the FSMS operates in a moderate-risk context (score 2). For product and process characteristics, the company dealt with high-risk raw materials (such as fresh raw fish) and final product groups (like fresh chilled/frozen fillets) which both require special storage conditions to prevent proliferation of micro-organisms including pathogens (median 3, mean 2.7, Table 3 and Figure 2A). Likewise, the national-wide study revealed medium-to-high risk (median 2.7, mean 2.4) product and process characteristics (Table 3). Both raw materials and final product groups are perishables (Jensen et al., 2010). Like other types of fish, Nile perch fish and fresh fillets have high water activity (0.98) and neutral pH, making them good media for microbiological growth (Erkan and Özden, 2008). Moreover, the production process is characterised by small batches with clear interference with people (due to low level of automation in filleting, skinning, and cleaning and disinfection). Besides, the production process has no intervention steps to reduce pathogens to acceptable levels. Under this

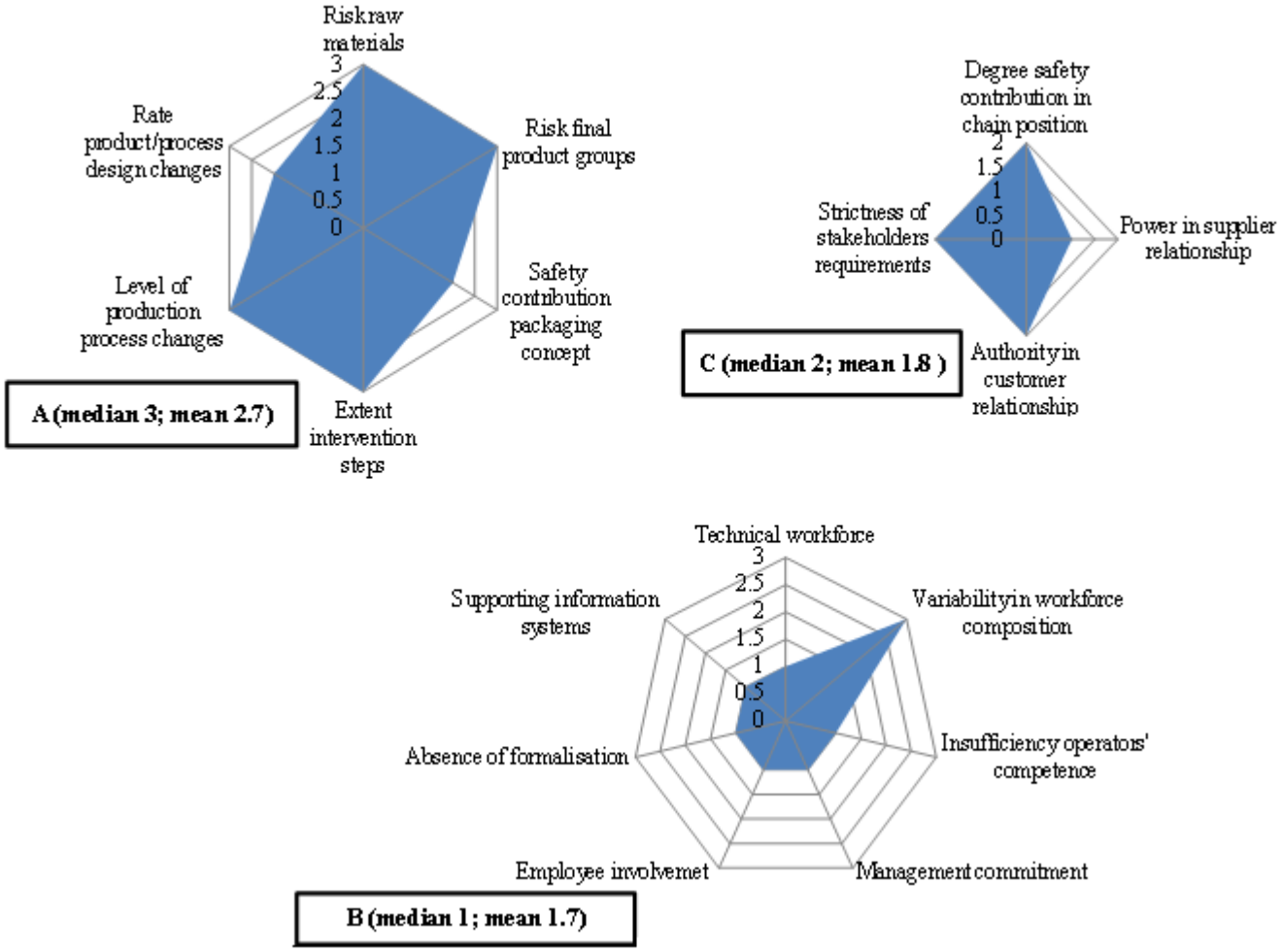

Figure 2. Levels of context riskiness (A) product and process characteristics, (B) organizational characteristics, (C) chain environment characteristics (numbers in brackets indicate median and mean scores).

context situation, it shows that this company is highly dependent on suppliers to ensure quality and safety of its products. Although the company is actively developing supplier specifications, it should also ensure that the preventive control strategies in the FSMS are at an advanced level.

With regards to organisational characteristics, all indicators scored 1 (low-risk level) except degree of variability in workforce composition, which scored 3 (high-risk level; Figure 2B). Like the national-wide study, this study also indicated low-to-medium (median 1, supportive-to-restricted) administrative conditions (Table 3). These administrative conditions support appropriate decision-making in the FSMS due to availability of competent technical staff (trained and experienced), management commitment (food safety/quality policy, food safety team, and financial support), high formalisation (procedures for every activity or operation) and availability of supporting information systems. However, high-turnover of employees and temporary operators throughout the year increase the chances of poor execution of food safety tasks due to continuous loss of company specific experience/skills. Recent studies observed that majority of fish companies in Tanzania (8/14) (Kussaga et al., 2014) and Kenya (7/9) (Onjong et al., 2014a) had moderate turnover of employees. High variability in workforce composition is also reported in a Vietnamese Pangasius processing company (Thi et al., 2014). As an intervention strategy, the company has to recruit permanent staff and review its remuneration packages and working conditions to enable workers to stay longer. Remuneration packages (like salaries/wages) and working conditions could either motivate workers to perform well and stay longer or frustrate them to quit the job (Mullins, 2007).

For the chain environment characteristics, as observed

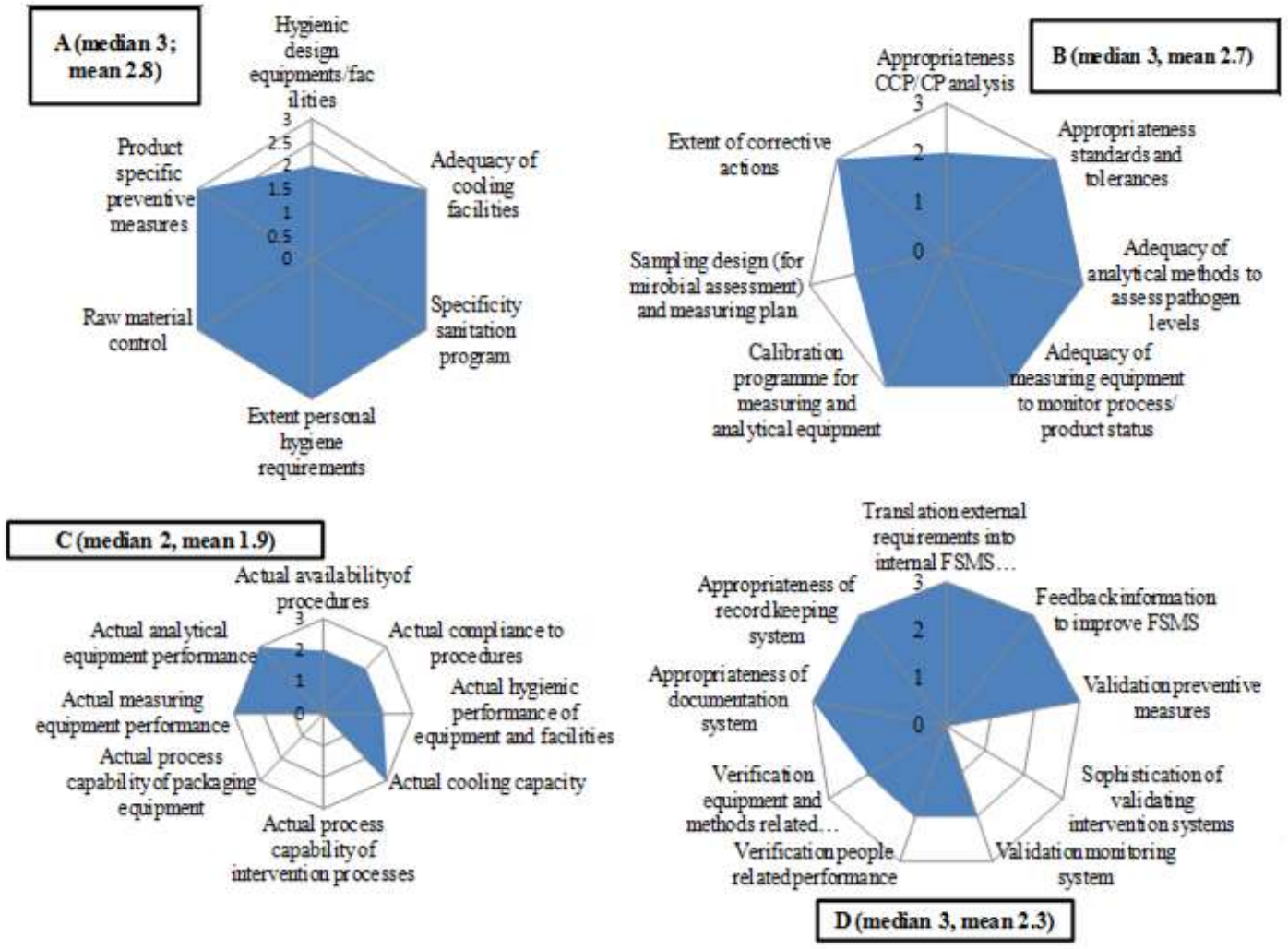

Figure 3. Levels of FSMS activities: (A) preventive measures; (B) monitoring system design; (C) operation of core safety control strategies; (D) assurance activities (numbers in brackets indicate median and mean scores).

in the nation-wide study, the company analysed in this study produced fresh chilled or frozen fillets which require further cooking at the final consumer; thus, it contributes to the final safety through prevention of contamination and growth of pathogens (score 2). With regards to supplier and customer relationships, the company is explicitly involved in the development of product specifications and audit suppliers QMS (score1). However, it has restricted authority in customers' relationships (as it could only discuss the product use by major customers but has no influence on their systems), and has to meet additional but similar QA requirements from stakeholders like eco-labelling, BRC, HACCP, and traceability (score 2; Figure 2C). Lack of influence on QMS/FSMS of major customers could result into unpredictable use and handling of the products (e.g., temperature abuse, unhygienic handling) compromising safety of the products.

Diagnosis of performance levels of core control activities

All indicators of preventive measures design scored 3 (advanced level) with exception to hygienic design of equipment and facilities, which scored 2, the average level (Figure 3A). In general, this study indicated advanced design of preventive measures (median 3, mean 2.8) as revealed in the nation-wide study (median 3, mean 2.7; Table 3) (Kussaga, 2015). This illustrates that critical equipment like cooling facilities comply with specific hygiene requirements (but not tested in the company specific production situation). Cooling facilities are very critical for food processing companies that do not apply intervention strategies (like heating, fermentation and drying); therefore, their performance need to be tested (Luning et al., 2008). Although offsite assessment revealed that other preventive measures

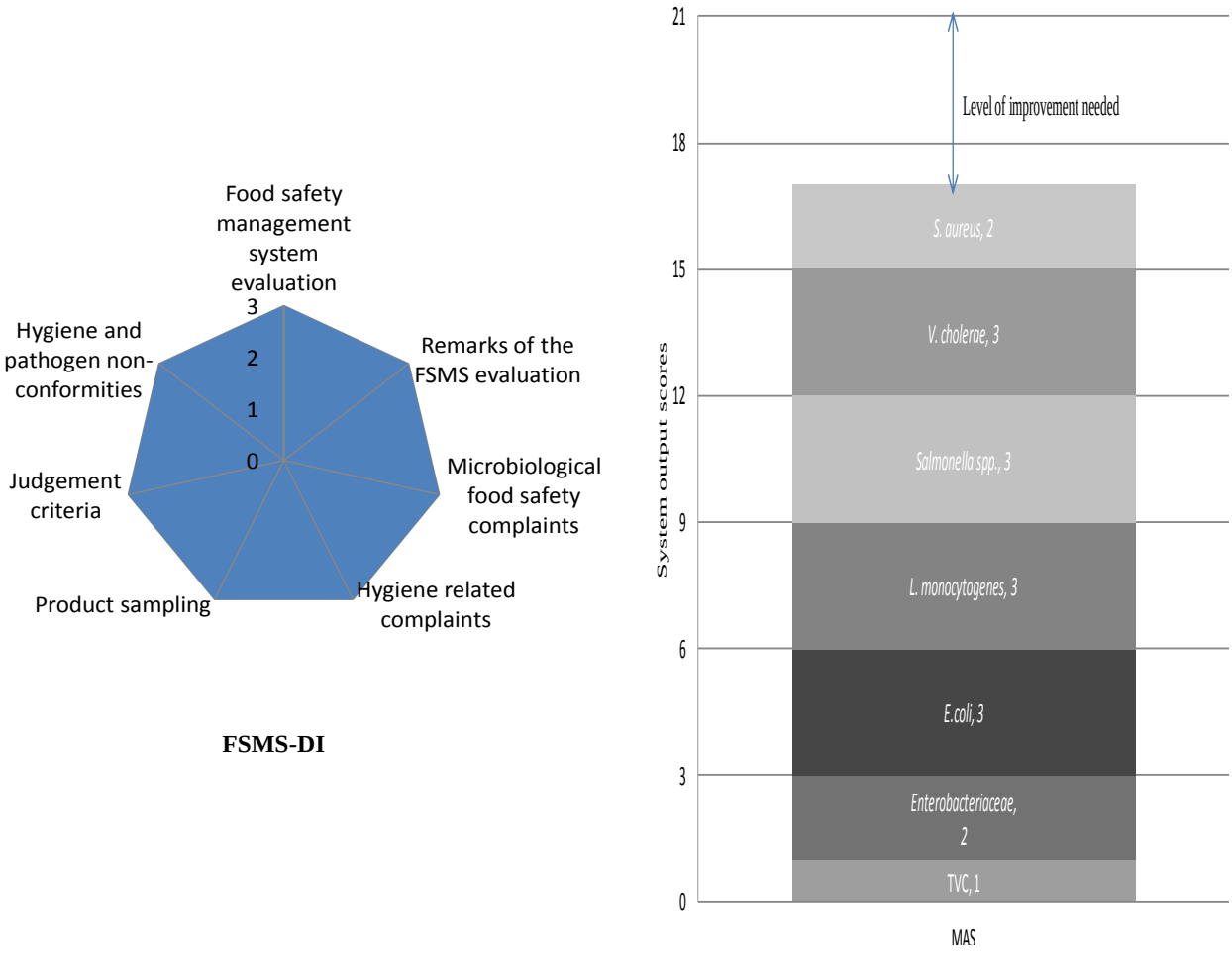

Figure 4. Levels of system output by FSMS-DI (the spider web diagram) and actual microbiological assessment (associated numbers are the scores for each parameter).

were at advanced level, onsite visit showed inadequate cleaning of conveyor belts, flaking out of the wall paints, and condensation from ceiling board, which could serve as potential sources of microbiological proliferation and contamination. In principle, it is required that any equipment in the processing area is included in the cleaning schedule. Moreover, all indicators of intervention processes scored 0 (were not included in calculating the overall FSMS score), because export fish companies in Tanzania process fresh and frozen fish products which do not apply physical intervention processes (like heating) and intervention methods (like fermentation) to reduce microbiological hazards to an acceptable level. Since no intervention processes were applied, the preventive strategies need to be at an advanced level to prevent cross contamination and growth of available micro-organisms (Luning et al., 2011a).

With exception to appropriateness of CCP/CP analysis and specificity of sampling design (for microbiological assessment) and measuring plan (scored level 2), the rest of the indicators of monitoring system design (corrective actions, standards tolerances, adequacy of analytical methods and measuring equipment, and calibration program) scored level 3 (Figure 3B). Similar situation appears in the nation-wide study which in overall indicated average-to-advanced design of monitoring system (median 3, mean 2.6; Table 3). Analysis of pathogens (like *Salmonella* spp. and *V. cholerae*) and chemical contaminants (pesticides including DDT) is performed by several accredited laboratories including the laboratory of the competent authority, the NFQCL, TBS, and Chemiphar laboratory in Uganda (for heavy metals like lead and mercury). The measuring equipment to monitor process/product status like thermometers were in-line (automated) for the chillers, plate freezers, and cold rooms, where the temperature measurements or variations could be easily seen and temperature records are retrievable. The company has a specific program for calibration and maintenance of thermometers. Normally thermometers are checked on daily basis to ensure

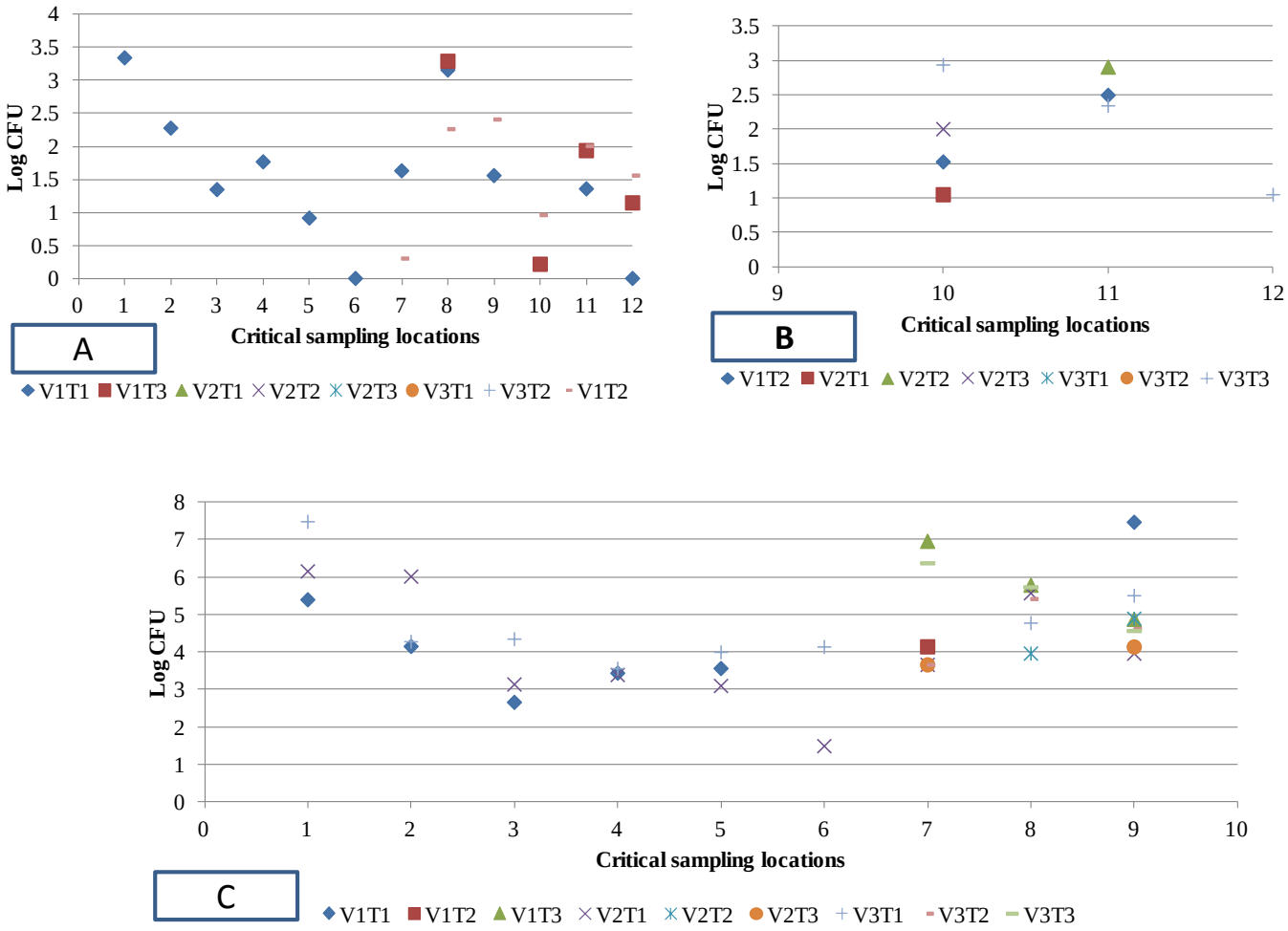

Figure 5. Distribution of (A) *Enterobacteriaceae*, (B) *S. aureus*, and (C) Total viable counts in all critical sampling locations along the frozen Nile Perch processing line. The results are expressed in Log CFU/25 cm^2 for contact surfaces and Log CFU/50 cm^2 for products (V1T1- V3T3 (indicate number of visits and times of sampling, e.g. V1T1-visit1 Time1 sampling, V1T2-Visit1 Time2, VIT3-Visit2 Time3, V2T1-Visit2 Time1, V2T2-Viisit2 Time2, V2T3,-Visit2 Time3, V3T1-Visit3 Time1, V3T3,-Visit3 Time2, and 3T3- Visit3 Time3 sampling).

proper freezing of fish products. For audit purposes, calibration and maintenance records are kept up-to-date. In addition, the food control authorities like TBS conduct calibration of measuring equipment (however, periodically). Moreover, the competent authority inspects fish companies on regular basis. A similar study in a *Pangasianodon hypophthalmus* processing company found that sampling design and measuring plan was at an average level (Noseda et al., 2013). Since analysis of CCP/CPs is done based on expert knowledge without actual testing, the company analysed in this study, could use additional scientific knowledge and experimental tests under the company production circumstances. In addition, the sampling design and measuring plan have to be typified by analysis of pathogen distribution in own food production process.

Like the national-wide study (median 3 and mean 2.2), the company analysed in this study indicated average design of the operation of control strategies (median 2, mean 1.9 and Table 3). Similarly, the actual process capability of intervention and packaging equipment scored level 0 (Table 3). This is because most fish companies produced fresh and frozen products, so no any physical intervention equipment used. Besides, the packaging concept was not aimed to control or reduce microbial contamination. Fish products were wrapped in plastic bags and packaged in Styrofoam and waxed-box cartons with plastic bag linings to protect them from contaminants (like dirt) and exclude oxygen to prevent oxidation. Moreover, actual availability of procedures, compliance to procedures and hygienic performance of equipment and facilities scored level 2 (Figure 3C). This shows that procedures were available at location though mostly paper-based and kept up-to-date on ad-hoc basis, tasks were executed based on habits and operators regularly controlled on compliance, and unexpected

contamination problems occur due to inappropriate equipment and/ or facilities. However, the company had stable cooling capacity and measuring equipment (level 3). The major measuring equipment used were thermometers and *p*H meters. In addition, the actual performance of analytical equipment scored level 3 because microbiological and chemical analyses were conducted at accredited laboratories of the competent authority for fish products (NFQCL) and national food control agencies (TBS and TFDA). Besides, the company had its own laboratory to conduct basic microbiological analysis (like *Enterobacteriaceae, E. coli,* and TVC) with exception to pathogens (*Salmonella* spp. and *V. cholerae*) and chemical contaminants (that is, dioxins and heavy metals like lead and mercury), which are analysed either within (NFQCL, TBS, and TFDA) or outside the country like Chemiphar (U) Ltd in Uganda (especially for heavy metals). Apart from monitoring of chlorine level in processing water (that is, the company has its own water treatment section), other chemical tests (heavy metals) are conducted for monitoring purposes as requested by the competent authority.

Diagnosis of performance levels of core assurance activities

In overall, the nation-wide study (median 2, mean 2.2) indicated similar situation as this study (median 3, mean 2.3 and Table 3). Five out of nine indicators of core assurance activities scored level 3 (Figure 3D). The company scored 0 in validation of intervention systems because no intervention processes were applied. Moreover, it scored level 2 in validation of monitoring system and verification of people- and equipment and methods-related performance. This shows that, however, validation of monitoring system was conducted on regular basis by external expert; it was based on comparison with regulatory documents without experimental trials. Likewise, it confirms that verification activities were conducted on regular basis by independent internal staff by analysing procedures, records and calibration activities. Therefore, this company could develop interventions towards advanced activity levels like scientific based and independent validation of monitoring systems and verification of people- and equipment and methods- related performance. However, as found in the national-wide study (Kussaga et al., 2014), the company proactively translated the external assurance requirements like new legislation (e.g., the EU) and evaluated on its own the critical production circumstances.

Diagnosis of system output by the FSMS-DI

All system output indicators scored level 3 (Figure 4) indicating good system output (median 3, mean 3 and Table 3). Similarly, a national-wide study revealed a relatively good system output with most indicators scoring level 3 (Table 3) (Kussaga et al., 2014). Based on the self-assessment, this fish company has comprehensive internal and external FSMS output assessment. The FSMS is audited by several accredited third parties including private and governmental (national food control agencies and the competent authority) audits, no major and/or minor remarks on the FSMS, and no customers' microbiological food safety and hygiene related complaints. Besides, the company had structured sampling for both the products and environment, and used combination of legal requirements/criteria and specifications by external parties and company established specifications to judge the microbiological results. Moreover, it had no non-conformities regarding microbiological food safety or hygiene indicators. Fish companies in Tanzania are inspected by the national food safety control authorities and audited by accredited third parties; the majority had specific sampling plans and none experienced microbiological food safety or hygiene non-conformities (Kussaga et al., 2014). The actual microbiological assessment of products, food contact surfaces, and hands/gloves of the personnel were performed to confirm the results of FSMS-diagnosis.

Diagnosis of actual microbiological output of the system

The actual microbiological assessment indicated a moderate-good (score 2 to 3) system output (Table 4 and Figures 4 and 5), which is relatively lower than the one obtained through the FSMS diagnosis (median 3, mean 3; Table 3 and Figure 4). Similar to actual microbiological assessment, a Tanzanian fish nation-wide study indicated an overall moderate-good system output (median 2.5, mean 2.2; Table 3) (Kussaga et al., 2014). This illustrates that although the FSMS-diagnosis revealed advanced activity levels, they are not sufficient to control certain microbiological parameters or deal with the current context risk-level. On the other hand, it reveals an overestimation of the level of design and operation of core control and assurance activities by the company during the self-assessment as it is opposed to the actual microbiological assessment. However, indicators of food safety (*Salmonella* spp., *L. monocytogenes,* and *V. cholerae*) and faecal hygiene (*E. coli*) were respectively below the detection levels (absence in 50 cm^2 for food products or 25 cm^2 for food contact surfaces) and quantification limit (<1 CFU in 50/25 cm^2) throughout the study (assigned score 3; Table 4). This indicates that the implemented FSMS activities are sufficient to control such microbiological parameters. This is also in agreement with the FSMS-diagnosis, which indicated an average FSMS (median 2.5, mean

2.2) operating under moderate-risk context (median 2, mean 1.9; Table 3). Taking into account that no intervention processes applied, the preventive measures which were the most important control strategies for this company were also at an advanced level (median 3, mean 2.8; Table 3 and Figure 3A).

On the contrary, Enterobacteriaceae were assigned overall score 2 (moderate system output) because were found on tables at trimming (3 out of 9 samples) and packaging (2/9) areas, and hands of personnel at trimming (3/9) and packaging (5/9) sections above the levels in the products handled at the respective areas (Table 4 and Figure 5). The FSMS-diagnosis has also shown restricted use of procedures (which were commonly paper based and not systematically kept up-to-date) as tasks execution was based on habits, and unexpected contamination occasionally occurs due to inappropriate equipment and facilities like flaking out of wall-paints (Figure 3C). Recent studies in fish processing companies in Vietnam (Thi et al., 2014) and Kenya (Onjong et al., 2014b) observed high variability of Enterobacteriaceae on food contact surfaces and hands of the personnel as well as fish products. According to literature, possible causes of Enterobacteriaceae contamination are inadequate procedures of slaughter, handling, packaging, and storage (Boari et al., 2008; Okonko et al., 2008, 2009) and ineffective cleaning of food contact surfaces like tables and equipment (Bagge-Ravn et al., 2003). Likewise, water and ice (Okonko et al., 2009; Shikongo-Nambabi et al., 2010; Mohamed et al., 2011), personnel (Mohamed et al., 2011), and reduced chlorine concentration of the dip after intensive use (Shikongo-Nambabi et al., 2011) or microbial build-up after an extensive use of the dip could be possible causes of contamination. *Staphylococcus aureus* scored level 2 because were observed on hands of personnel at receiving (4/9 samples), trimming (2/9) and packaging (1/9, Table 4; Figure 5) above the microbiological guidelines in the fish industry (Table 2), indicating inadequate personal hygiene. Also, FSMS diagnosis revealed high turnover of employees and execution of tasks were based on habits, indicating that good manufacturing and hygienic practices (like personal hygiene, hand washing, use of aprons/hair covers) were not exactly followed. Previous studies reported *S. aureus* on workers hands and fishery products (Simon and Sanjeev, 2007; Mohamed et al., 2011; Onjong et al., 2014b; Thi et al., 2014). Food handlers are also known to be potential sources of staphylococcal food contamination (Okonko et al., 2009; Adedeji and Ibrahim, 2011; Mohamed et al., 2011).

Total viable counts exceeded the limits in raw materials (1/3 samples) and tables at trimming (7/9) and packaging (7/9) sections. The huge variations in TVC was noted in products (2.4 to 7.5 log CFU/cm^2) and working tables (<1-7.5 log CFU/cm^2) (assigned score 1, Table 4 and Figure 5). However, high prevalence of TVC on working tables suggests that the company has inadequate pre-requisite programs (PRPs) particularly, the raw material purchasing specification and cleaning and disinfection. Although water could be another route of contamination, the possibility of contamination through processing water is minimal as the company routinely monitors microbiological quality and chlorine level in the water. Besides, the company has its own source of water and treatment is done and monitored by the company. Though the company dealt with high-risk raw materials (which could be contaminated from the source and along the chain, and require special storage conditions; Figure 2A), there were no intervention processes applied (as the 5 ppm concentration of chlorine could not reduce microbiological levels to an acceptable level). This chlorine concentration (5 mg/L) is also far below the EU levels of chlorine (250 mg/L in form of chloride) required in drinking water (European Union Commission, 1998). Furthermore, the company had restricted hygienic design of equipment and facilities and no independent verification of equipment/methods and people related performance. According to literature, skin or fillet of freshly caught fish may contain microbial load ranging from 2 to 6 log CFU/cm^2 (Olafsdóttir et al., 1997). The bacterial loading on freshly caught fish reflects the environment from which it was caught, rather than the fish species (Al-Harbi and Uddin, 2005). Also, other studies noticed TVC beyond the set standards in raw fish (Okonko et al., 2009; Shikongo-Nambabi et al., 2010; Onjong et al., 2014b), fresh fish-fillets (Chytiri et al., 2004; Onjong et al., 2014b), working tables (Okonko et al., 2009; Onjong et al., 2014b) and hands of the personnel (Okonko et al., 2009). Thus, raw materials, food contact surfaces, and hands of personnel could be the sources of TVC (Chytiri et al., 2004; Shikongo-Nambabi et al., 2010; Shikongo-Nambabi et al., 2011). Furthermore, this company (including other Tanzanian fish exporting companies) occasionally receives notifications and border rejections of their products due to failures to meet microbiological standards of the export market (Food and Veterinary Office, 2007; Kadigi et al., 2007; Rapid Alert System for Food and Feed, 2009a; Day et al., 2012; Kussaga et al., 2014; Rapid Alert System for Food and Feed, 2014a). In general, improving the PRPs would address the food safety problems reported in this study.

Conclusions

Although the design and operation of FSMS activities sufficiently controlled some microbiological parameters, the actual microbiological assessment indicated slightly low system output as compared to the FSMS diagnosis. The actual microbiological assessment found variable and high counts of TVC in raw materials, final products and food contact surfaces as well as Enterobacteriaceae in food contact surfaces.

Table 2. Microbiological specifications of fish products, food contact surfaces, and hands of the personnel.

Microorganisms	Maximum limit (CFU)			
	Tanzanian Standards[a]	East African Standards[b]	USFDA[d]	Ghent University guidelines[e]
Fresh fin fish				
Total viable counts	m = 10^6 CFU/g, M = 10^7 CFU/g	M = 10^6 CFU/g	-	-
E. coli	m = 5 CFU/g, M = 10^2 CFU/g	M = 10^1 CFU/g	-	-
Enterobacteriaceae	-	M = 10^2 CFU/g	-	-
L. monocytogenes	-	-	Absent in 25 g	-
Salmonella spp.	Absent/g	Absent in 25g	Absent in 25 g	-
V. cholerae	-	Absent in 1g	Absent in 25 g	-
Frozen fin fish[b] or fillets[f]				
Total viable counts	m = 10^6 CFU/g, M = 10^7 CFU/g	M = 10^6 CFU/g	-	-
E. coli	m = 5 CFU/g /g, M = 10^2 CFU/g	M = 10^1 CFU/g	-	-
Enterobacteriaceae		M = 10^2 CFU/g	-	-
L. monocytogenes		-	-	-
Salmonella spp.	Absent	Absent in 25 g	-	-
V. cholerae		Absent in 1 g	-	-
Food contact surfaces (working tables)				
Total viable counts	-	-	-	**
E. coli	-	-	-	**
Enterobacteriaceae	-	-	-	**
L. monocytogenes	-	-	-	Absent in the tested area
Salmonella spp.	-	-	-	Absent in the tested area
V. cholerae	-	-	-	Absent in the tested area
Hands of the personnel				
E. coli	-	-	-	**
Enterobacteriaceae	-	-	-	**
S. aureus	-	-	-	Below limit (10 CFU/25 cm^2) of quantification

[a](Tanzania Standard, 1988), [b](East African Community, 2010a), [c](European Union, 2005), [d](U.S. Food and Drug Administration, 2009), [e](Sampers et al., 2010), [f](East African Community, 2010b); **Same as product handled in the respective area.

Table 3. Median and mean scores of context factors, control and assurance activities, and system output of fish processing companies in Tanzania.

Context factor and core control or assurance activities	Median and Mean (in brackets) of all (14) fish companies*	Median and mean (in brackets) of single company
Context characteristics		
Product and process characteristics	2.7 (2.4)	3.0 (2.7)
Organisation characteristics	1 (1.5)	1 (1.3)
Chain-environment characteristics	2 (1.7)	2 (1.8)
Overall context-riskiness	*2 (1.9)*	*2 (1.9)*
Core control and assurance activities		
Preventive measures design	3 (2.6)	3 (2.8)
Intervention measures	0 (0.87	0 (0.8)
Monitoring system	3 (2.6)	3 (2.7)
Actual operation of core control strategies	3 (2.2)	2 (1.9)
Assurance activities	2 (2.2)	3 (2.3)
Overall FSMS performance	*2.5 (2.2)*	*3 (2.2)*
System output	3 (2.7)	3 (3.0)

*Adapted from Kussaga (2015).

Table 4. Detailed MAS results indicating microbial parameters analysed at each CSL, frequency of detection/quantification, and assigned and overall system output scores.

Critical sampling location (CSL)	Detection of food safety indicators			Quantification of indicators of hygiene (CFU/50 or 25 cm^2)**			
	Absent (A)/Present (P) in 50 or 25 cm^2			Faecal hygiene	Personal hygiene	Overall process hygiene	
	LIST[a]	SALM[b]	VIBRIO[c]	ECOL[d]	STAP[f]	ENTE[e]	TVC[g]
1. Raw fish (before washing)	A	A	A	<1	NA	<1-3.3	5.4-**7.5** (1/3)[h]
2. Washed raw fish	A	A	A	<1	NA	<1-2.3	4.1-6.0 [i]
3. Trimmed fillets	A	A	A	<1	NA	<1-1.3	2.7-**4.3**
4. Washed fillet	A	A	A	<1	NA	<1-1.7	3.4-**3.6**
5. Bagged fillet	A	A	A	<1	NA	<1	3.1-**4.0**
6. Packaged frozen fillet	A	A	A	<1		<1	<1-**4.1**
7. Tables at receiving section	A	A	A	<1	NA	<1-1.6	<1-6.9
8. Tables at trimming section	A	A	A	< 1	NA	<1-**3.3 (3/9)**	<1-**5.8** (7/9)
9. Tables at packaging section	A	A	A	<1	NA	<1-**2.4 (2/9)**	<1-**7.5** (7/9)
10. Operator's hands- receiving section	NA	NA	NA	<1	<2 -**2.9(4/9)**	<1-1.0	NA
11. Operator's hands- trimming section	NA	NA	NA	<1	< 2-**2.9 (2/9**)	<1-**2.3 (3/9)**	NA
12. Operator's gloves- packaging section	NA	NA	NA	< 1	<2	< 1-**1.6 (5/9)**	NA
Total samples not detected with pathogens or microorganisms below or within the legal limits	45/45	45/45	45/45	72/72	20/27	59/72	26/45
Total samples detected with pathogens or microorganisms exceeding the legal limits	0/45	0/45	0/45	0/72	7/27	13/72	19/45
System output assigned score	**3**	**3**	**3**	**3**	**2**	**2**	**1**
Overall system output score	**17/21 (score 2-3)**						

[a] *L. monocytogenes;*[b] *Salmonella* spp.; [c] *V. Cholerae;* [d] *E. coli;* [e] *Enterobacteriaceae;* [f] *S. aureus;* [g] Total viable counts; [h] number of samples exceeding the limit in all samples analysed within a particular CSL;[i] lowest and highest CFU counted in all three visits within a specific CSL; NA - not applicable; ** The results are expressed in log CFU/50 cm^2 for products and log CFU/25 cm^2 for contact surfaces (filtration tray, filling machine) and hands of personnel. Bolded numbers indicate samples that exceeded legal limits or guidelines. Tanzania standards were used to interpret results for TVC and *E. coli* in raw fish and frozen fillets; East African standards for *L. monocytogenes, Salmonella* spp., *E. coli* and *Enterobacteriaceae* in frozen fish fillets, European Union for *Salmonella* spp., *V. cholerae* and *L. monocytogenes* in raw fish; Ghent University guidelines for *Enterobacteriaceae, Salmonella* spp. *V. cholerae*, and *L. monocytogenes* on food contact surfaces and *S. aureus* on hands of the personnel (Table 2).

Currently, there are no EU requirements set for such parameters, providing an opportunity for this Nile perch processing company to continue exporting to the EU as pathogen levels are within the EU standards. However, higher levels of Enterobacteriaceae indicate possibilities of health issues as these are regarded as indicators of process hygiene, inadequate processing and post processing contamination. If there is poor process hygiene there is a chance of introducing pathogens to the process, or when the heating process was inadequate survival of pathogens is likely. Some members of the family Enterobacteriaceae (e.g. *Shigella* spp.) are also responsible for causing foodborne diseases. The level of context riskiness could be reduced through automation of the production process (like filleting, packaging, and sanitation) to reduce personnel interferences, recruitment of trained and experienced personnel on permanent basis, and specify product-use by major customers (storage and distribution conditions). The levels of FSMS activities could be enhanced through re-designing of equipment (like automation) and

facilities (re-painting and filling cracks on the walls and floors), improving sanitation programme (including all equipment in the cleaning and sanitation schedule), changing sampling design and measuring plan (by analyzing pathogen distribution in the production process), improve procedures (specifically designed for user, easily accessible, well understood and internalised), independent validation (experimental trials, well established and documented) and verification (supported by scientific evidence and data from own food production process). Therefore, fish companies are required to improve the level of the design and operation of the FSMS activities and reduce the level of context riskiness to guarantee good system output that will ultimately reduce microbiological notifications on fish export.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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ABBREVIATIONS

FSMS, Food safety management system; **FSMS-DI,** food safety management system - diagnostic instrument; **CSL,** critical sampling location; **TVC,** total viable counts; **TIFPA,** Tanzania Industrial Fish Processors Association; **QA,** quality assurance; **FDA-BAM,** U.S. Food and Drug Administration-Bacteriological Analytical Manual; **MAS,** Microbial Assessment Scheme; **QMS,** Quality Management System; **NFQCL,** National Fish Quality Control Laboratory; **TBS,** Tanzania Bureau of Standards; **TFDA,** Tanzania Food and Drugs Authority.

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Full Length Research Paper

Orange-fleshed sweet potato based complementary food provides sufficient vitamin A for infants aged 6-12 months

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Vitamin A deficiency is a major public health problem in developing countries, specifically Ghana. The high beta-carotene content of orange-fleshed sweetpotato (OFSP) may help alleviate vitamin A deficiency because beta-carotene is converted to vitamin A when consumed. It is hypothesized that complementary food formulated with OFSP, rice, soybean and cowpea or peanut would contain >50% of the estimated average requirement (EAR) of vitamin A for infants 6 to 12 months of age. Three different blend formulations (OFSP Rice-1, OFSP Rice-2 and OFSP Rice-3) were prepared. OFSP Rice-1 was prepared with 50% rice, 30% OFSP and 20% soybeans flours as control; OFSP Rice-2 was prepared with 45% rice, 30% OFSP, 20% cowpea flours and 5% vegetable oil; and OFSP Rice-3 was prepared with 55% rice, 20% OFSP, 20% soybeans flours and 5% peanuts. Each formulation was evaluated for its nutritional quality, sensory characteristics and pasting properties using standard AOAC methods, a 9-point hedonic scale, and Brabender viscoamylograph, respectively. All three formulations met >50% EAR for energy (386.2 to 391.8 kcal/100 g), protein (10.4 to 16.9 g/100 g), carbohydrates (68.4 to 72.2 g/100 g), iron (3.6 to 7.6 mg/100 g) and beta-carotene (2112 to 7879 µg/100 g). OFSP-Rice2 had a significantly higher concentration of beta-carotene (7879.20 µg/100 g). OFSP-Rice3 had the highest acceptability score of 8.0, highest peak viscosity (41.5 BU), highest viscosity after holding (37.0 BU), and the highest final viscosity (53.5 BU). Rice with 20 or 30% OFSP may provide >50% EAR of vitamin A for infants 6 to 12 months of age. This may provide an acceptable vitamin A rich food that could help alleviate vitamin A deficiency in Ghana.

Key words: Complementary food, orange-fleshed sweetpotato (OFSP), vitamin A, broken rice.

INTRODUCTION

Vitamin A deficiency is a major public health concern in developing countries. It is associated with night blindness, xeropthalmia, skin and hair diseases, growth retardation and impaired immune system (Dada et al., 2002;

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McCaffery et al., 2003). In Africa, 32% of children under the age of five are estimated to be vitamin A deficient, with a 1.5% prevalence of xerophthalmia within the same age group (UN SCN, 2004). In Ghana, vitamin A is of particular concern, since the prevalence of vitamin A deficiency is 72% (GHS, 2005; GDHS, 2008; WHO, 2002). A diverse diet including foods such as fruits, leafy green vegetables, and animal products can provide sufficient vitamin A. Additionally, foods rich in beta-carotene such as orange-fleshed sweetpotato (OFSP) provide sufficient dietary vitamin A (Low et al., 2008). However, infant diets in low income countries consist largely of starchy staples such as maize, millet and sorghum and lack the vitamins and minerals needed for good health and development (WHO/UNICEF, 2012). A lack of vitamin A during these critical growth periods contribute to growth-stunting during the first 2 years of life, with potential adverse consequences for child development and later adult health, highlighting the need to provide infants with food adequate in vitamin A to prevent the risks associated with vitamin A deficiency. The present study was designed to investigate the use of orange-fleshed sweetpotato, rice and other locally available ingredients as potential complementary food to help combat vitamin A deficiency among children 6 to 12 months of age.

Several funded programs have been initiated by both local researchers and international organisations to help solve this problem of micronutrient deficiency. Programs such as promotion of complementary foods with cereal-legume mix (Annan et al., 1995; Amagloh et al., 2011), provision of high oral doses of vitamin A (Bruno et al., 2001), micronutrient fortification of cereal flours (WFP Report, 2012), use of sprinkle powder (Zlotkin et al., 2001), and lipid-based supplementation (Adu-Afarwuah et al., 2008; Adu-Afarwuah et al., 2010) have all been employed to address this problem. Although, these interventions have been effective, they are limited in reach and often expensive, especially for rural areas where majority of the poor live. Therefore, it is important for all stakeholders to consider new approaches that may involve locally available raw materials.

One such approach is the use of the biofortified root crop, orange-fleshed sweetpotatoes (OFSP) as a way of enhancing the nutritive value of infant foods. Earlier work conducted among children in Peru found OFSP to be a good ingredient for complementary food (Espinola et al., 1998). Others have also found improvement in vitamin A status after children were fed diets containing OFSP (Jaarsveld et al., 2005; Amagloh et al., 2012). Again, work by Bonsi et al. (2014) found a high acceptability of OFSP blends with roasted maize meal. However, the use of rice, especially broken rice fractions and OFSP as a complementary food has not been explored in Ghana. In Ghana, like many African countries, the use of rudimentary tools and practices affect the quality of the milled rice. The resultant product usually contains unhusked grains, bran and husk fractions, making it less attractive and less competitive on the market, however high in micronutrients (Appiah et al., 2011). Using broken rice fractions together with OFSP as a composite blend in complementary food may provide an alternative use for rice cultivated in Ghana and improve the beta-carotene content of infant food, thus improving vitamin A status of infants. It is hypothesized that complementary food formulated with OFSP, rice and soybean, cowpea or peanut would contain >50% of the estimated average requirement (EAR) of vitamin A for infants 6 to 12 months of age

The aim of this study was therefore to produce three different blend formulations from OFSP flour, broken rice flour, soybean, peanut and/or cowpea and evaluate their nutritional quality, sensory characteristics and pasting properties.

MATERIALS AND METHODS

Orange-fleshed sweetpotatoes (OFSP) tubers and broken rice fractions (*Oryza sativa*) were the major ingredients for all the formulations. OFSP were obtained from Tuskegee University, Tuskegee, Alabama, while the broken rice fraction was obtained from rice milling center in Afefe in the Volta Region of Ghana. Other ingredients used for the blend formulations such as soybeans (*Glycine max*), peanuts (*Arachishypogaea*), cowpea (*Vignaunguiculata*) and vegetable oil were purchased from a local market in Accra, Ghana.

Samples preparation

The OFSP was peeled, washed and sliced to about 2 mm using an electric slicer. OFSP slices were blanched in water at 90°C for 5 min, and cooled immediately in cold water. The blanched OFSP was dried in an oven at 50°C for 16 h. Milling was done using the pin mill followed by the hammer mill into fine flour.

Low grade broken rice fractions was cleaned and sorted to remove chaff and immature grains before milling. The rice was milled into fine flour using a hammer mill. The procedure for aflatoxin elimination in peanut (CRSP, IUFoST, 2012) was followed to sort out the immature, shriveled, discolored and damaged kernels from the clean kernels. This involved heat blanching of the groundnuts in a preheated oven at 140°C for 15 min. The peanuts were allowed to cool and thereafter, the reddish seed coats manually removed. The peanuts were then roasted in an oven at 140°C for 30 min with intermittent turning every 10 min. For blends that contained peanut, a proportion of the roasted peanut that would give a 5% of peanut in the final blend was mixed with specific weight of the broken rice fraction before milling. This was done in order to obtain a uniform mixture.

Full-fat soy flour and cowpea flour were obtained following a method from Plahar et al. (1997). Cleaned soybeans or cowpea were soaked in water for one hour and blanched for 20 min in hot water. The hot water was drained from the soybean with a sieve and immediately refreshed with cold water to avoid over blanching. The blanched soybean was allowed to cool, spread thinly on a stainless steel tray and dried in an oven at 60°C for 8 h. The disc attrition mill (Hunt No. 2A Premier Mill, Hunt & Co., UK) was used to dehull the dried soybeans. The soybean was then winnowed and milled in the hammer mill to obtain fine flour.

Cowpea was dehulled manually by hand after blanching in hot

Table 1. Formulations and product characteristics of OFSP-rice blends.

Blend formulations	Product characteristics and limiting sensory attribute
OFSP Rice-1	Yellowish-orange flour which when reconstituted and cooked becomes creamy yellowish porridge.
OFSP Rice-2	Yellowish-brown flour which when reconstituted and cooked becomes deep yellowish porridge. Beany flavor
OFSP Rice-3	Light-yellowish orange flour which when reconstituted becomes a creamy yellowish porridge.

OFSP Rice-1: rice flour 50%: OFSP 30%: soybean 20%; OFSP Rice-2: rice flour 45%: OFSP 30%: cowpea 20%: Oil 5%; OFSP rice-3: rice flour 55%: OFSP 20%: soybean 20%: peanut 5%.

water for 20 min and dried in an oven at 60°C for 8 h. Dehulled cowpea was milled using a hammer mill to obtain fine flour. The three weaning blends were prepared with appropriate portions of the various flours weighed separated and mixed thoroughly using a manual mixer (WFP, 2012).

Blend formulation

Based on work done by Annan et al. (1995), the blends (Table 1) were formulated with ingredients that will provide vitamin A, and also meet at least 50% of the estimated dietary allowance (EAR) of vital nutrients. Again, work done by Bonsi et al. (2014), found an acceptability of 20 to 30% of OFSP in maize meal. Based on this, 20 and 30% OFSP was chosen for this work. Three different blend formulations were prepared from orange-fleshed sweetpotatoes flour, broken rice fraction flour and soybean flour with or without peanut and OFSP, broken rice and dehulled cowpea. Flour from broken rice fractions was the main energy source. This was combined with OFSP flour and soybeans, a source of protein and fat. In other formulations, dehulled cowpea was used as the protein source. In order to meet the Codex Alimentarius Commission (CAC) criteria for fat content of complementary foods, oil was added to the formulation containing cowpea during manual mixing of the flours in that formulation. Peanut was also added to increase the fat content, and to improve aroma. In formulations containing peanut, the peanut was mixed with the broken rice before milling.

Proximate composition

Nutritional analysis to determine proximate composition (energy, carbohydrates, protein, fat, ash) and mineral/vitamin composition (iron, vitamin A, calcium and phosphorus) were done using standard procedures (AOAC, 2000). Carbohydrates were calculated by difference. Energy values were determined using Atwater factors 3.47, 8.37 and 4.00 for protein, fat and carbohydrates, respectively (Eyeson and Ankrah, 1975).

Carotenoid analysis

Carotenoid analysis was carried out using the method of Rodriguez-Amaya and Kimura (2004). This involved extraction with cold acetone using a mortar and pestle, partitioned to petroleum ether and taking the absorbance at 450 nm.

Calculation

$$\text{Total carotenoid content (µg/100 g)} = \frac{\text{A x Vol. (mL)} \times 104 \times 100}{\text{A1\% 1 cm} \times \text{Sample wt (g)}}$$

Total carotenoid content (µg/100)/6 = µg RE/100 g

Where: A= absorbance, Vol. = 50 ml A1%1cm = Absorbance co-efficient of β-carotene in PE (2592).

Pasting properties

Pasting characteristics of the various formulations were determined with a Brabender Viscoamylograph (Model VA-VE, Brabender Instruments, South Hackensack, NJ) equipped with a 700 cm-g sensitivity cartridge. A 10% slurry (db) of each flour was prepared with distilled water and the slurry heated uniformly (1.5°C per min) from 25 to 95°C, held to cool at the same rate to 50°C (Shuey et al., 1982). The resulting amylograms from triplicate determinations gave the pasting temperatures, peak viscosities, viscosity at 95°C, paste stability, ease of cooking and set back viscosities. Ease of cooking was calculated as the difference between time to reach gelatinization temperature and time to obtain maximum viscosity during heating (Adeyemi et al., 1987).

Consumer acceptability studies

To determine acceptability of porridge prepared from the various formulated OFSP-Rice meal, 60 care-givers, with children between 6 and 12 months were recruited from child-care/weighing centers in the La Nkwantanan-Madina District of Accra, Ghana. A total of 5 weighing centers were visited. Mothers who consented to be part of the study were recruited. Mothers were made to rank their acceptability of the various porridges based on colour, appearance, taste, aroma, mouth feel, consistency and overall acceptability on a 9-point hedonic scale, with 1 representing “dislike extremely” and 9 representing “like extremely” (Larmond, 1977). Permission was sought from the Administrative Director of the University Hospital. Mothers were also made to sign a consent form before the study was carried out.

Data analysis

Data was analyzed using statistical package for social sciences (SPSS) version (17.1.01). Significantly different means were separated using Fishers least significant difference (LSD), assuming a level of significance at $p<0.05$.

RESULTS

Product characteristics and blend formulations

The different blend formulations and product

Table 2. Mean sensory scores for OFSP rice-blends from consumer acceptability test**.

Sensory characteristics	OFSP Rice-1	OFSP Rice- 2	OFSP Rice-3
Appearance	6.82 ± 1.99[a]	7.05 ± 1.85[a]	7.07 ± 1.52[a]
Colour	6.63 ± 1.71[a]	6.49 ± 1.86[a]	6.70 ± 1.64[a]
Aroma	6.37 ± 1.81[a]	6.28 ± 1.79[a]	6.67 ± 1.79[a]
Taste	7.07 ± 1.62[a]	6.68 ± 1.97[a]	7.16 ± 1.56[a]
After taste	6.84 ± 1.78[ab]	6.46 ± 1.94[a]	7.32 ± 1.38[b]
Consistency	7.56 ± 1.44[a]	7.19 ± 1.82[a]	7.40 ± 1.49[a]
Mouth feel	6.84 ± 1.77[a]	6.96 ± 1.91[a]	7.39 ± 1.44[a]
Acceptability	7.80 ± 1.37[a]	7.36 ± 1.9[a]	8.00 ± 0.96[b]

Means within a row not followed by the same superscript letter(s) are significantly different ($p<0.05$). **Interpretation of scores: 1=dislike extremely; 2= dislike very much; 3=dislike moderately; 4=dislike slightly, 5= neither like nor dislike; 6=like slightly; 7= like moderately; 8= like very much; 9=like extremely. OFSP Rice-1: Rice flour 50%: OFSP 30%: Soybean 20%; OFSP Rice-2: Rice flour 45%: OFSP 30%: Cowpea 20%: Oil 5%; OFSP Rice-3: Rice flour 55%: OFSP 20%: Soybean 20%: Peanut 5%

characteristics of the three blends, as perceived by selected expert panel, are shown in Table 1. The product colour ranged from light-yellow to yellowish-brown flour and when reconstituted, resulted in a creamy yellowish to deep yellowish porridge.

Acceptability studies

Table 2 gives the results of consumer acceptability studies for porridge prepared from the three formulated OFSP-Rice blends. After-taste was the only sensory characteristics that showed significant differences between the three blends. OFSP Rice-3 had a significantly higher score for aftertaste than OFSP Rice-2 but not significantly different from OFSP Rice-1.

Appearance, colour, aroma, taste, consistency and mouth feel did not show significant differences between the three products. OFSP-Rice 3 showed a significantly higher acceptability than the other two formulations: OFSP Rice-1 and OFSP-Rice 2. The mean scores of sensory attributes of all three products ranged from “like slightly” to “like very much”. Out of the 60 caregivers who were interviewed, 43.3% preferred OFSP Rice-3, 38.3% preferred OFSP Rice-1, while 18.3% preferred OFSP Rice-2. OFSP Rice-3 was the most preferred blend

Nutrient composition of OFSP-rice blends

The proximate composition, beta-carotene levels, vitamin C and mineral content of the OFSP rice blends are shown in Table 3. OFSP Rice-1 (control) had a significantly higher content of protein, ash, vitamin C and iron. OFSP Rice-2 was significantly high in energy, carbohydrates, beta-carotene and zinc. OFSP Rice-3 was significantly high in fat and moisture content, which may be due to addition of peanut. As the quantity of rice increased and OFSP decreased (OFSP Rice-3), the beta-carotene content decreased significantly, as compared to the control (OFSP Rice-1). OFSP Rice-2 had a relatively higher beta-carotene content. This could be due to addition of oil which may be fortified with vitamin A (Gain, 2007). Addition of soybean (OFSP Rice-1 and OFSP Rice-3) resulted in a blend with relatively higher protein content than cowpea (OFSP Rice-2). This is because the protein content of soybeans is higher than that of cowpea. A combination of peanut and soybeans as a source of fat gave a significantly higher fat content than when oil or soybean alone was used as the source of fat. The energy, carbohydrates, protein, iron and beta-carotene contents of all 3 blends met more than 50% of EAR for infant. Fat, vitamin C, calcium and zinc contents were slightly lower than the recommendations set by CAC.

In all three OFSP-Rice formulations, ash levels fell within the recommended range of <3%. However, the ash content in OFSP-Rice 2 was significantly lower ($p<0.05$) than the other two formulations. This could be due to the soybean and peanut content of the OFSP-Rice-1 and OFSP-Rice 3, respectively which increased the ash content.

Pasting properties

The pasting properties of OFSP rice samples are presented in Table 4. Differences were observed, among the blended formulae in their behavior during heating and cooling. Among the three formulations, OFSP Rice-3 had the highest peak viscosity, highest viscosity after holding, and final viscosity, while OFSP Rice-1 exhibited much lower values of peak viscosity, pasting temperature and final viscosity.

Table 3. Proximate composition, mineral, vitamin and carotene content of OFSP- rice blends.

Nutrient composition	OFSP Rice-1	OFSP Rice-2	OFSP Rice-3	EAR[1] (7 to 12 month)
Energy (kcal/100 g)	386.24±0.78[a]	391.78±1.43[b]	390.41±0.21[b]	400kcal
Carbohydrate (g/100 g)	66.85±0.28[a]	72.19±0.15[b]	66.42±0.78[a]	95g/d
Fat (g/100 g)	5.69±0.49[a]	6.82±0.25[b]	7.05±0.01[b]	30g/d
Protein (g/100 g)	16.90±0.19[a]	10.42±0.49[b]	15.32±0.10[c]	9.6g
Ash (g/100 g)	2.44±0.06[a]	1.88±0.28[b]	2.20±0.21[c]	<3%[2]
Moisture (g/100 g)	8.13±0.02[a]	8.70±0.08[b]	9.02±0.09[c]	<5%[2]
β-carotene (µg/100 g)	2649.60[a]	7879.20[b]	2112.50[c]	-
RE (µg/100 g)	441.60[a]	1313.20[b]	352.10[c]	400µg/d
Vitamin C (mg/100 g)	3.36±0.63[a]	2.13±0.15	1.91±0.16[b]	25mg/d
Iron (mg/100 g)	7.57±0.16[a]	7.49±0.56[a]	3.67±0.22[b]	11.6mg/d
Zinc (mg/100 g)	0.51±0.00[a]	1.49±0.21[b]	1.34±0.25[b]	6.9mg/d
Calcium (mg/100 g)	33.03±4.68[a]	28.22±2.36[a]	24.85±2.37[a]	417mg/d

Values are means of triplicate determination ± standard deviation. Means within a row not followed by the same superscript letter(s) are significantly different ($p<0.05$). [1]Estimated Average Requirement.[2]Recommended ranges of nutrients not EAR (Codex, 2010). OFSP Rice-1: Rice flour 50%: OFSP 30%: Soybean 20%; OFSP Rice-2: Rice flour 45%: OFSP 30%: Cowpea 20%: Oil 5%; OFSP Rice-3: Rice flour 55%: OFSP 20%: Soybean 20%: Peanut 5%

Table 4. Pasting properties of OFSP-rice blends.

Parameter	OFSP Rice-1	OFSP Rice-2	OFSP Rice-3
Pasting Temp (°C)	79.6±4.5[a]	75.5±1.0[a]	82.6±0.2[a]
Pasting time (min)	20.2±3.1 [a]	17.5±0.7[a]	22.3±0.1[a]
Peak viscosity(BU)	18.0±2.8[a]	19.5±2.1[a]	41.5±0.7[b]
V Hold at 90°C (BU)	17.5±3.5[a]	19.0±2.8[a]	37.0±0.0[b]
Final viscosity(BU)	30.0±3.1[a]	30.5±4.9[a]	53.5±3.6[b]
Break down(BU)	0.5±0.70[a]	0.5±0.7[a]	3.5±0.7[b]
Set back(BU)	27.0±3.6[b]	25.0±2.1[b]	20.5±2.3[a]

Means bearing different superscripts along a row are significantly different ($p \leqslant 0.05$). OFSP Rice-1: Rice flour 50%: OFSP 30%: Soybean 20%; OFSP Rice-2: Rice flour 45%: OFSP 30%: Cowpea 20%: Oil 5%; OFSP Rice-3: Rice flour 55%: OFSP 20%: Soybean 20%: Peanut 5%

DISCUSSION

The focus of this study was to explore the use of broken rice fraction and orange-flesh sweetpotato as potential food ingredients that can add value to complementary foods. The fat content of peanut and/or vegetable oil aid in the absorption of the vitamin A and improved aroma, while soybean and/or cowpea serves as protein source for the formulated complementary food.

Nutrient content of food that meet at least 50% of the recommended daily intake are said to be adequate in that nutrient (Codex, 2010). The energy, carbohydrates, protein and vitamin A contents of all the three formulated blends were very high and met the recommended amount set for infant foods. Rice was the main source of carbohydrates in this formulation. The carbohydrate content of OFSP Rice-2 was significantly different from the control (OFSP Rice-1) but there was no significant difference when compared with OFSP Rice-3. Even though, the rice content of the control was 5% less, there were some contributions of carbohydrate from the OFSP, thus making the total carbohydrate content in the control vs. OFSP Rice-3 about 80:75% which may account for the insignificant difference. Protein is important, especially during the weaning period to prevent protein-energy malnutrition, which is usually observed among children in developing countries (Achidi et al., 2016). Protein source came from either soybean or peanut or cowpea. As compared with the control (OFSP Rice-1), when soybean was replaced with cowpea (OFSP Rice-2), there was a significant decrease in protein; however, this was more than the EAR set for proteins. Similar observations were made when the same quantity of soybeans was added. The observation in this study implies that the developed blend products with addition of rice and soybean or cowpea may be a good source of protein for complementary foods (Welch et al., 2000; Broughton et al., 2003; Amjad et al., 2006), and would

Table 5. Nutrient composition of broken rice and soybean.

Nutrient composition	Low grade broken rice fraction	Soybean
Energy (Kcal/100 g)	354.5	447.5
Carbohydrate (g/100 g)	76.94	36.79
Fat(g/100 g)	0.70	18.10
Protein (g/100 g)	10.12	34.37
Ash (g/100 g)	0.84	5.14
Moisture (g/100 g)	11.20	5.60
Vitamin C (mg/100 g)	0.60	1.40
Iron (mg/kg)	20.25	6.50
Zinc (mg/kg)	13.62	49.70
Calcium (mg/100 g)	20.00	339.30
Phosphorus (mg/100 g)	161.60	218.40

supply the needed protein-energy requirements to meet infants' growth demands.

Guidelines set by Codex Alimentarius Commission (CAC) seek to ensure that complementary foods contain adequate amounts of vitamin A andiron. There was a significant improvement in both vitamin A levels and iron levels in all three formulations. As the quantity of rice increases with a decrease in OFSP (OFSP Rice-3), the beta-carotene content decreased significantly, as compared to control (OFSP Rice-1). However, with the same quantity of OFSP and a decreased portion of rice (OFSP Rice-2), there was a significantly higher beta-carotene content. This result was not expected since the same quantity of OFSP was in each formulation. However, the observed difference in OFSP Rice-2 could be due to addition of vegetable oil which may be fortified with vitamin A. Fortification of vegetable oil with vitamin A is a recommended practice in Ghana to help combat vitamin A deficiency (Gain, 2007). The beta-carotene content in all the three formulations were high enough to meet recommendations set by CAC. This indicates that substituting rice with either 20 or 30% of OFSP will significantly improve the beta-carotene content which will subsequently improve vitamin A status. These results are similar to results obtained by Bonsi et al. (2014). It also supports observations by Tchum (2009) who showed that bio-fortification of complementary foods such as maize meal and millet flour with local sources of pro-vitamin A carotenoids can enhance vitamin A status.

Iron content of OFSP Rice-3 was relatively lower as compared to the control. There was no significant difference between iron content of OFSP Rice-2 when compared with control. Iron is a very important nutrient which improves the cognitive function of children (Lozoff et al., 1998; Beard et al., 2003). Complementary foods must contain the right amount of iron for infants 6 to 12 month of age because the iron content in breast milk may no longer be sufficient to meet infant demands during this period (Dupont, 2003). In this study, iron content of all three formulations met more than 50% of the EAR. This may have come from contributions from soybeans, cowpea and the broken rice fractions. Soybeans and cowpea may also contribute good amounts of iron (Broughton *et* al., 2003; White and Broadley, 2005). Broken rice fractions may have contributed to the high iron content of these products since it has lot of bran which is rich in iron as shown in Table 5. Despite this, high phytate levels in rice, soybean and cowpea may inhibit its bioavailability (Welch et al., 2000; Hurrel, 2004). Vitamin C is known to enhance absorption of non-heme iron from cereals (Hallberg, 1989). In this study, the vitamin C content of all the three products was very low. As such, enrichment of the blends is recommended with sources of vitamin C to enhance the absorption of iron in these formulations. Some local vitamin C sources that can be explored are oranges, mangoes and pineapples.

Apart from vitamin C, fat and zinc contents, were lower than recommendations set by CAC. As compared to the control (OFSP Rice-1), addition of oil improved the fat content significantly but not in amounts that met 50% of the EAR. It is however important to add oil to weaning formulations when cowpea is used as protein source because cowpea has insignificant amount of fat (Nagai et al., 2009). This study recommends addition of vegetable oil above 5% in order to meet guidelines set by CAC as well as the EAR for infants 6 to 12 months of age. When peanut was used as source of fat, there was significant improvement in fat content when compared with control. Even though the fat content in peanut formulation (OFSP Rice-3) was slightly higher when compared with oil as a source of fat (OFSP Rice-1), there was no significant difference between the two. The significant increase in fat content observed for OFSP Rice-3 as compared to control was as a result of adding both soybeans and peanut. Addition of peanuts and soybean may be a better source of fat than soybean or vegetable oil alone as the main fat contributor.

Zinc content was low in all three formulations. However,

zinc content of OFSP Rice-2 and OFSP Rice-3 were significantly higher when compared with control (OFSP-Rice 1). None of the formulations met 50% EAR for zinc. Zinc is an important nutrient which helps with growth, development and cognitive function of children (Salgueiro et al., 2001). Zinc deficiency is of particular concern because it can lead to long-term deficits in growth, immune function, cognitive and motor development, behavior, and academic performance (Black, 1998; Brown, 2009; WHO, 2013). As such, complementary food should include the right amount of zinc to help with child growth and development. Some important sources of zinc that can be explored to improve complementary foods includes mushrooms, nuts and seed and dark chocolate.

Despite the low nutrient content of OFSP Rice-3, it recorded the highest acceptability score of 8.0 indicating “like very much”. Even though there was no limiting sensory attributes for both OFSP Rice-3 and control (OFSP Rice-1), addition of groundnut significantly improved the acceptability of the formulations (OFSP Rice-3) when compared with the control. The formulations with cowpea and oil (OFSP Rice -2) gave the least acceptability mean of 7.4. Even though this mean score indicates “like moderately”, the significant effect for OFSP Rice-2 was on the aroma and aftertaste. Again, aftertaste and acceptability were the only sensory attributes that showed significant differences between the three products. This low acceptability of OFSP Rice-2 could be due to the beany flavor and taste of the cowpea which panelists thought as unpleasant. Even though all three formulated products had a high acceptability scores, the most preferred formulation based on most sensory attributes was OFSP Rice-3.

Colour and appearance are important sensory attributes which affect the perception of other attributes, such as aroma, taste and flavor (Hutching, 1999). Colour, aroma and taste can therefore affect the way children perceive food. Parents often determine sensory attributes on behalf of their children, and may know whether food will be liked or disliked by their child. The orange colour of the OFSP gave the porridge a nice and pleasant colour which ranged from creamy yellowish to deep yellowish porridge.

Differences were observed, among the blended formulae in their behaviour during heating and cooling. Among the three formulations, OFSP Rice-3 had the highest peak viscosity, highest viscosity after holding, and final viscosity while the control (OFSP Rice- 1) exhibited much lower values of peak viscosity, pasting temperature and final viscosity. This might be due to higher value of rice in the formulation and higher rate of absorption and swelling of starch granules. The results suggest that the samples will behave differently during cooking and processing. Additionally, differences in the protein composition of the peanut and cowpea as well as the soybean in these formulations could account for the pasting behaviours (Batey and Curtin, 2000; Morris et al., 1997). The prepared formulae largely displayed rather low peak viscosities, in the range of 18.0 to 41.5 BU. Having a low viscosity value, these composite flours could be incorporated into certain food products in higher concentrations without becoming too viscous (Cornell, 2004). The low viscosity of these blend flours is also beneficial for infant nutrition since they cannot tolerate solid diet due to their digestive system and eating skills that are not yet fully developed (Walker et al., 1999; Muyonga et al., 2001). The lower breakdown of these composite flours might produce a less cohesive paste which would be beneficial during food processing (Moorthy, 2004).

Conclusions

This study has demonstrated that broken rice fraction can be used with orange flesh sweetpotato to provide a nutritionally adequate and culturally acceptable complementary food for Ghanaian children 6 to 12 months of age with >50% of EAR for vitamin A. The addition of OFSP makes it a useful ingredient that can help improve vitamin A content of complementary foods. The use of broken rice fractions, which otherwise would be used as animal feed or left as waste, may be a cheaper alternative in infant feeding. Women in rice-growing communities must be encouraged to use broken rice fractions with OFSP as a composite blend in complementary food. This may provide an alternative use for the rice cultivated in Ghana as well as boost the socio-economic status of farmers and individual entrepreneurs, especially women in the local rice value chain.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of storage methods on the nutritional qualities of African Catfish *Clarias gariepinus* (Burchell, 1822)

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The aim of this study was to evaluate the effect of storage methods on the nutritional qualities of African Catfish (*Clarias gariepinus*). Fresh fish samples were obtained from Johnybeth Farm, Ilesa, Nigeria. Fish specimens were divided into two equal parts. One part was stored fresh in a freezer at -6°C the other was smoked at 65 ± 5°C until equilibrium moisture content was attained, and was further divided into two parts. One part of the smoked fish was stored in continuous heated environment at 40 ± 4°C while the second part was packaged in polyethylene bags and stored at -6°C. Samples were analyzed over a period of six weeks. Nutrients (protein, lipid and carbohydrate), non-nutritional (ash, fibre) and trace element (Ca, Fe, Zn,) content and spoilage parameters (peroxide, pH, TVN, PV, TBA and FFA) were determined using standard methods. The crude protein ranged between 40.53 and 74.00%, lipid 5.46 and 21.71%, ash 2.59 and 8.57%, fibre 0.0 and 2.36%, carbohydrate 0.15 and 38.86%. Peroxide value ranged between 4.60 and 16.02 meq, O2/kg FFA 0.17 and 1.92%, TVN 4.10 and 23.97 mgN/100g, TBA 0.18 and 1.42 mg MDA/g and pH 6.52 and 7.80. Ca ranged between 0.03 and 1.62, Fe 0.28 and 2.27, Zn 0.30 and 2.59, mgkg ^{1} respectively. There was significant reduction in the nutrient, non-nutrients and trace elements while spoilage parameters increased during the six weeks storage period at (P<0.05). However smoked fish stored in heated environment has less reduction in quality.

Key words: Freezing, smoking, nutrients, quality indices, *Clarias gariepinus*

INTRODUCTION

Fish is a potential source of animal protein available in the tropics and has been widely accepted as a good source of protein and essential nutrients for the maintenance of a healthy body (Fawole et al., 2007). Fish compared to other human dietary items, are excellent sources of highly digestible essential nutrients considering

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considering their amino acid (Louka et al., 2004). In addition, fish provides a good source of vitamins, minerals (Bashir et al., 2012). Minerals are important for vital body functions such as acid, base and water balance. Fish is highly perishable commodity recording considerable losses in quality before consumption; their susceptibility to deterioration has been the main obstacle in preservation. Locally fish spoilage has been known to be influenced to a large extent by high ambient temperatures, and inadequate infrastructure for post-harvest processing and landing. According to Adesehinwa et al. (2005), captured fisheries which provides over 60% of total domestic production per annum, have been hampered largely due to post harvest losses estimated at 30-50% of total catches. In Africa postharvest loses, are around 5% of the total artisanal productions while for the West African Region at between 10 and 20% (Ward and Jefferies, 2000). Hence concerted effort in adoption and improvement of preservation such as refrigeration, freezing, salting, brining (wet salting), icing, smoking, glazing, drying, frying to reduce or avoid losses due to quality deterioration and spoilage (Tairu et al., 2017). The successful application of this techniques results in the conservation of desirable qualities in stabilized fish products.

Most processing methods serve not only to conserve the fish but also to alter their nutrient levels either positively or negatively. Reports exist in the agro industry that smoking is not only a conservation method but also a flavour, aroma and coloration improving method which are attributes sought by consumers. Smoking is the most popular method of fish preservation in many developing countries (Kumolu-Johnson et al., 2010; Emere and Dibal, 2013). Most consumers of fish in Nigeria consume smoked fish. It is relished for its taste and aroma as well as longer shelf life as a result of the combined effects of dehydration, antimicrobial and antioxidant activities of several smoke constituents mainly: formaldehyde, carboxylic acids and phenols (Serkan et al., 2010). Thus, making it is an important ingredient in the Nigerian traditional diet (Foline et al., 2011; Kiin-Kabiri et al., 2011; Akise et al., 2013).

While freezing, has been known to preserve the quality for a longer period and also minimum deterioration in product colour, flavor and texture. Most consumers in tropics, lack access to freezers, and subsequently stored smoked fish at room temperature in the kitchen. The aim of freezing of food items is to combine shelf life extension with maintenance of sensory and nutritional characteristics. Previous works by (Flick, 2010) on fish quality recommend that freezing of smoked fish in other to preserve quality. At low temperature (below 3°C), micro-organisms become inactive, enzymatic activity also slows down, thus biochemical activities decreases (Flick, 2010). Consequently, the fish remain free from spoilage for longer duration. The aim of this study was intended to determine patterns and rates of fish quality deterioration in (fresh/freeze = FFF), under recommended (smoke/ freeze= SFF) and under conditions more commonly encountered at homes (smoke/warm =SHF).

MATERIALS AND METHODS

Twenty-four pieces of catfish, *Clarias gariepinus*, each weighing 450 ± 5 g were purchased from Johnny-Beth Fish Farm at Ilesa. Osun State, Nigeria. The fish used for this study were cropped and sorted based on size the same day. Standard Unit (S.U.) Turkey Cold Room at Oba's market Akure was used for frozen storage. The cold room operates at -6°C and the sample was stored for six weeks. Portable smoking kiln used was obtained from Food Science and Technology Laboratory of the Federal University of Technology, Akure, Nigeria.

Sample preparation

The fish samples were killed immediately after capture, carefully degutted and washed with clean water to remove blood and slime according to the standard method described by Ogbonnaya and Ibrahim (2009). The samples were divided into two equal parts. 12 fresh fish were put in polyethylene nylon and stored in a freezer (FFF); 12 fish were smoked at 65± 5^{o}C for 24 h and was divided into two portions of 6 fish as a batch. One batch of the smoked fish was stored in continuous heated environment (SFH) at 40 ± 4°C, while the second part was packaged in polyethylene nylon and stored in thermo thermocool thermostatic freezer (SFF) preset at -6°C. Storage was carried out for six weeks, while samples were taken on weekly basis for analyses. Each sample was divided into three for replicates.

Proximate analyses

Moisture, crude protein, fat, ash, and crude fibre contents were determined according to the standard method of (AOAC, 2000).

Chemical indices analyses

The samples were grounded into powder with a Kenwood blender. The samples was determined for total volatile base (TVN), peroxide value (PV), thiobarbituric acid value (TBA), free fatty acids (FFA) according to (AOAC, 2005). While the pH was determined using a pH meter (Jenway 3015 model, Cole Pamers Co., USA).

Mineral analyses

The minerals such as calcium, zinc and iron were determined on aliquots of the solutions of ash, by using atomic absorption spectrophotometer (210 VGP Buck Scientific Inc., USA).

Statistical analysis

Statistical analysis was performed on the replicate data by one-way analysis of variance (ANOVA) laid in completely randomised design using SPSS 17.0 (SPSS Inc., USA). Separation of the mean was

Table 1. Proximate composition (%, dry basis) of fresh frozen fish.

Storage periods (Wks)	Protein (%)	Lipid (%)	Ash (%)	Fibre (%)
0	74.00^{a}±0.50	15.75^{a}±0.64	7.40^{a}±0.19	2.36^{a}±0.43
1	73.72^{a}±0.03	15.60^{a}±0.05	7.30^{a}±0.24	1.89^{a}±0.61
2	71.64^{b}±0.04	13.88^{b}±0.63	5.97^{b}±0.02	1.27^{b}±0.70
3	63.27^{c}±0.03	12.70^{c}±1.08	5.21^{c}±0.13	0.51^{c}±0.02
4	51.56^{d}±0.05	8.38^{d}±0.07	3.32^{d}±0.24	0.32^{c}±0.02
5	51.00^{d}±1.26	6.61^{e}±0.04	3.34^{d}±0.23	0.19^{c}±0.01
6	40.53^{e}±0.07	5.46^{f}±0.97	2.59^{e}±0.46	0.14^{c}±0.02

*SD = Standard Deviation ∗Means ± SD are values of triplicate determination. ∗Mean with different superscript in the same column for each parameter are significantly (p<0.05) different.

Table 2. Proximate composition (%, dry basis) of smoked fish stored in heated environment**.**

Storage periods (Wks)	Protein (%)	Lipid (%)	Ash (%)	Fibre (%)
0	70.91^{a}±0.71	21.71^{a}±0.21	6.34^{c}±0.05	0.89^{a}±0.75
1	66.42^{b}±0.23	21.73^{a}±0.28	6.35^{c}±0.13	0.36^{b}±0.01
2	63.50^{c}±0.14	21.75^{a}±0.16	6.40^{c}±0.13	0.14^{b}±0.01
3	61.79^{d}±0.17	21.70^{a}±0.42	7.01^{b}±0.53	0.03^{b}±0.02
4	55.86^{e}±0.79	21.65^{a}±0.01	7.23^{b}±0.16	0.01^{b}±0.01
5	52.63^{f}±0.04	20.60^{b}±0.10	8.21^{a}±0.03	0.01^{b}±0.00
6	51.77^{g}±0.08	20.57^{b}±0.05	8.57^{a}±0.01	0.01^{b}±0.01

*SD = Standard deviation ∗Means ± SD are values of triplicate determination. Mean with different superscript in the same column for each parameter are significantly (P<0.05) different.

determined by the Duncan New Multiple Range Test (DNMRT) at p<0.05 level of significance.

RESULTS

Proximate compositions of fish samples

Fresh frozen fish (FFF)

The result of the proximate composition of fresh frozen fish (FFF) is presented in Table 1. From results, at Wk 0 (zero time), the protein, lipid, ash and fibre contents were 74.00, 15.75, 7.40 and 2.36%, respectively. During storage, these contents insignificantly difference at Wk 1, but significantly decreased (P≤0.05) from Wk 2 to Wk 6 of storage. Notably, the fiber content was non-significant different from 3rd to 6th Wk.

Smoked fish stored in heated environment (SFH) at 40 ± 4°C

Freshly smoked fish (Wk 0) had the highest protein content 70.91% which decreased significantly (p<0.05) during storage from 66.42% (Wk 1) to 51.77% (Wk 6). The lipid content was 21.71% at Wk 0. However, the lipid content was not significantly different from Wk 1 to 4th Wk but differed significantly from 5 to 6 Wks. Ash content of 6.34% (Wk 0) was not significantly different from 1st and 2nd Wks but increased significantly to 8.57% in Wk 6. The fibre content significantly (p<0.05) decreased during storage period (0.89 to 0.1%) but 1st to 6th Wk were non-significantly different at (p<0.05) (Table 2).

Smoked fish stored in a freezer (SFF) at -6°C

The protein content smoked fish stored in freezer decreased during storage periods in the range of 70.91% (Wk 0) to 41.30% (Wk 6) (Table 3). The lipid content at Wk 0 was 21.71% was non-significantly different from 1st to 4th Wks but differed significantly in 5th and 6th Wks. However, lipid decreased during storage in the range of 21.71 (Wk 0) to 17.86% (Wk 6). Ash content decreased insignificantly during storage and ranged from 6.34% (Wk 0) to 5.45% (Wk 6). Fibre content decreased significantly during storage in the range of 0.89% (Wk 0) to 0.01 (Wk 6), however, 1st to 6th Wks were non significantly different from each other and ranged from 0.03 to 0.01%(Table 3).

Table 3. Proximate composition (%, dry basis) of smoked fish stored in a freezer (-6°C).

Storage periods (Wks)	Protein (%)	Lipid (%)	Ash (%)	Fibre (%)
0	70.91^{a}±0.71	21.71^{a}±0.21	6.34^{abc}±0.05	0.89^{a}±0.75
1	60.13^{b}±0.03	21.70^{a}±0.31	7.43^{a}±0.45	0.03^{b}±0.01
2	55.58^{c}±0.04	20.73^{a}±0.33	7.40^{a}±0.70	0.02^{b}±0.00
3	55.29^{c}±0.01	20.62^{a}±0.22	6.76^{ab}±1.04	0.02^{b}±0.01
4	52.42^{d}±1.22	20.45^{a}±1.04	5.95^{bc}±0.71	0.02^{b}±0.01
5	44.50^{e}±0.04	18.65^{b}±1.42	5.63^{c}±0.84	0.01^{b}±0.01
6	41.30^{f}±0.05	17.86^{b}±0.38	5.45^{c}±0.25	0.01^{b}±0.00

SD = Standard deviation; ∗Means ± SD are values of triplicate determination; Mean with different superscript in the same column for each parameter are significantly (P<0.05) different.

Table 4. Quality indices of FFF during storage periods.

Storage periods (Wks)	PV (meq O_2/kg)	FFA (%)	TVN (mg N/100 g)	TBA (mg MDA/g)	pH
0	4.60^{e}±0.20	0.17^{e}±0.03	4.10^{g}±0.36	0.18^{d}±0.04	7.80^{a}±0.10
1	4.87^{de}±0.15	0.25^{d}±0.02	6.43^{f}±0.40	0.23^{d}±0.03	7.60^{ab}±0.20
2	5.03^{d}±0.15	0.34^{c}±0.04	10.60^{e}±0.30	0.48^{c}±0.01	7.52^{b}±0.21
3	5.70^{c}±0.10	0.35^{c}±0.01	13.77^{d}±0.87	0.53^{c}±0.03	7.39^{b}±0.23
4	6.37^{b}±0.15	0.47^{b}±0.04	15.97^{c}±0.74	0.73^{b}±0.02	6.70^{c}±0.10
5	6.60^{b}±0.20	0.92^{a}±0.03	19.77^{b}±0.57	0.74^{b}±0.02	6.57^{c}±0.03
6	7.37^{a}±0.15	0.96^{a}±0.01	21.00^{a}±0.30	1.08^{a}±0.03	6.52^{c}±0.05

Values with the same letter in the same column are not significantly different (P≤0.05). PV = Peroxide value, FFA= free fatty acid, TVN = Total volatile nitrogen, TBA= Thiobarbituric acid.

Changes in quality indices of fresh frozen fish (FFF)

The peroxide value (PV) (meq O_2/kg) of fresh fish ranged between from 4.60 (Wk 0) to 7.37 (Wk 6), this increased steadily during storage periods. However, 1st Wk was non significantly different (p>0.05) from 2nd Wk. Also, the same trend occurred between 4 and 5th Wk. In general, PV significantly increased between zero time and 6th Wk (4.60 and 7.37 meq O_2/kg). In general, FFA significantly increased during the storage periods (1st - 6th Wks), with exception, non-significant difference between (2nd -3rd Wks) and (5-6th Wks). The free fatty acid (FFA) was 0.17% (Wk 0) increased significantly (p<0.05) during storage to 0.96% (Wk 6). Total volatile nitrogen (TVN) (mgN/100 g) of Wk 0 was 4.10. TVN increased significantly as storage progressed from 4.10 (zero time) to 21.00 (6th Wk). TBA values (mg MDA/g), generally, significantly increased (p<0.05) during storage periods, where were 0.18 (0 Wk) to 1.08 (6th Wk) with exception non-significant difference between (0-1st Wks), (2nd - 3rd Wks) and (4-5th Wks). pH values significantly decreased during storage in the range of 7.80 to 6.52. (Table 4

Quality indices changes of smoked fish stored in heated environment (SFH)

Table 5 showed that the PV (meq O_2/kg) of fresh fish (Wk 0), increased steadily during storage from 6.30 to 7.55 meq O_2/kg (6th Wk). There were non-significant difference in PV between (zero time -1st Wk) and (4th and 5th Wks). FFA (%) of Wk 0 increased during storage from 0.43 to 1.42 (6th Wk). FFA values were non-significant difference during the first three weeks of storage periods. TVN values (mg N/100 g) significantly (p<0.05) increased from 5.30 (Wk 0) to 9.80 (Wk 6). TBA values ranged from 0.25 (Wk 0) to 0.99 (Wk 6). pH decreased during storage in the range of 7.30 (zero time) to 6.45 (6th Wk).

Quality indices changes of smoked fish stored in a freezer (SFF) (-6°C)

The peroxide value (PV) of fresh fish (Wk 0), was not significantly different (p<0.05) from Wk 1 but differed significantly from Wk 6. PV (meq O_2/kg) significantly increased during storage period from 6.30 (Wk 0) to 16.02 meq O_2/kg (6th Wk) (Table 6). FFA values (%) increased significantly during storage period from 0.43 (Wk 0) to 1.92 (6th Wk). TVN (mg N/100g) increased significantly (p<0.05) as storage proceeded from 5.30 to 23.97 (6th Wk) (Table 6). TBA (mg MDA/g) significantly increased during storage from 0.25 mg/g (Wk 0) to 1.42 mg/g (6th Wk) (Table 6). pH decreased significantly during storage from 7.60 (Wk 0) to 6.52 (6th Wk). However, Wk 0

Table 5. Changes in quality indices of smoked fish stored in heated environment (40 ± 4°C).

Storage periods (wks)	PV (meq O_2/kg)	FFA (%)	TVN (mg N/100 g)	TBA (mg MDA/g)	pH
0	6.30^{c}±0.30	0.43^{e}±0.03	5.30^{e}±0.30	0.25^{f}±0.02	7.30^{a}±0.20
1	6.33^{c}±0.08	0.45^{e}±0.01	5.53^{e}±0.15	0.34^{e}±0.04	6.80^{b}±0.30
2	6.54^{c}±0.19	0.47^{e}±0.02	6.37^{d}±0.12	0.37^{de}±0.02	6.60^{bc}±0.20
3	6.80^{bc}±0.40	0.54^{d}±0.02	6.53^{d}±0.15	0.40^{d}±0.01	6.57^{bc}±0.04
4	7.17^{ab}±0.07	0.92^{c}±0.03	7.30^{c}±0.20	0.62^{c}±0.05	6.52^{bc}±0.03
5	7.26^{ab}±0.65	1.22^{b}±0.02	9.27^{b}±0.12	0.93^{b}±0.02	6.47^{c}±0.02
6	7.55^{a}±0.16	1.42^{a}±0.03	9.80^{a}±0.10	0.99^{a}±0.03	6.45^{c}±0.02

Values with the same letter in the column are not significantly different ($P \leq 0.05$). PV = Peroxide value, FFA= free fatty acid, TVN = Total volatile nitrogen, TBA= Thiobarbituric acid.

Table 6. Changes in quality indices of smoked fish stored in a freezer (-6°C).

Storage periods (Wks)	PV (meq O_2/kg)	FFA (%)	TVN (mg N/100 g)	TBA (mg MDA/g)	pH
0	6.30^{f}±0.30	0.43^{g}±0.03	5.30^{g}±0.30	0.25^{e}±0.02	7.60^{a}±0.20
1	6.93^{ef}±0.17	0.62^{f}±0.05	7.20^{f}±0.44	0.41^{d}±0.09	7.56^{ab}±0.01
2	7.36^{e}±0.54	0.85^{e}±0.06	9.80^{e}±0.40	0.65^{c}±0.02	7.52^{ab}±0.03
3	8.96^{d}±0.18	1.21^{d}±0.02	16.53^{d}±0.31	0.69^{c}±0.04	7.43^{b}±0.03
4	11.09^{c}±0.30	1.58^{c}±0.08	17.47^{c}±0.49	0.71^{c}±0.02	6.57^{c}±0.02
5	14.00^{b}±1.00	1.71^{b}±0.04	21.64^{b}±0.15	1.21^{b}±0.02	6.55^{c}±0.04
6	16.02^{a}±0.42	1.92^{a}±0.06	23.97^{a}±0.23	1.42^{a}±0.03	6.52^{c}±0.05

Values with the same letter in the column are not significantly different ($P \leq 0.05$). PV = Peroxide value, FFA= free fatty acid, TVN = Total volatile nitrogen, TBA= Thiobarbituric acid.

was significantly different ($p<0.05$) from 7.60 (Wk 0) to 6.52(6^{th} Wk) (Table 6).

Rate of change in the storage indices of the fish

The percentage of increase or decrease and the rates of increase (slopes of regression) in these storage indices plots of the increase in PV, FFA, TVN, TBA, pH, protein and lipids contents of fish stored as FFF, SFF and SFH for six weeks are presented in Table 7.

In general, there was increment in all the parameters. The percentage of increase were higher in the FFF which increased by 464.71% (0.14) in FFA, 412.20% (2.96) in TVN and 500% (0.14) in TBA. This was followed by smoked fish SFF with a percentage increase of 346.51% (0.27) in FFA, 352.26% (3.31) in TVN and 468.00% (0.19) in TBA and 16.56% (0.22) in pH, while SFH has the lowest increase of 230.23% (0.18) in FFA, 84.91% (0.78) in TVN and 296.00% (0.13) in TBA and 13.18% (0.12) in pH.

The PV increased fastest in the sample SFF than the other two treatments. This was also confirmed by the SFF which showed the highest % increase of 154.29% while FFF and SFH were 60.22% and 19.22% respectively. SFF also had the highest rate of increase in PV (1.69) while FFF and SFH had 0.47 and 0.23, respectively.

However, pH, protein and lipids contents decreased. The percentage of decrease in pH -16.41% was higher in FFF, followed by -14.21% in SFF and -11.64% in SFH. The rate of decrease in protein was faster in FFF kept in the freezer which decreased by 45.23% followed by smoked fish kept in the freezer (41.76%) while it was slowest in smoked fish kept in the heated environment (26.99%).

This observation was confirmed by the rates of decrease in pH which was highest in FFF (-16.41) followed by SFF (-14.21) while SFH was the slowest (-11.64), protein was highest in FFF (-45.23) followed by SFF (-41.76) while SFH was the slowest (-26.99). The lipid contents decreased by 65.33% in FFF, 17.73% in SFF and 5.25% in SFH.

The slopes of the plots also follow the same trend. The rate of reduction was faster in FFF (-1.94), followed by SFF (-0.64) and SFH (-0.21). This shows that there was a higher decrease in pH, lipid content of the fresh fish than the smoked fish.

Mineral content of fresh frozen fish (FFF)

The calcium content ($mgkg^{-1}$) of Wk 0 sample was significantly ($p<0.05$) higher than stored samples and

Table 7. Percent increase/decrease and rates of increase/decrease of oxidative parameters and protein/lipids of frozen fish (FFF), heated environment (SFH) and smoked fish in freezer (SFF).

Parameters	Regression Equation	% Increase/Decrease in 6 weeks	R^2
FFF			
PV	y =0.47x+4.39	60.22	0.97
FFA	y =0.14x+0.09	464.71	0.87
TVN	y =2.96x+ 4.23	412.20	0.99
TBA	y =0.14x+ 0.14	500.00	0.95
pH	y = -0.27x +7.99	-16.41	0.92
Protein	y = -5.93x + 78.60	-45.23	0.93
Fat	y = -1.94x + 17.02	-65.33	0.95
SFH			
PV	y =0.22x + 6.18	19.84	0.97
FFA	y =0.18x + 0.25	230.23	0.87
TVN	y =0.78x +4.81	84.91	0.92
TBA	y =0.13x +0.17	296.00	0.90
pH	y =-0.12x+7.04	-11.64	0.58
Protein	y = -3.31x +70.34	-26.99	0.98
Fat	y = -0.21x + 22.00	-5.25	0.95
SFF			
PV	y =1.68x + 5.06	154.29	0.94
FFA	y =0.27x + 0.40	346.51	0.99
TVN	y =3.31x+ 4.64	352.26	0.98
TBA	y =0.19x+0.21	468.00	0.92
pH	y = -0.25x+7.89	-14.21	0.86
Protein	y = -4.40x + 67.51	-41.76	0.93
Fat	y = -0.64x + 22.17	-17.73	0.69

decreased during storage in the range of 0.15 (Wk 0) to 0.03 (6^{th} Wk) (Table 8). There was no significant difference ($p>0.05$) within 1^{st} Wk (0.07) too 6^{th} Wk (0.03). Week 0 sample had the highest iron content. During storage, iron level ($mgkg^{-1}$) decreased significantly ranging from 0.97 (Wk 0) to 0.28 (6^{th} Wk). However, 5^{th} Wk was not significantly different ($p>0.05$) from 6^{th} Wk. A high level of zinc was 0.57 (zero time). During storage, the zinc level significantly decreased ($p<0.05$) from 0.57 (0 Wk) to 0.19 (6^{th} k). These result showed that frozen storage reduced the mineral content of fresh *C. gariepinus*.

Mineral content of smoked fish stored in heated environment (SFH)

Table 9 revealed that freshly smoked fish had calcium level ($mgkg^{-1}$) increased significantly ($p<0.05$) during storage where 0.40 (Wk 0) to 1.62 (6^{th} Wk). Iron content in increased significantly ($p<0.05$) from zero time Wk 0 (1.13) to Wk 6 (2.27). Also, zinc level ($mgkg^{-1}$) increased significantly ($p<0.05$) from 0.90 (0 Wk) to 2.14 (6^{th} Wk).

Mineral content of smoked fish stored in a freezer (SFF)

Calcium level ($mgkg^{-1}$) decreased significantly ($p<0.05$) from 0.40 (0 Wk) to 0.04 (6^{th} Wk). The same trend for iron level ($mgkg^{-1}$) from 1.13 (0 Wk) to 0.55 (6^{th} Wk). Zinc level ($mgkg^{-1}$) increased slightly from Wk 0 0.90 (0 Wk) to 2.59 (1^{st} Wk) (Table 10).

DISCUSSION

The crude protein content of the fresh catfish 74.00% was higher than smoked catfish 70.91%. However, this value was higher than the value of 53.10% recorded by Ogbonna and Ibrahim (2009), 68.17% by Agbabiaka et al. (2012) and 68.40% reported by Olayemi et al. (2011).

Table 8. Mineral content ($mgkg^{-1}$) of fresh frozen fish (FFF).

Storage periods (Wks)	Ca	Fe	Zn
0	0.15^{a}±0.15	0.97^{a}±0.35	0.57^{a}±0.03
1	0.07^{b}±0.02	0.67^{b}±0.02	0.52^{b}±0.03
2	0.06^{b}±0.02	0.55^{c}±0.03	0.50^{b}±0.02
3	0.06^{b}±0.40	0.49^{d}±0.04	0.30^{c}±0.03
4	0.05^{b}±0.05	0.40^{e}±0.02	0.24^{d}±0.01
5	0.05^{b}±0.05	0.30^{f}±0.01	0.22^{d}±0.04
6	0.03^{b}±0.03	0.28^{f}±0.01	0.19^{d}±0.04

Values with the same letter in the same column are not significantly different ($p<0.05$). Ca = calcium, Fe = Iron, Zn = zinc.

Table 9. Mineral content ($mgkg^{-1}$) of smoked fish stored in hot environment (SFH).

Storage periods (Wks)	Ca	Fe	Zn
0	0.40^{f}±0.05	1.13^{c}±0.03	0.90^{c}±0.02
1	0.44^{ef}±0.04	1.28^{c}±0.04	1.83^{b}±0.03
2	0.51^{de}±0.03	1.70^{b}±0.04	2.00^{ab}±0.70
3	0.57^{cd}±0.05	1.90^{b}±0.02	2.40^{a}±0.02
4	0.63^{c}±0.01	1.92^{ab}±0.03	2.47^{a}±0.02
5	0.94^{b}±0.04	2.10^{ab}±0.50	2.35^{a}±0.05
6	1.62^{a}±0.05	2.27^{a}±0.06	2.14^{ab}±0.02

Values with the same letter in the same column are not significantly different ($P\leq0.05$). Ca = calcium, Fe = Iron Zn = zinc.

Table 10. Mineral content ($mgkg^{-1}$) of smoked fish stored in a freezer.

Storage period (Wks)	Ca	Fe	Zn
0	0.40^{a}±0.05	1.13^{a}±0.03	0.90^{c}±0.02
1	0.40^{a}±0.04	1.11^{a}±0.01	2.59^{a}±0.60
2	0.35^{a}±0.05	1.09^{a}±0.02	2.55^{a}±0.03
3	0.25^{b}±0.05	1.04^{ab}±0.04	2.22^{a}±0.03
4	0.09^{c}±0.04	0.88^{b}±0.24	1.33^{b}±0.06
5	0.08^{c}±0.01	0.58^{c}±0.02	0.70^{c}±0.04
6	0.04^{c}±0.04	0.55^{c}±0.05	0.63^{c}±0.04

Values with the same letter in the same column are not significantly different ($P\leq0.05$). Ca = calcium, Fe = Iron, Zn = zinc.

Smoking and frozen storage reduced the percentage protein content of the samples. In support of present findings, Beklevik et al. (2005) in sea bass (*Dicentrarchus labrex*) and Siddique et al. (2011) in *Puntius sp.* reported significant decrease in protein content during frozen storage. They attributed this protein loss due to the leaching effect of amino acids and water soluble protein leaching out with melting ice. Arannilewa et al. (2005) noted that protein decreased with increasing duration of frozen storage with fresh samples not frozen having higher protein content. Disadvantages such as product dehydration, rancidity, drip loss and product bleaching have an overall effect on the quality of frozen food. According to Saeed and Howell (2002), proteins exposed to oxidizing environments are very susceptible to chemical modification, such as amino acid destruction, peptide scission and formation of protein-lipid complexes that results in decrease in protein content. Eyo (2001)

attributed this loss to gradual degradation of the initial crude protein to more volatile products such as Total Volatile Bases (TVB), Hydrogen sulphide and Ammonia. Saliu (2008) reported that frozen storage reduced the percentage protein content in *Malapterurus electricus*, *Synodontis clarias*, *Chrysichthys nigrodigitatus*, *C. gariepinus* and *sarotherodon melanotheron* species. Audrey et al. (2006) observed that smoking cause nutrient loss due to associated heat flow of gases and interaction of the smoke components with protein. During storage, reduction in protein was corroborated with increase in TVN. However, SFH was higher in protein than SFF and FFF at the end of six weeks storage period. The values of 15.7 and 21.7% were obtained for lipids in the fresh and smoked samples were similar to those earlier reported by Ogbonna and Ibrahim (2009), Olayemi et al*.* (2011) and Agbabiaka et al. (2012). FFF and SFF showed a greater decrease in lipid content during storage when compared with the SFH. This result was supported by Arannilewa et al*.* (2005) in Tilapia; Žoldoš, et al*.* (2011) in Alaska Pollack (*Theragra chalcogramma*); Siddique et al*.* (2011) in *Puntius sp*; Roopma et al. (2012) in *Labeo rohita* and Saliu (2008) who found a significant loss in total lipid content during frozen storage. These workers attributed this loss due to oxidation of lipid. Reduction in lipid content could be attributed to oxidation of poly-unsaturated fatty acids (PUFA) contained in the fish tissue to products such as peroxides, aldehydes, ketones and free fatty acids (Gueraud et al., 2010). There might be high risks of rancidity during prolonged storage due to the fatty nature of fish (Horner 1992). This was confirmed by higher peroxide value and free fatty acid profiles of the stored products. Daramola et al. (2007) also reported that reduction in lipid is associated with higher PV and FFA in five different species of smoked freshwater fish: Bony tongue, *Heterotis niloticus*, African carp, *Labeo coubie*, Snake fish, *Parachanna obscura*, Nile Tilapia, *Oreochromis niloticus* and African mud catfish, *C. gariepinus* during storage at ambient temperature for 8 weeks.

Ash values 7.4 and 6.34% recorded for fresh and smoked samples were also similar to those earlier reported by Ogbonna and Ibrahim (2009), Olayemi et al*.* (2011) and Agbabiaka et al. (2012), respectively. Salán et al. (2006) reported that the increase in ash content when fish are smoked is due to loss of moisture. The decrease in ash content during FFF and SFF during storage is in agreement with Beklevik et al*.* (2005) who working on sea bass fillets and Okoyo et al*.* (2009) on Nile perch reported a decrease in total ash content during frozen storage. But Arannilewa et al*.* (2005) observed that the ash content remained almost the same throughout the 60 days of frozen storage of tilapia. The decrease in ash content was attributed to the drip loss (Beklevik et al*.,* 2005).

Akinneye et al. (2010) did not detect fibre in the three fish species (*H. niloticus, Sardinella spp*. and *Bonga spp*.) that were subjected to oven and sun drying. Ogbonnaya (2009) recorded 1.91, 1.06 and 1.45% in fresh, kiln-dried and electric-dried respectively in *O. niloticus*. Fibre helps and speeds up the excretion of waste and potentially carcinogenic substances from the human body, preventing them from sitting in the intestine or bowel for too long, rid of wastes and potentially carcinogenic substances such as hormones and cholesterol, both of which can contribute to disease (Monroe et al., 2007; Gann et al., 2003).

Peroxide value is a primary indicator of oxidation of fat (rancidity) (Adeyemi et al*.,* 2013). Results presented here indicate that preservation method increased peroxide values meq O_2/kg to 7.37 in FFF, 7.55 in SFH and 16.02 in SFF. The peroxide values corresponding to incipient spoilage are usually in the order of 20-40 meq O_2/kg ml/kg. However, Connell (1995) reported that when peroxide value is above 10-20 meq O_2/kg, fish develop rancid taste and smell. Thus, it can be concluded that the values from this study are still within acceptable limit of spoilage, although SFF was fastest in deterioration.

Accumulation of secondary oxidation products was measured by determining the thiobarbituric acid value (TBA) (Pegg, 2004). The initial value of TBA 0.18 mg MDA/g (fresh fish Wk 0), suggesting that limited lipid oxidation occur during post-mortem handling. From this result, TBA slightly increased during smoking (0.25 mgMDA/g in smoked fish Wk 0) and also during subsequent storage periods in SFF and in SFH. The highest increase in SFF than in FFF and SFH are in agreement with Goulas and Kontominas (2005), who reported that the initial TBA value of 0.23 mg MDA/g in Chub mackerel (*Scomber japonicus*). The increase in TBA value during the smoking procedure may be attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids as a result of smoking. This result was also in agreement with results reported by Mohamed and Atef (2012) who observed increase in TBA value of Grass Carp (*Ctenopharyngodon idella*) fillets after smoking. The combined effects of smoking and frozen storage could be attributed to the high values of TBA in smoked fish stored in freezer. However, the results from this study contrast with Bugueno et al. (2003) where no changes in TBA value of smoke brined salmon under vacuum until 25 days of smoke brined; and that of Gómez-Estaca et al. (2007) on cold smoked dolphin fish.

Free Fatty Acid (FFA), a tertiary product of rancidity, increased during storage. FFA is a measure of hydrolytic rancidity- the extent of lipid hydrolysis by lipase action (Farzana et al., 2014). Hydrolysis of glycerol-fatty acid esters is one of the important changes that occur in fish muscle lipids during post-harvest with the release of free fatty acids (Chaijan et al., 2006). Generally, the formation

of FFA in fish oil during storage is related to the initial lipid content, the lipolytic activity and temperature. It was noted that FFA increased in (FFF, SFH and SFF). Eyo (1993) reported that in most fish oils, rancidity is noticeable when the FFA (calculated as oleic acid) is between 0.5-1.5%. Total volatile nitrogen TVN increased in the limit of acceptability of fish is reported to be 30 mg N/100 g by Connell (1995) while Kirk and Sawyer (1991), suggested that a value of 30-40 mg N/100 g as the upper limit. Beyond this level, white fish and prawns are regarded as unacceptable. However, result from this study shows that the three methods of storage still have their final TVN within acceptable limits, since they all have values less than 30 mg N/100 g.

pH decreased during storage in all the three samples. Eyo (1993) stated that pH is an indicator of the extent of microbial spoilage in fish and that some proteolytic microbes produce acid after decomposition of carbohydrate, thereby increasing the acid level of the medium. The pH value is a reliable indicator of the degree of freshness or spoilage. Decrease in the pH level is due to the fact that carbohydrate of the fish was fermented to acids. Daramola et al. (2007) observed that a decrease in the pH level of smoke-dried fish species: Bony tongue, *H. niloticus*, African carp, *L. coubie*, Snake fish, *P. obscura*, Nile Tilapia, *O. niloticus* and African mud catfish, *C. gariepinus* stored at ambient temperature after 8 weeks. This showed that fresh frozen fish has the tendency to spoil faster than smoked fish as indicated by its higher percentage increase of -16.41%.

Smoking was a better preservative technique for fish than freezing of fresh fish. However, when the two samples of smoked fish were compared, the rate of increase was faster for PV, FFA, TVN and TBA in the smoked fish kept in the freezer than the one kept in heated environment. This was contrary to recommendation that smoked fish should be kept under frozen condition (Flick, 2010). Granata (2012) recommended that smoked fish should be handled, packaged and stored much like fresh fish. It should be kept frozen or under refrigeration just above freezing temperatures. If storage temperatures rise above 3°C, there is a risk that *Clostridium botulinum* may grow and produce toxins in some types of smoked fish. Mold growth on smoked fish can be retarded if it is package in a porous material such as cloth or paper towelling. This prevents “sweating,” a process in which moisture moves from the fish to the inside of the bag, causing a wet spot where mould can grow. This can be a problem if warm, plastic wrapped fish is put in a refrigerator.

The initial calcium (Ca), iron (Fe) and zinc (Zn) values of the smoked fish were significantly ($P \leq 0.05$) higher than fresh fish. This result is in accordance with Beyza and Ozeren (2009) who observed significant increase in mineral contents of cooked African catfish using different cooking treatments (baking, grilling, microwaving and frying). The changes with respect to frozen period in all the minerals evaluated could be attributed to drip loss and dehydration that is associated with frozen storage (Arannilewa et al*.,* 2005). Calcium is good for growth and maintenance of bones, teeth and muscles (Turan et al., 2003). Normal extra cellular calcium concentrations are necessary for blood coagulation and for the integrity, intracellular cement substances (Okaka and Okaka, 2001). Iron is an important constituent of haemoglobin (Onwordi et al., 2009). The presence of zinc in the fishes could mean that the fishes can play valuable roles in the management of diabetes, which result from insulin malfunction (Okaka and Okaka, 2001). Iron and Zinc (micro elements) are important in trace amounts, but they tend to become harmful when their concentrations if the tissues exceed the metabolic demands (Ako and Salihu, 2004).

Conclusion

This study has shown that at any of the storage conditions- heated environment and frozen storage, there were significant differences in the quality of fresh and smoked *C. gariepinus*. There was reduction in the proximate, mineral while chemical quality changes increased during the six weeks storage period. However smoked fish stored in heated environment has less reduction in quality. There is need for more attention and necessity for microbiological study as well as safety.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

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Chemical compositions of traditional alcoholic beverages and consumers' characteristics, Ethiopia

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Commercially available home brewed alcoholic beverages of *Areki and Tej* from Addis Ababa and other regional cities of Ethiopia were analysed for alcohol concentration, methanol level and other additives. Surveys were also carried out on the rate of alcoholism and the socio-demographic characteristics of the consumers. The chemical properties of the sampled beverages showed that home distilled *Areki* and fermented *Tej* drinks can pose health threats due to their high alcoholic strength and undesirable additives. Methanol concentration was found significantly below the highest limit to causing harm to human health. Close to a third of the observed *Tej and Areki* users have exhibited symptoms of alcoholism. Factors related to gender and reasons for drinking were significantly associated with alcohol abuse. The introduction of community-based intervention to reduce the rate of alcoholism in Addis Ababa is strongly suggested. Commercial vending houses should be subjected to acceptable regulations in their mode of production and delivery mechanisms. Applicable strategies for effective management and supervision of traditional alcohol consumption and to reduce alcoholism and risks of health menace are recommended. Further studies on other health influencing substrates deserve supporting.

Key words: Homemade alcohols, *Tej* and *Areki*, community survey, alcoholism, chemical compositions, Ethiopia.

INTRODUCTION

Background

There are numerous traditional alcoholic beverages which are locally produced and consumed among native peoples of many countries around the world. Such types of drinks are very commonly produced in a variety of

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ways as home-made beverages in many African countries (WHO, 2004). Home-brewed beverages have several names mostly reflecting the areas where they are produced but most of them are produced in almost similar ways by distillation and fermentation of grain cereals, fruits and/or vegetables (Shale et al., 2014). Distillation is a more complex time consuming method requiring specialized equipments to produce potent alcoholic beverages. Some of the most culturally important traditional beverages in Africa are: `tella` in Ethiopia (Shale et al., 2014) ` thobwa` in Malawi (Matumba et al., 2011), `burukutu` in Nigeria (Sawadogo-Lingani et al., 2010) `tonto` in Uganda (Mwesigye et al., 1995) and `muratina` in Kenya (Aka et al., 2014). These drinks are very popular, perhaps the most widely consumed beverage types because of the nutritional, therapeutic, social and religious values attached to them (Solange, 2014). Indeed, studies also confirmed that fermented products in general can improve sensory qualities and nutritional values by enriching the product with essential proteins and vitamins (Steinkraus, 1986).

Like in other parts of the world, alcohol has been used in Africa for centuries and played an important role in the culture and local economy. Given the abundance of raw materials, varieties of fermented drinks are produced and consumed for various reasons including component of standard diet, a means to settle disputes, facilitating marriages, to hold festivities after a successful hunt or harvest, and for hygienic or medicinal reasons (Platt, 1955). Furthermore, home brewed beverage production and local trade have also provided livelihood for many urban and rural poor, particularly women, for whom it is often the primary means of supporting their families (Adelekan et al., 2008). Despite all these significant economic and cultural importance traditional alcohol drinks have in common in many African societies, there are growing concerns on hygienic handling of raw materials, production procedures and qualities of the final products and rise of alcoholism. Considerable evidences demonstrate that some of the popular traditional alcohol drinks produced in Africa are contaminated with bacteria (Holzapfel, 2002) and contain harmful impurities and adulterants (Sanni, 1993). For example, member of the bacteria types such as Staphylococcus, Escherichia, and Salmonella have been reported to be present in a number of traditional food products and beverage drinks in Africa (Lues et al., 2011). In extreme cases, death might result as well, as the case in Kenya in November 2000 where 140 people died and many went blind and hospitalized after consuming the traditional drink called Kumi Kumi. These drinks are very popular among Kenyan poor neighborhoods and contain harmful substances such as car battery solution, methanol and other dangerous additives (Mureithi, 2002). Similar alcohol poisoning cases have also been reported from Zimbabwe and Somalia (Riley et al., 1999).

The other concerning aspect in many African countries is, the rapidly changing drinking culture. In traditional African society drinking alcohol has been an occasional and communal activity, associated with particular communal festivals (Room, 2014; Room et al., 2002). People do not drink alone or for the sake of drinking only, but these days this values are changing due to expansions of urbanization together with income inequality, along with the tendency to drink excessively inexpensive illegal and/or homemade high alcoholic beverages to alleviate their stress and have good time (Room et al., 2002). According to the latest WHO (2014) global status report on alcohol consumption showed that about 30% of all alcohol consumed globally is unrecorded. This rate, however, is much higher in Eastern Mediterranean, South East Asia and Africa (56, 69 and 31%).

Ethiopia is a country rich in history, ethnicity, and cultural and crop genetic diversity. Traditional alcohol beverages are widely consumed among different ethnic groups of the country as a prominent part of the local traditions of major social events including public holidays, wedding, funerals and other forms of festivities. Some of the most widely consumed home-brewed beverages are Tella, Shamita, Tej, Borde, and Areki. Of these traditionally fermented beverages of Tej, Tella and Areki are the most preferred drinks for big festive occasions.

The WHO global status report on alcohol and health released in 2014 showed that in Ethiopia the volume of unrecorded alcohol consumption is estimated to be 3.5 L of pure alcohol per capita indicating the highest consumption rate compared to other African countries such as Nigeria (1 L),Uganda(1.5 L) and Angola (1.6 L) (WHO, 2014).

As impurities and adulterations are the case in many African countries (Mureithi, 2002; Riley et al., 1999) there are some complaints from regular customers in the study cities that some vending houses use certain plant roots and cement to increase the alcoholic potency of beverages (Yohannes et al., 2013). Such kind of complaint is much more pronounced in the drinks of Areki and Tej, essentially in major cities such as Addis Ababa. Correspondingly, since Areki and Tej drinks are inexpensive compared to industrially produced beer or wine drinks, there is a tendency among the public that those who drink Tej and Areki are considered as alcoholics.

Ethiopian traditional fermented produces are not sufficiently studied.There are quite few works on Ethiopian traditional fermented beverages including data on fusel oil and alcohol content of Tej (Bekele, 2001).The ethanol level,pH and sensory,evaluation of Tej,Areki and Tella were also reported by Yohannes et al. (2013).These studies have, however, methodological drawbacks while quantifying the level of ethanol in Tej and Areki. More

Table 1. Sample allocation.

Characteristics of districts	Total number of districts	Total number of households (as of 2011 census)	Sample size
Low income areas	222 (65%)	35,520	704/54,720*35,520 = 457
Mid-High income areas	120 (35 %)	19,200	704/54,720*19,200 = 247

importantly, they lack information on health relevant substrates such as methanol or other additives with respect to human health. Consequently, there is limited comprehensive and reliable data on the quality of Ethiopian traditional alcoholic beverages in the literatures.

The overall goal of this study is thus to describe the current traditional alcohol consumers' characters in *Kirkos* sub city of Addis Ababa through the analyses of alcohol strength, methanol level and other additives in Tej and Areki beverages from human health perspective. The specific objectives of the study are to:

1. Examine the concentration of ethanol, methanol and other additives of *Tej* and *Areki* drinks.
2. Characterize users of traditional alcohol drinks in *Kirkos* sub city of Addis Ababa, and
3. To determine the rate of alcoholism and the associated factors among observed consumers of *Tej* and *Areki.*

MATERIALS AND METHODS

Study design

The study is quantitative and cross sectional in design. The survey is designed to examine toxicological relevant substrates and characterize current consumers.

Study area and sampling methodology for community survey

The community survey was conducted in *Kirkos* sub city of Addis Ababa. The sub city was selected based on the availability of vending houses and composition of varying socio-economic classes. A stratified and simple random sampling design was employed. Districts were designated as Mid-High and Low income areas based on local knowledge and using Probability Proportional to Size (PPS) and random selection of households.

Sampling frame

The sampling frame of the study was obtained from Ethiopian Statistical Agency 2010/11 census and stratified as districts. Since there was no complete income distribution data in the country, the lead investigator decided to use the aggregate housing appearances of the districts as an alternative way to stratify households. For instance, in area where there are many houses built from mud, woods, plastic and old government built houses, , would be considered as low income areas.

Sample size and allocation

In computation of the sample size, it was estimated 20% of adults living in *Kirkos* sub city have had traditional alcohol beverages in recent time, with 10% margin of error, at 95% level of confidence, design effect of 2, non-response adjustment of 10% and an average household size of 6 is incorporated (Table 1). Based on the above assumptions, a total of 704 households were studied.

Inclusion/exclusion criteria

The exclusion criteria have been age and mental or physical illness. Upon entry to household any member of the household (aged above 18 and mentally aware) who had consumed traditional alcoholic beverages such as *Tej* and/or *Areki* at least once in the past 12 months were requested to fill up the survey form.

Data collection method and instruments

A face to face self-administered questionnaire was utilized. The household data obtained through two structured questionnaires, that is, (1) CAGE was employed to identify symptoms of alcoholism and (2) other questionnaires based on literature reviews such as socio-demographic characteristics, income status, motives and drinking behavior were used to identify relevant variables.

Methods of analysis

The Statistical Package for Social Science (SPSS) version 21 was applied for the entire analyses. Particularly, frequencies and percentages were used to summarize the socio-demographic characteristics and prevalence of alcoholism. Multiple logistic regression analysis was used to identify factors associated with alcoholism. As the dependent variable (alcoholism) is a categorical variable, application of such analysis is appropriate. A series of Chi-square test were also used to examine the crude association of predictor variables with the outcome variable.

Ethical approval

An approval for this study has been granted by Addis Ababa Regional Health Bureau prior to survey implementation.

Survey of sample alcoholic beverages

Samples and sampling sites

A total of four *Tej* and five *Areki* alcohol drinks were collected from

Table 2. Characteristics of *Areki* samples.

***Areki* samples**	**Color**	**Notation**	**Odor/taste**
Terra Areki1	Clear	A1	Alcoholic, earthy
Terra Areki2	Clear	A2	Alcoholic, earthy
Terra Areki3	Clear	A3	Alcoholic, earthy
Terra Areki4	Clear	A4	Alcoholic, earthy
Yekosso Areki5	Amber	A5	Sweet, strong, earthy

Table 3. Characteristics of *Tej* samples.

***Tej* samples**	**Color**	**Notation**	**Odor/taste**
Tej Sample1	Light yellow	T1	Fruity, strongly fermented
Tej Sample2	Yellow brownish	T2	Fermented, whey, acid
Tej Sample3	Dark yellow brownish	T3	Sweet, acid, whey
Tej Sample4	Green yellow brownish	T4	Sweet, pineapple, acid

Addis Ababa city, Sululuta town and regional cities of Gojam, and Debre Birhan. The samples of each drink were collected by sterilized plastic bottles, screw-caped and then the *Tej* drinks were separately kept in a refrigerator until analyses were conducted. The vending houses were selected randomly.

Areki is locally found often as colourless and clear distilled alcoholic beverage, which is made from fermented product of *Yereki-tinsis*. *Yereki-tinsis* is a mixture of pounded *Gesho* leaves (*Rhamnus prinoides*) and cereal flours of sorghum, wheat or maize.

Areki A1 to A4 have similar colour and odour. *Areki* A5 is a bit amber in colour and smells earthier than the others (Table 2).

Tej is home-brewed fermented honey wine (mead). It is prepared by mixing honey, water, and leaves of *Gesho* (*Rhamnus prinoides*).

Tej samples T1 to T4 have different shades of yellow color and all the samples have residue at the bottom of the bottle which could be from the raw material used for production (Table 3).

Used chemicals

Ethanol and methanol standard (purity higher 99.9%), acetonitrile (HPLC grad) hexane and ethylacetate (both purity above 99.8%) were purchased from Sigma Aldrich.

Methods

GC/FID measurements were undertaken with an Agilent 6890 series GC/MS with an DB-WAXETR thin-film capillary column (30 m* 0.25 µm). 1 µl was injected with a split of 30:1, T(injection) = 200°C. Helium as mobile phase had a flow of 1 ml/min. T(detector) = 250°C. Temperature gradient: 60°C hold for 0.5 min to 85°C with 4.5°C/min. GC/MS characterisation was undertaken with an Agilent 6890 N coupled with an Agilent MS 5973 inert. A HP5-MS column (30 m, 0.25 µm, 0.25 micron) was used as stationary phase. 1 µl was injected at a split of 10:1. Injection temperature was 200°C, Helium was used with 1 ml/min as mobile phase. Temperature gradient: 50°C hold for 1 min with 5°C/min to 200°C(hold for 1 min).

Experiments

Samples were centrifuged for 1 min by 12000 rpm to remove residues. The *Areki* samples were stored in PET bottles for some weeks in a dark room. The *Tej* samples were stored in PET bottles in the refrigerator protected by light for some weeks. pH measurements were performed with a single-rod measuring cell at standard conditions.

For GC/FID quantification the samples were mixed with acetonitrile as internal standard and measured directly. Standards with different concentrations for ethanol and methanol quantification were produced and acetonitrile was added to as internal standard.

For GC/MS identification à 350 µl of each sample was extracted with 350 µl hexane. And second extraction was made with 350 µl sample and 350 µl acetic acid ethyl ester. The mixture was shaken at room temperature for 10 min at 1500 rpm. The organic upper phase was transferred into a vial for measurement.

RESULTS AND DISCUSSION

Response rates

Initially, a representative sample size for the study was estimated 704. Of the 704 approached, 665 residents responded correctly, having an overall response rate of 94%.

Socio-demographic characteristics of current consumers

The study consisted of all permanent residents of *Kirkos* sub city, whose ages were 18 years and above, had consumed traditional alcoholic drinks at least once in the past 12 months. Nearly forty percent of the consumers' age ranges between 25 and 39 year and were not married. Majority of the respondents were male, living in less affluent area, having a regular income for their living. Close to half of the participants have already completed their primary school education (Table 4).

Table 4. Demographic and socioeconomic characteristics of observed consumers.

Characteristics	Number (%)
Age groups	
18- 24	63 (9.6)
25 - 39	258 (39.2)
40 - 54	166 (25.2)
>55	172 (26.1)
Gender	
Male	492 (74.3)
Female	170 (25.7)
Marital status	
Single	264 (40)
Married	241 (36.5)
Divorced	79 (11.9)
Widowed	76 (11.4)
Living area	
HighMid housing	241(36.3)
Low housing	422(63.7)
Educational BG	
No education	104 (15.8)
Primary school education	299 (45.4)
Secondary school education	125 (22)
University/college	111 (16.8)
Income status	
Has regular income	456 (71)
Has no regular income	186 (29)
Free time activities	
Has health benefits	486 (75.8)
Has no health benefits	155 (24.2)
Reasons for drinking Alc.	
Helps me interact with others	98 (14.8)
Low selling price	51 (7.7)
For health benefits	99 (15)
Cultural/religious ceremonies	128 (19.3)
Helps me cope with stress	118 (17.8)
Uses as food appetizer	47 (7.1)
For enjoyment	121 (18.3)
# **Alcohol dependence**	
Yes	187 (28.3%)
No	473 (71.7%)

Alcohol abuse prevalence rate

Assessing the rate of alcoholism among the current users in *Kirkos* sub city of Addis Ababa was attempted using CAGE questionnaire (Ewing, 1984), as a result, close to a third (28.3%) of the respondents have indicated symptoms of alcoholism. The result is comparable with previous findings on hazardous alcohol uses in regional cities of Butajira and Soddo (Kebede, 1999; Tefera, 2016) in Ethiopia. Compared to previous report on hazardous alcohol use from Addis Ababa, however, the rate was much higher (Kebede, 1999). It should be noted that different devices could have contributed to differing results: The study by Kebede (1999) in Addis Ababa utilized Composite International Diagnostic Interview (CIDI) to identify cases with alcohol dependence whereas in the current study CAGE was used for identification. It could also be due to a huge time gap between the two studies year of implementation. Since late 1990`s Ethiopia particular Addis Ababa has under gone through a serious of demographic, economic and socio-political changes. Above all there is an ever increasing income inequality, high unemployment and poverty alongside with high availability of cheap home brewed alcoholic drinks and locally grown stimulant Khat/Chat (Catha edulis) may have exacerbated the level of substance abuse. Similar literatures from various countries have indicated that both production and consumption of unrecorded beverages tend to flourish during economic crisis, high unemployment, corruption and lack of social or economic stability (Moskalewicz, 2000; Tomkins, 2007). In this study we also found out that all Areki sample drinks contained very high alcohol strength consequently contributing to high levels of alcoholism.

Model building for alcoholism

To help us identify factors associated with hazardous alcohol use, we developed a model specific to the population. This objective is fulfilled using a model building procedure described by Hosmer and Lemeshow (Bursac et al., 2008).

The screening for alcoholism was carried out using cross culturally validated CAGE questionnaire. The acronym stands for 4 yes/no questions consisting (1) have you ever felt that you need to Cut down on your drinking? (2) Have people Annoyed you by criticizing your drinking? (3) Have you ever felt bad or Guilty about your drinking? (4) Have you ever had a drink first thing in the morning to steady your nerves or to get rid of a hangover (Eye-opener)? The total score can range from 0 up to 4: Four being “yes” to all items. The recommended cut-off for CAGE is ≥2 to screen for alcohol abuse (Ewing, 1984). In addition, the questions have gone through several adjustments to ensure its cultural and content validity.

Bivariate analysis and variables for multivariate analysis

Initially, all socio-demographic and remaining behavioural variables were defined as potential predictors of alcoholism. The results of chi-square have showed that all the variables included have met the specified

Table 5. Bivariate analysis.

Characteristics	CAGE		Total (%)	*p*- value
	No	Yes		
Age groups				
18- 24	39 (61.9%)	24 (38.1%)	63 (100%)	
25 - 39	165 (64.2%)	92 (35.8%)	257 (100%)	
40 - 54	125 (76.2%	39 (23.8%)	164 (100%)	0.000*
>55	142 (83.5%)	28 (16.5%)	170 (100%)	
Gender				
Male	328 (67.2%)	160 (32.8%	488 (100%)	0.000*
Female	142 (84%)	27 (16%)	169 (100%)	
Marital status				
Single	168 (64.1%)	94 (35.9%)	262 (100%)	
Married	175 (73.2%)	64 (26.8%)	239 (100%)	0.000*
Divorced	58 (73.4%)	21(26.6%)	79 (100%)	
Widowed	69 (92%)	6 (8%)	75 (100%)	
Living area				
HighMid housing	187 (78.9%)	50 (21.1%)	237 (100%)	
Low housing	285 (67.7 %%)	136 (32.3%)	421 (100%)	0.002*
Educational BG				
No education	75 (72.1%)	29 (27.9%)	104 (100%)	
Primary education	199 (67.5%	96 (32.5%)	295 (100%)	
Secondary education	110 (76.4%)	34 (23.6%)	144 (100%)	0.11*
University/college	86 (77.5%)	25 (22.5%)	111(100%)	
Income status				
Has regular income	348 (77%)	104 (23 %)	452 (100%)	
Has no regular income	107 (57.8%)	78 (42.2%)	185 (100%)	0.000*
Free time activities				
Has health benefits	370 (76.8%)	112 (23.2%)	482 (100%)	
Has no health benefits	89 (57.8%)	65 (42.2%)	154 (100%)	0.000*
Reasons for alcohol use				
Helps me interact	64 (66.7%)	32 (33.3%)	96 (100%)	
Low selling price	27 (54%)	23 (46%)	50 (100%)	
For healthy benefits	89 (90.8%)	9 (9.2%)	98 (100%)	
Cultural/religious Re.	111 (86.7%)	17 (13.3%)	128 (100%)	0.000*
Helps me cope with stress	63 (53.4%)	55 (46.6%)	118 (100%)	
Uses as food appetizer	37 (78.7%)	10 (21.3%)	47 (100%)	
For enjoyment	79 (65.8%)	41 (34.2%)	121 (100%)	

statistical criteria, *p*-value <0.25 (Table 5).

Factors associated with alcoholism

One of the objectives of this study was to identify factors associated with hazardous alcohol use. Accordingly, the result of the study identified four factors that were significantly associated with alcoholism. Gender particularly males were 2.4 times more likely than females to abuse alcoholic drinks (OR = 2.4, 95% CI: 1.296 - 3.618, p < 0.005). This finding is in consistent with previous literatures which show the association between being male and alcohol abuse (Alem, 1999; Razvodovsky, 2013; Tefera, 2016). Besides, in present survey majority of the respondents were males in their early adulthoods. Consequently, there is a higher vulnerability to chew locally found stimulant such as Khat/Chat (Catha edulis); alongside to consume a potent alcoholic drinks such as Areki in order to relieve from temporary depressive feelings created by chewing the Khat. According to recent reports some 80-90 and 10-60% East African males and females, respectively, chew Khat on daily basis (Odenwald et al., 2005).

Table 6. Main effects.

Variable	Odds ratio	95% confidence interval	*p*-values
Sex	2.165	1.296 - 3.618	0.003
Reasons for alcohol use	3.741	2.214 - 6.322	0.000
Income status	0.625	.407 - .960	0.032
Free time activities	0.615	3.98 - .951	0.029

Table 7. pH measuring.

Sample	pH	Sample	pH
A1	4.00	**T1**	3.60
A2	3.70	**T2**	3.40
A3	3.90	**T3**	3.50
A4	4.10	**T4**	3.40
A5	5.30		

Individuals' motives for consuming traditional alcoholic drinks were the second major risk factor associated with alcohol misuse. Those respondents who responded that they prefer drinking homemade alcoholic drinks because of their relative cheap prices were 3.7 times more likely than those who have not given similar reasons to suffer from alcohol use related problems such as alcoholism (OR = 3.741, 95% CI: 2.214 - 6.322, p < 0.005). Given this result, it is possible to infer that cheap homemade alcoholic drinks have contributed to the rise of alcoholism in the community. Literatures in similar topic have also indicated that availability of unrecorded alcohol in many countries have been linked to people of lower socio-economic strata and alcohol dependence (Adelekan et al., 2008; Gamburd, 2008).

Factors such as income status and free time activities are identified as protective factors against alcoholism. The percentages of individuals who reported that they have had some kind of regular monthly income source for their livelihood were less likely to abuse alcohol drinks, in comparison with those who do not have reliable income source (OR = 0.625, 95% CI: .407 - .960, P< 0.005). This finding could be related to the fact of being unemployed and employed; being unemployed is often associated with greater alcohol consumption and drinking surrogate alcohol drinks (Razvodovsky, 2013; Tomkins, 2007). In other words, individuals without fixed income source are related feelings of boredom and lack of economical means to satisfy one's basic needs may cause them to have a grief and sorrow feelings, leading to increased alcohol use to help them cope with the unpleasant situations. In contrast, employed people are those likely with some kind of responsibilities as such, they may drink less often because of tight time schedule or to avoid potential job loss (Peirce et al., 1994). In connection with, majority of the respondents surveyed in this study were employed and choose to pass their spare time in healthy beneficial ways such as reading books, doing physical exercise, spending time with family and visiting cultural and historical sites etc. Similarly, individuals who reported spending their free time by engaging in activities which could enhance either mental or physical health were 0.615 time or 61.5% less likely to become an alcoholic person than those who have not indicated health benefiting activities as their hobby (OR = 0.615, 95% CI: 3.98 - .951, P<0.005). This finding shows a potential intervention strategy against the widespread substance use problem in the city in general (Table 6).

Chemical analysis of the sampled drinks

The aim was to quantify ethanol and methanol of two different homebrewed alcoholic beverages produced in Ethiopia via gas chromatography flame ionization detection (GC-FID). *Tej* is a fermented honey wine with an expected ethanol concentration of around 8% (v/v). *Areki* is distilled liquor with an expected concentration of circa 40%. In addition, the pH is measured and additional volatile components are determined via gas chromatography mass spectrometry (GC/MS).

pH

The pH is more acidic than expected for the *Areki* samples. A1-A4 are in the same pH range, the dark A5 alcohol is the less acidic of all the samples.

T1 to T4 are also in the similar pH range and more acidic than the strong *Areki* liquor. It was supposed that T1 was over fermented, but the pH did not indicate it, maybe the high carbon dioxide content is typical for some of these products (Table 7).

GC/MS identification

Table 8 shows the main volatile components of the different samples. The MS spectrum was compared with the National Institutes of Standards and Technology (NIST) databank. Only when the measured spectrum had a match over 90% with the databank, the found molecule will be listed in Table 8. The results of the extraction with hexane and acetic acid ethyl ester were combined to get the whole volatile molecules composition.

In every sample phenylethanol was found. It is a flavour component which occurs in Rosaceae, Asparagaceae and Caryophyllaceae. In this case the yeast can be the source of this molecule. It decomposes through light and

Table 8. Characterization of aroma components in alcoholic beverages (Range, 2009).

Sample	Molecule	CAS	Smell[1]
A1	2-Phenylethanol	60-12-8	Rose
	Hexanoic acid ethyl ester	123-66-0	Fruity
	Octanoic acid ethyl ester	106-32-1	Fruity, sweet, pineapple
	Decanoic acid ethyl ester	110-38-3	Sweet waxy apple
	Palmitic acid ethyl ester	628-97-7	Mild waxy fruity creamy
	Stearic acid ethyl ester	111-61-5	Mild waxy
	Isoamylacetate	123-92-2	Sweet, banana
	Dodecanoic acid ethyl ester	106-33-2	-
A2	Isoamylacetate	123-92-2	Sweet, banana
	Hexanoic acid ethyl ester	123-66-0	Fruity , produced at fermentation
	Phenylethanol	60-12-8	Rose , Decomposition under air/light
	Octanoic acid ethyl ester	106-32-1	Fruity, sweet
	Decanoic acid ethyl ester	110-38-3	Sweet waxy apple
	Dodecanoic acid ethyl ester	106-33-2	-
	Palmitic acid ethyl ester	628-97-7	Mild waxy fruity creamy
	Stearic acid ethyl ester	111-61-5	Mild waxy
A3	Phenylethanol	60-12-8	Rose , Decomposition under air/light
	Octanoic acid ethyl ester	106-32-1	Fruity, sweet
	Dodecanoic acid ethyl ester	106-33-2	-
	Palmitic acid ethyl ester	628-97-7	Mild waxy fruity creamy
	Decanioc acid ethyl ester	110-38-3	Sweet waxy apple
	Isoamylacetate	123-92-2	Sweet, banana
	Butanoic acid ethyl ester	105-54-4	Fruity juicy
A4	Phenylethanol	60-12-8	Rose, decomposition under air/light
	Butanoic acid ethyl ester	105-54-4	Fruity juicy
	Isoamylacetate	123-92-2	Sweet, banana
	Hexanoic ethyl ester	123-66-0	Fruity, produced at fermentation
	Decanoic acid ethyl ester	110-38-3	Sweet waxy apple
	Octanoic acid ethyl ester	106-32-1	Fruity, sweet
	Palmitic acid ethyl ester	628-97-7	Mild waxy fruity creamy
	Dodecanoic acid ethyl ester	106-33-2	-
A5	α-Pinen	80-56-8	Fresh camphor pine
	Phenylethanol	60-12-8	Rose, Decomposition under air/light
	Octanoic acid ethyl ester	106-32-1	Fruity, sweet
	α-Guaiene	3691-12	Sweet woody peppery
	Aromadendrene	14682-34-9	-
	Caffeine	58-08-2	-
	Palmitic acid ethyl ester	628-97-7	Mild waxy fruity creamy
	d-Limonene	5989-54-8	Pine herbal terpene
	Decanoic acid ethyl ester	110-38-3	Sweet waxy apple
	Linolic acid butyl ester	2634-45-9	-
T1	Butandiole		
	Phenylethanol	60-12-8	Rose, decomposition under air/light
	Butandioic acid monoethyl ester		
	Caffeine	58-08-2	-
	Palmitic acid ethyl ester	628-97-7	Mild waxy fruity creamy

Table 8. Contd.

T2	Butandiole	584-03-2	-
	Phenylethanol	60-12-8	Rose, decomposition under air/light
T3	Butandiole	584-03-2	-
	Phenylethanol	60-12-8	Rose, decomposition under air/light
	(4-Hydroxy)Phenyl ethanol	501-94-0	Mild sweet floral taste
	Palmitic acid ethyl ester	628-97-7	Mild waxy fruity creamy
	Butandioic acid ethyl ester	105-54-4	Fruity juicy
T4	Butandiole	584-03-2	-
	Phenylethanol	60-12-8	Rose, decomposition under air/light
	(4Hydroxy) Phenyl ethanol	501-94-0	Mild sweet floral taste
	Palmitic acid ethyl ester	628-97-7	Mild waxy fruity creamy

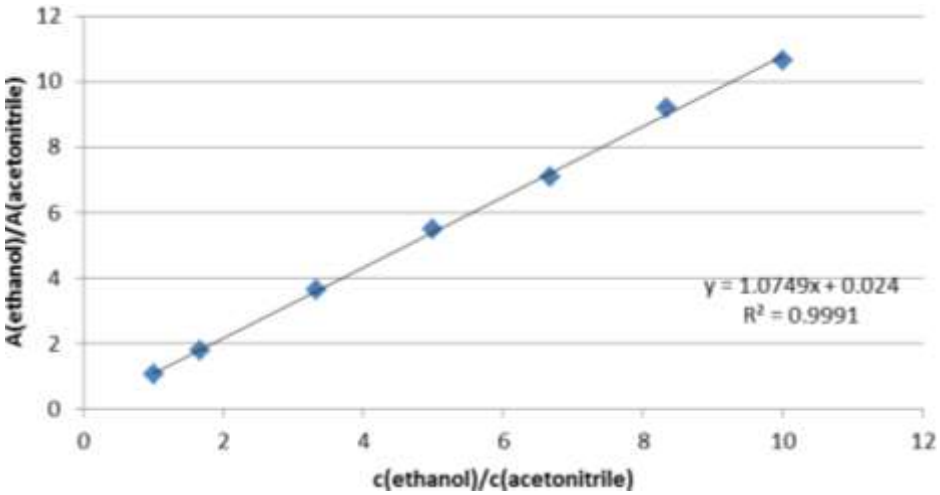

Figure 1. GCFID calibration of ethanol quantification with acetonitrile as internal standard; A = peak area.

oxygen so that the taste of the product can be influenced by storing conditions. In *Areki* liquor (A1-A4) many long-chain esters are found which can be produced at fermentation. They all taste fruity and give the product a fuller bouquet. Even though the samples are the same sort of product, they have a different odour composition which is caused by different raw-products and different producers. *Areki* A5 which has a dark colour has as some terpenes in it, mainly limonene and α-pinene, found in nearly every essential oil. The *Tej* alcohols also have some esters in them which smell fruity which also are produced during fermentation of the organic raw-material. Surprisingly, in A5 und T1 caffeine was found as principal component. The experimenters were not able to elicit why it has been used. Through further investigations it could also be possible to find even more flavour components.

Ethanol quantification

As homemade alcoholic beverages are produced at home without standardized procedures, thus the alcohol concentrations differ among samples significantly. Although there are some easy methods to measure the ethanol concentration via density as reported by Yohannes et al. (2013) which was not accurate. The experimenter chose GCFID to determine via internal standard the ethanol concentration in % (v/v). Standards between 3 and 30% (v/v) were prepared. The *Areki* samples A1 to A5 were diluted 1:1 with water due to the high ethanol concentration. *Tej* T1 to T4 were centrifuged and measured directly (Figure 1)(Table 9).

As expected *Areki* A1 to A4 have high ethanol content but it varies from producer to producer. *Areki* A5 has the highest concentration of 51.1% (v/v).*Tej* T1 to T4 have

Table 9. Ethanol concentration in different alcoholic beverages used calibration results of Figure 1.

Sample	c(ethanol)/ % (v/v)	Practical limit of error (f= 5, 99.5%)
A_1	44.9	±0.5
A_2	48.9	±0.5
A_3	48.1	±0.5
A_4	51.1	±0.5
A_5	44.8	±0.5
T_1	12.4	±0.5
T_2	12.3	±0.5
T_3	12.1	±0.5
T_4	13.0	±0.5

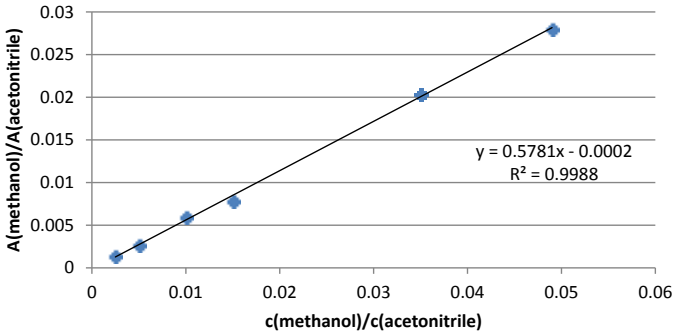

Figure 2. GCFID calibration of methanol standards with acetonitrile as internal standard.

Table 10. Results of methanol quantification with results of calibration in Figure 2.

Sample	c(methanol) /% (v/v)	Practical limit of error (f= 5, 99.5%)
A_1	0.187	±0.004
A_2	0.150	±0.004
A_3	0.167	±0.004
A_4	0.062	±0.004
A_5	0.021	±0.004
T_1	0.020	±0.004
T_2	0.008	±0.004
T_3	0.010	±0.004
T_4	0.006	±0.004

lower ethanol concentration by reasons of production. They vary not so much as the *Areki* liquors. The alcoholic strength of *Areki* samples showed much more variations than that of the *Tej* drinks. The greater variation in alcoholic strength in *Areki* samples might be due to the specific ingredients and methods of preparation of the source locality. Previously, similar research also observed significant variations in pH and alcohol contents among the samples considered (Yohannes et al., 2013). In addition, in this study, *Areki* drinks contained an average alcoholic strength of 47.56% which is higher than previously reported between 33.95 and 39.9% v/v (Yohannes et al., 2013) and even much higher than the maximum allowable limits for similar spirit drinks in Europe (Regulation, 1989). High alcoholic drinks such as *Areki* have a great potential to cause detrimental health effects through the huge increment of alcoholism, as it was identified in this study third of current consumer showed symptoms of alcoholism. On other hand, the *Tej* drinks had an alcoholic strength between 12.1 and 13% v/v which corresponds to the previous reports (Bekele, 2001; Yohannes et al., 2013) however, in *Tej* sample drinks caffeine was also identified which raises the possibility for the presence of other undesirable additives if further investigation is carried out.

Methanol quantification

Sometimes it is possible that methanol and other higher alcohols could be present in the final product of homemade alcoholic drinks. Methanol can be highly concentrated in the first fractions of distillation. It is normal to throw away the first fractions to avoid severe health risk due to methanol intoxication. Higher alcohols like glycols and long chained alcohols can be present in distillation fractions after the ethanol one. When high concentrated alcoholic beverages are produced and more fractions are used as usual, the final product can be contaminated with potential toxics.

In the samples methanol concentrations were determined with the same method as for ethanol. Standards staggered with acetonitrile are made with a concentration range between 0.1 and 0.005% (v/v) (Figure 2)(Table 10).

Methanol was found in all samples. The distilled beverage samples have relatively higher concentrations than that of *Tej* beverages. Methanol concentration for *Areki* beverages ranged between 0.021 and 0.187 % (v/v) whereas in *Tej* drinks concentration of 0.006 and 0.020% (v/v) were identified. The detected methanol both in *Tej* and *Areki* samples drinks were considerably below the maximum limit suggested by European Union for methanol concentration (Regulation, 1989). Nevertheless, the *Areki* sample beverages on average contain higher methanol concentration which speaks for the possibility that producers might have used the first fractions at the distillations. The GCFID and MS measurements have shown the possible presence of other alcohols which can also lead to health risks in high

concentrations. With further investigation these possible dangerous alcohols can be identified and quantified.

Although many more contaminates remained to be investigated, this finding at least confirmed slightly that Ethiopian homebrewed beverages of *Tej* and *Areki* have no serious threat to human health .

Although our results did not demonstrate a serious health threats to the consumers, we strongly recommend that the Ethiopian public health authorities introduce control on vending houses by examining regularly the qualities of the drinks and hygiene practices to ensuring safe consumption of the beverages. Such approach would at least help to reduce public health problems related to homemade alcoholic beverages.

Conclusion

The study has attempted to provide a comprehensive insight into the qualities of Ethiopian home-brewed drinks along with the characteristics of consumers from human health perspective. The qualities of homebrewed *Tej* and *Areki* samples were relatively safe except for high ethanol contents in *Areki* drinks, and an additive of caffeine that was also detected in *Tej* and *Areki* beverages, call for further investigations. To protect the public from alcohol related contaminants concerned government organs must put in place policies to ensure qualities and safety of traditional alcoholic drinks at least in selected vending houses in major cities of the country.

Overall, *Tej* and *Areki* drinks are mainly consumed by low socioeconomic class of the society. The prevalence rate of alcoholism among the studied consumers calls for some kind of interventions. Being male and using homebrewed alcoholic drinks because of its relative cheap price were predictor factors of alcoholism while having a regular monthly income and passing spare times with health beneficial activities were protective factors against alcohol abuse.

There are several methodological limitations to this study. First of all, the prevalence of alcoholism in *Kirkos* sub city shows just a portion of the situation and cannot be generalized to the entire Addis Ababa city and the country Ethiopia as a whole. Although the findings reflect the experiences of the specific part of the city of Addis, future inclusive research results could be obtained by working on larger sample sizes across the entire capital city shading light into alcohol consumption and its impacts on the health of city residents. Additional data could be collected and analysed across the nation toportray trends of alcoholism in the different regions ofEthiopia. Similarly, the chemical analysis of the sample drinks were performed from very small samples that may lack the representativeness of home brewed alcoholic drinks qualities in Addis Ababa and in Ethiopia as a whole.

We believe that our study has provided the basis for future research on the prevalence of alcoholism by addressing the basic concerns regarding the qualities of home brewed alcoholic beverages in Ethiopia. As there is inadequate information in the country on home brewed alcoholic drinks in relation to health outcomes, our findings would serve a basis for further investigation in the near future.

It is highly recommend that future research should focus on other health relevant quality parameters such as level of lead, acetaldehyde and bisphenol on representative sample from cross-sections of rural and urban regions of Ethiopia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of *Mucuna* species (*Mucuna pruriens var. pruriens*) flour and protein concentrate on quality of beef sausages

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This study aimed at substituting animal proteins by *Mucuna* flour and protein concentrate in beef sausage. *Mucuna pruriens* var *pruriens* grains were first transformed into flour (500 µm) from which the protein concentrate was obtained. The extraction process was done in an aqueous medium at pH 4.5. Following the characterization of the flour and protein concentrate, 5 sausages samples (3 units per sample) with varying rates of incorporation of flour and protein concentrates (25, 50, 75 and 100%) as well as a reference made with 100% beef were produced. Quality of emulsion sausages was evaluated in terms of physicochemical and functional characteristics. The results obtained showed that protein content of *Mucuna* flour and protein concentrate was significantly different ($p < 0.05$), with values of 29.92 ± 0.51 and 59.74 ± 0.32%, respectively. For the functional properties, water retention capacity (333.32 g/100 g/100 g), emulsifying capacity (60.44%), jellification capacity (12 g/100 g) and foaming capacity (52.38%) were higher in the flour sample; whereas the oil retention capacity (290.33 g/100 g DMC) was higher in the protein concentrate. Incorporation rate had a significant influence ($p<0.05$) on the physicochemical and functional properties of the sausage samples produced, since the technological yield was observed to vary from 73.10±0.71 (S_{25}) to 99.49±0.05% (S_{100}), hardness from 3.43 ± 0.35 (S_{100}) to 3.94 ± 0.05 N (S_{25}) and the water retention capacity varied from 33.34±0.79 (S_{25}) to 51.93±0.045% (S_{100}). These parameters were observed to increase with an increase in the rate of substitution.

Key words: *Mucuna* flour, *Mucuna* protein concentrate, beef sausage, functional characterizations.

INTRODUCTION

Meat and meat products are nutritionally rich, providing a wide range of nutrients, such as proteins, fats, minerals and vitamins (Cosgrove et al., 2005). They have long been considered as a highly desirable and nutritious food, and have become a mass consumer product throughout the world with the highest consumption rates being recorded in industrialized western countries.A significant percentage of the recommended dietary

requirements for proteins, vitamins-B, magnesium, iron and zinc are contributed by red meat and poultry (Pearson and Brooks, 1978). With the growing need for products with less fat or calorie content, it becomes necessary to develop meat products that are pertinent to consumer demand. Hence, basic formulations tend to evolve to account not only food safety concerns but also for changing economic conditions, raw material availability, consumer trends, and adaptation from one region to another (James and Lamkey, 1998). Several studies have demonstrated possible use of food hydrocolloids such as carrageenan, cellulose gum, konjac flour, guar gum and xanthan gum as fat replacements or the use of poultry meat as replacement for red meat in reduced-fat meat products (Troutt et al., 1992; Chin et al., 1998; Andrès et al., 2006; Bhattacharyya et al., 2007). Konjac flour, a glucomannan polysaccharide gum, has been used as a fat replacer in low-fat prerigor fresh pork sausages (Osburn and Keeton, 1994), low-fat bolognas (Chin et al., 1998), reduced-fat pork sausages (Akesowan, 2002a) and Thai traditional minced and preserved pork products (Akesowan, 2002b). In the same trend, soy proteins, one of non-meat proteins, are widely used as meat binders because of their several functionalities such as water-holding, binding and emulsifying properties (Arrese et al., 1991). Ahn et al. (1999) showed that addition of soy proteins resulted in better binding and texturization of sausages. Upon incorporation into comminuted meat, soy proteins improve physical and chemical properties of processed meat products such as frankfurters and ground meat patties (Alvarez et al., 1990). However, beany flavour of soy proteins also limits their expanded applications in foods (Ho et al., 1997). But concerns about high-cholesterol, allergens, animal welfare, the food industries impact on the environment, and high food costs have, however, resulted in an increased interest in using legume protein as meat replacers in foods (Hughes et al., 2011).

Recent studies on *Mucuna pruriens var. pruriens*, another legume promoted by smallholder farmers in Africa, showed rich protein content (23 to 35%) (Mugendi et al., 2010) but remain a minor food crop due to the presence of antinutrients (Ngatchic et al., 2013). The effects of these anti-nutritional factors such as antitrypsin factors, tannins, anticoagulants, phytates and 3,4-dihydroxy-L-phenylalanine (L-Dopa) (Ravindran and Ravindran, 1988; Rosenthal et al., 1989), on the body are known as the causes of poor protein digestibility, reduce food intake, nutrients availability and can provoke deleterious effects on many organs (Esenwah and Ikenebomey, 2008). Some vegetables proteins, either soy or pea, were used as fillers or extenders to enhance the texture, stability of emulsion, replace the fat and to lower the cost (Edanane and Bernard, 2014). Since Mwasaru et al. (1999), Rangel et al. (2004) and Ngatchic et al. (2013) showed that techniques employed to obtain protein concentrates or isolates are effective in the elimination of the antinutrients in *Mucuna* seed, flour and protein concentrate can be incorporated in food preparations.

Therefore, the purpose of this study was to determine the effect of *Mucuna* flour and protein concentrate on physicochemical and textural properties of beef sausages.

MATERIALS AND METHODS

Beef semi-membranous muscle (top round), beef bump fats and casings (sheep gut of about 20 mm diameter) were obtained from local processors (Ngaoundere, Cameroon). Beef muscles were trimmed of all visible extra-muscular fat and connective tissue before storage at 4°C for 72 h. Sheep guts were separated immediately after slaughtering, emptied, rinsed and stored in brine at 4°C until sausages were produced. Seed of *M. pruriens* were purchased from local markets in Ngaoundere (Cameroon) and manually separated from infested seeds impurities.

Preparation of *Mucuna* bean flours

The flours were produced from seeds legumes according to the method of Kaptso (2008). The seeds were soaked at ambient temperature (25°C) for overnight in tap water with bean to water ratio of 1 to 10 (w/v). After soaking, seeds were dried for 24 h at 50°C and dehulled manually. The dehulled seeds were ground to flour using a hammer mill and sieved with the 500 µm mesh sieve and stored in polyethylene bags at 4°C until analysis.

Preparation of protein concentrate

The *Mucuna* protein concentrate was prepared according to the process described by Wolf (1970) with minor modifications. Defatted *Mucuna* flour were dispersed in de-ionized water (1:10, w/v) at room temperature and the pH of the dispersion adjusted to 4.5 by addition of 1N HCl, stirred using a magnetic stirrer for 1 h. The slurry was then centrifuged (10,000 g, 30 min, 4°C) in a CR22G centrifuge (Hitachi Koki Co., Hitachinake, Japan). The precipitate was washed with de-ionized water, re-dissolved in de-ionized water, neutralized to pH 7.0 with 1 N NaOH at room temperature, and then freeze-dried.

Beef sausage processing

The emulsion sausage was prepared from a comminuted mixture of meat, fat, salt, condiments, spices mixtures and *Mucuna* flour a

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protein concentrate at a level of 0, 25, 75 and 100% of substitution of meat batter. The beef lean meat (2 × 2 × 2 cm^3 cut) cured with nitrite salts (NaCl:$NaNO_2$ = 99.4:0.6) and beef bump fats (2 × 2 × 2 cm^3 cut) were ground in a grinder (Manurhing type cutter: C-301, France) for 10 min, and then ice cubes were added and further comminuted for 5 min. As the mix absorbed the moisture received from molten ice, the other ingredients like salt, spices, condiment, and starches were added and chopping was further continued for 5 min and the end temperature in range of 14 to 16°C. Entire mix was filled in the stuffing machine and casing (sheep gut of about 20 mm diameter) was used for filling sausage. The finished sausage was cooked in sausage cooker for 20 min at 110°C temperature. Cooked sausages were exposing to chilled water and packed in nylon-polyethylene bags. The finished sausages were stored at 4°C for future study.

Proximate analysis of *Mucuna* flour, concentrate and sausages

All sausages, *Mucuna* flour and proteins concentrates were analysed for moisture, fat and ash contents according to the methods of AOAC (2000), numbers 950.46, 960.39 and 920.153, respectively. The protein content of samples was determined by the micro-Kjeldhal method (2000) through the use of the protein-nitrogen coefficient of 5.30 (Sze-Tao and Sathe, 2000). Carbohydrates were determined according to the method of Fisher and Stein (1961). The contents were expressed on a dry weight basis for *Mucuna* flour and protein concentrates and on fresh weight basis for sausages. Each analysis was done in triplicate, and data were reported as means ± standard deviation.

Bulk density

The bulk density was determined according to the method described by Adeleke and Odedeji (2010).

Functional properties of *Mucuna* flour and protein concentrate

Emulsifying activity (EA)

EA was determined according to the method described by Naczk et al. (1985) with some modifications. Samples (3.5 g) were homogenized at a speed of 10,000 rpm for 1 min at room temperature (about 25°C) in 25 ml de-ionized water. The sample solution was mixed with 25 ml of soybean oil followed by homogenization at a speed of 10,000 rpm for 1 min. Finally, the emulsion was centrifuged at 1300 g for 5 min. All analysis was performed in triplicate. Emulsifying activity was determined by:

$$\text{Emulsifying activity } (\%) = \frac{\text{Height of emulsified layer}}{\text{Height of the contents of the tube}} X\ 100$$

Emulsion stability (ES) was measured by re-centrifugation followed by heating at 80°C for 15 min and then expressed as follows:

$$\text{Emulsion stability } (\%) = \frac{\text{Height of remaining emulsion layer}}{\text{Height of original emulsified la}}\ X\ 100$$

Foaming capacity (FC) and foam stability (FS)

FC and FS were based on the method described by Sze-Tao and Sathe (2000) with minor modifications. 3 g samples were dispersed in 100 ml of de-ionized water. The solutions were stirred at a speed of 10,000 rpm for 2 min. The blend was immediately transferred into a 250 ml graduated cylinder. The volume was recorded before and after stirring. FC was expressed as the volume (%) increased due to stirring. For the determination of FS, foam volume changes in the graduated cylinder were recorded at 30 min of storage. All analysis was performed in triplicate. Foam capacity and foam stability were then calculated according to the following formulae:

$$\text{Foam capacity } (\%) = \frac{\text{volume after whipping} - \text{volume before whipping (mL)}}{\text{volume before whipping (mL)}}\ X\ 100$$

$$\text{Foam stability } (\%) = \frac{\text{volume after standing} - \text{volume before whipping (mL)}}{\text{volume before whipping (mL)}}\ X\ 100$$

Fat absorption capacity (FAC)

FAC was determined using the method described by Phillips et al. (1988) with minor modifications. 1 g of sample was weighed into 15 ml pre-weighed centrifuge tube and thoroughly mixed with 5 ml soybean oil. The emulsion was incubated at room temperature (about 25°C) for 30 min and then centrifuged at 5000 g for 30 min at 25°C. Then the supernatant was carefully removed, and the tube was reweighed. All analysis was performed in triplicate. FAC (gram of oil per gram of sample) was determined by:

$$FAC = \frac{F2 - F1}{F0}$$

where F0 is the weight of the dry sample (g), F1 is the weight of the tube plus the dry sample (g), and F2 is the weight of the tube plus the sediment (g).

Water absorption capacity (WAC)

WAC was determined using the method described by Rodriguez-Ambriz et al. (2005) with minor modifications. 1 g of sample was weighed into 15 ml pre-weighed centrifuge tube. Then, 10 ml of distilled water was added in small increments to the tube under continuous stirring with a glass rod. After being held at room temperature (about 25°C) for 30 min, the tube was centrifuged at 2000 g for 20 min. In the end, the amount of added distilled water resulting in the supernatant liquid in the test tube was recorded. All analysis was performed in triplicate. WAC expressed as grams of water per gram of sample, was calculated by:

$$WAC = \frac{W2 - W1}{W0}$$

where W0 is the weight of the dry sample (g), W1 is the weight of the tube plus the dry sample (g), and W2 is the weight of the tube plus the sediment (g).

Least gelation concentration (LGC)

LGC was estimated according to the method described by Deshpande et al. (1982). Samples of starch, 2 to 18% (w/v), were prepared in test tubes with 5 ml distilled water. The starch suspensions were mixed with a Vari-whirl mixer for 5 min. The test tubes were heated for 30 min at 80°C in a water bath, followed by rapid cooling under running cold tap water. The test tubes were

Table 1. Chemical composition and density of flour and protein concentrate of *Mucuna*.

Composition	*Mucuna*	
	Flour	Protein concentrate
Dry matter (DM)	88.47 ± 0.11[a]	91.47 ± 0.90[b]
Apparent density (g/cm^3)	0.67 ± 0.04[b]	0.43 ± 0.04[a]
Lipides (g/100 g DM)	1.63 ± 0.26[a]	2.33 ± 0.51[b]
Ash (g/100 g DM)	3.20 ± 0.02[b]	2.16 ± 0.01[a]
Proteins (g/100 g DM)	29.92 ± 0.51[a]	59.74 ± 0.32[b]
Crude fibres (g/100 g DM)	6.54 ± 0.22[a]	10.93 ± 0.22[b]
Carbohydrates (g/100 g DM)	20.66 ± 1.95[b]	16.85 ± 3.30[a]

Values with different letters within the same row differed significantly ($p<0.05$).

further cooled at 4°C for 2 h. LGC was determined as that concentration when the sample from the inverted test tube did not fall down or slip.

Properties of sausages

pH determination

Raw and cooked sausages (10 g) were homogenised with 90 ml of distilled water and the pH was determined with a pH-meter (Eutech Cybernetics, Cyberscan 1000, Singapore) (AOAC, 1980).

Water holding capacity (WHC)

The Tsai and Ockerman (1981) press technique was used with some modification to measure the WHC of the raw sausages. A sample (0.5 g) was placed between 2 sheets of filter paper (Whatman no. 1, stored over saturated KCL) which was placed between two Plexiglas sheets and pressed for 30 min under 1 kg load. The area of pressed meat and a spread juice was measured and the water holding capacity was calculated as follows:

$$free\ water\ (\%) = \frac{(Total\ surface\ area - meat\ film\ area)(mm^2) \times 6.11}{Total\ moisture\ (mg)\ in\ meat\ sample} \times 100$$

WHC = 100 – % Free Water

Cooking yield

After formulating sausages and placing them in casings of known weight, the sausages were weighed and then place in the sausage cooker. At the end of the cooking period, they were left to cool and then weighed again.

$$Cooking\ yield = \frac{raw\ weight}{cooked\ weigh} \times 100$$

2-Thiobarbituric acid (TBA) values

The degree of lipid oxidation of the raw and cooked beef sausages was determined by the TBA cold extraction method, described by Wite et al. (1970). The results are expressed as mg malonaldehyde/kg of sausages.

Texture measurement

The texture, based on a compression test, was measured using texturometer (Brookfield Texturometer LFRA 4500 TA 115). The resistance to a given deformation (compression), characteristic of the hardness were measured and expressed in Newton (N). During the compression test, samples were cut in squares of 30 mm and a thickness of 10 mm, then placed on the stage of a texturometer and an aluminium cylinder probe with a 25 mm diameter, at a speed of 1.0 mm/s with a trigger of 5 g was used to compress the product to 60% of its original thickness. Two parameters were recorded: peak load and final load. The equal values of peak load and final load for a sample means it texture homogeneity.

Statistical analysis

The effect of each treatment was analyzed from the different preparations. Data were subjected to analysis of variance and the differences among means were obtained using Duncan's multiple range test (significance $p<0.05$) using Statgraphics plus 5.0 software.

RESULTS AND DISCUSSION

Physico-chemical characterization of *Mucuna* flour and protein concentrate

The proximate composition and bulk density of *Mucuna* flour and protein concentrate is shown in Table 1. *Mucuna* flour was used as starting material for the preparation of *Mucuna* protein concentrate. Protein content of *Mucuna* flour and protein concentrate was significantly different ($p < 0.05$), with values of 29.92 ± 0.51 and 59.74 ± 0.32%, respectively.

The protein content increased by about 50% from flour to protein concentrate. The same trend was observed for lipid and crude fibres while its density, ash and

Table 2. Functional properties of flour and proteins concentrates of *Mucuna*.

Properties	Samples	
	Flour	Proteins concentrates
Least gelation concentration (%)	12[a]	20[b]
Water absorption capacity (g/100 g DM)	333.32 ± 6.78[b]	279.78 ± 2.04[a]
Fat absorption capacity (g/100 g DM)	116.58 ± 2.05[a]	290.33 ± 4.72[b]
Emulsion activity (%)	60.44 ± 0.10[b]	56.33 ± 1.72[a]
Emulsion stability (%)	58±0.51[b]	41±0.51[a]
Foaming capacity (%)	52.38 ± 0.36[b]	27.61 ± 0.64[a]
Foam stability (%)		
After 10 min	45.71 ± 0.85	20.00 ± 0.85
After 30 min	39.04 ± 0.36	14.28 ± 0.85
After 60 min	23.80 ± 0.54	11.42 ± 0.85

Values with different letters within the same row differed significantly ($p<0.05$).

carbohydrate contents decreased significantly ($p < 0.05$) from 0.67 to 0.43, 3.20 to 2.16 and 20.66 to 16.85%, respectively (Table 1). The variation observed between the two samples may be attributed to the extraction method used.

In order to evaluate if a protein is applicable and suitable in certain food systems and food products, it is important to characterize the functionalities of the protein (Kinsella, 1982; Vaclavik and Christian, 2003). Some functional properties of flour and proteins concentrates of *M. pruriens* var. *pruriens* are presented in Table 2. LGC, used as the index of gelation, indicated that *Mucuna* flour exhibited high gelation ability than *Mucuna* protein concentrate, 12 and 20%, respectively. The difference observed here can be linked to the difference in carbohydrate content (20.66± 1.9 and 16.85± 3.30%, respectively) of the two samples. According to Lawal and Adebowale (2005), gel strength depends on strength of intra-granular binding forces within swollen starch granules. In this respect it can be believed that intra-granular bonding forces are higher in *Mucuna* flour. This observation corroborated the results of water absorption capacity.

WAC of both samples were significantly different ($p < 0.05$), with flour having the highest WAC (333.32 ± 6.78 g/100 g). This was consistent with other studies (Prinyawiwatkul et al., 1997). Protein water absorption capacity of proteins is a function of several parameters, including size, shape, steric factors, conformational characteristics, hydrophilic-hydrophobic balance of amino acids in the protein molecules as well as lipids, carbohydrates and tannins associated with proteins. Carbohydrates contain hydrophilic parts, such as polar or charged side chains, which can enhance WAC (Jitngarmkusol et al., 2008). Flour water absorption capacity was enhanced, as the flour carbohydrate content (20.66 ± 1.95%) was significantly higher ($p < 0.05$) than that of protein concentrates (16.85 ± 3.30%). WAC value of *Mucuna* flour and protein concentrates were not significantly different than 3.551 g/g of commercial soy protein isolate reported by Zhu et al. (2010).

FAC of both samples were significantly different ($p < 0.05$), with protein concentrate having more than twice FAC (290.33 ± 4.72 g/100 g) than flour (116.58 ± 2.05 g/100 g). The differences in fat absorption capacity between the samples might be due to the presence of more non-polar amino acids in protein concentrates than in flour, and also due to the partial denaturation of proteins with exposure of hydrophobic amino acid groups during the protein concentrate production process. The presence of several non-polar side chains may bind the hydrocarbon chains of fats, thereby resulting in higher absorption of oil (Sathe et al., 1982). El Nasri and El Tinay (2007) reported that surface area and hydrophobicity improve fat absorption capacity and also high protein content shows high FAC. Campell et al. (1992) reported that FAC increased as protein content increased in sunflower and soy protein products. FAC values of Mucuna proteins concentrates was higher than 2.434 g/g of commercial soy protein isolate reported by Zhu et al. (2010). The ability of protein to bind fat is very important for applications as meat replacement and extenders, principally because it enhances flavour retention, and reputedly improves mouth feel (Ogunwolu et al., 2009). Results obtained indicated that Mucuna protein concentrates have good oil absorption capacity. High FAC of Mucuna protein concentrate renders it a good ingredient in cold meat industry, particularly for sausages, where the protein usually bridges the fat and

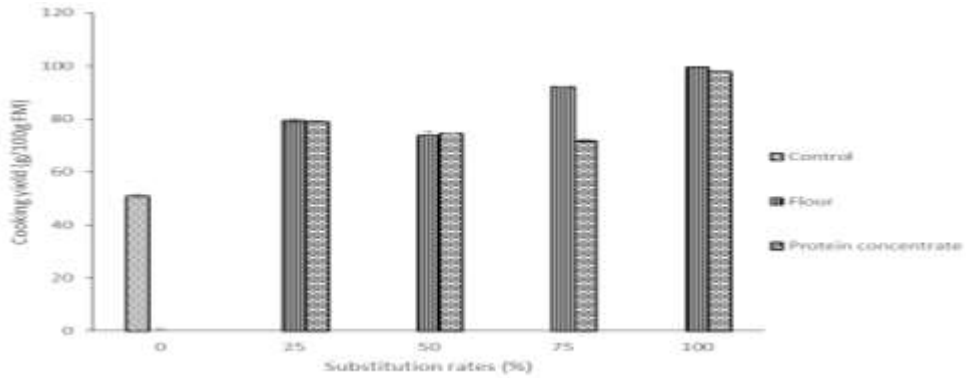

Figure 1. Influence of substitution rate of Lean meat by *Mucuna* flour and *Mucuna* protein concentrate on cooking yield of sausages.

Table 3. Influence of substitution rate of Lean meat by *Mucuna* flour and *Mucuna* protein concentrate on pH of sausages.

Parameter	Stat	Substitutions rates				
		S0	S25	S50	S75	S100
Flour	Raw	5.93±0.05^{a}	5.23±0.05^{b}	5.13±.05^{bc}	5.13±0.05^{bc}	5.03±0.05^{c}
	Cook	6.06±0.11^{a}	5.83±0.05^{b}	5.76±0.05^{b}	5.53±0.05^{b}	5.83±0.05^{c}
Protein Concentrate	Raw	5.93±0.05^{a}	5.50±0.00^{c}	5.63±.00^{bc}	5.66±0.05^{c}	5.83±0.11^{ab}
	cook	6.06±0.11^{a}	5.66±0.05^{c}	6.06±0.05^{b}	6.13±0.05^{b}	6.33±0.15^{a}

Values with different letters within the same row differed significantly ($p<0.05$).

water in order to obtain good products. EA of *Mucuna* flour and protein concentrate, was significantly different ($p < 0.05$) with values of 60.44 ± 0.10 and 56.33 ± 1.72%, respectively. The results were in agreement with Chove et al. (2001), who stated that the emulsifying capacity of proteins tends to decrease as protein concentration is increased and this was also consistent with the results reported on winged bean protein concentrate (Sathe et al., 1982), sunflower protein isolate (Lin et al., 1974) and cashew nut protein concentrate and isolate (Ogunwolu et al., 2009). The same trend was observed for ES.

FC of flour was significantly higher than that of protein concentrate, with values of 52.38 ± 0.36 and 27.61 ± 0.64%, respectively. FS of flour was significantly higher than that of protein concentrates, and this stability decrease with time (Table 2). The results suggested that the flour had a more flexible protein structure in aqueous solutions and interacted strongly at the air-water interface to form more stable foams when compared with the protein concentrates.

Cooking yield of sausages with *Mucuna* flour and protein concentrate at different rates as replacement for beef meat is shown in Figure 1. Cooking yield values exhibited a significant ($p<0.05$) increasing trend with incorporation of *Mucuna* flour and protein concentrate as compared to control. Indeed, Chin et al. (1998) reported that textural modifying ingredients, such as non-meat proteins or gums would be added in low-fat meat products to retain added water not to loss during cooking. At the same incorporation rate of *Mucuna* flour and protein concentrate, no significant differences ($p>0.05$) were observed on cooking yield of sausages except at 75 % incorporation rate. This was probably link the difference of WAC and FAC of *Mucuna* flour and protein concentrate. The beneficial effect of *Mucuna* flour and protein concentrate on reducing purge loss was similar to that reported by Chin et al. (2000) who found that low-fat bolognas containing 1% soy protein isolate had reduced purge loss after processing and during refrigerated storage.

The pH affects many functional properties such as color, flavor and texture of food, although the pH of food is important for microbial growth. The variations of pH of raw and cooked sausages with different substitutions rates of *Mucuna* flour and protein concentrate are shown in Table 3. Incorporation of *Mucuna* flour influence

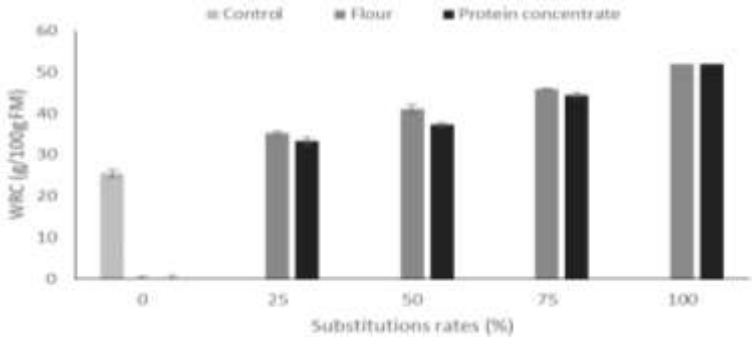

Figure 2. Influence of substitution rate of Lean meat by *Mucuna* flour and *Mucuna* protein concentrate on water retention capacity of cooked sausages

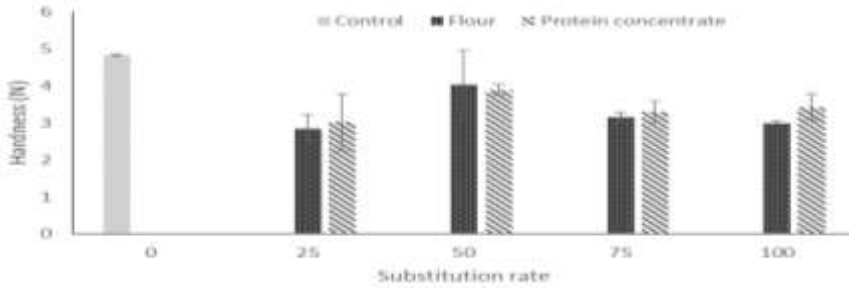

Figure 3. Influence of substitution rate of lean meat by *Mucuna* flour and *Mucuna* protein concentrate on cooked sausages hardness

significantly pH of raw and cook sausages ($p<0.05$), the same trend was observed for sausages incorporated with *Mucuna* protein concentrate. The decrease of pH with the different substitution could be mainly due to the pH value of *Mucuna* flour and protein concentrate.

There is a general increase in pH of sausages after cooking compared to the raw. The increase of pH in sausages after cooking may be due to either the hydrolysis of protein during cooking with the release of peptide and amino acids leading to the increase of pH of the medium or the loss of short chains organic acids produced during maturation of meat released during cooking. The increase in pH of cooked samples compared to the raw samples has been reported by Manish et al. (2007) using sodium alginate as a fat replacer in pork patties.

WRC is important for the formation of gels and emulsions. WHC of the control (25.49±0.85 g/100 g FM) was significantly lower ($P<0.05$) than that of the substituted samples (Figure 2). These results clearly show that the substituted samples, which consisted of 25 to 100% substitution of *Mucuna* flour and protein concentrates of lean meat in sausages had a higher ability to hold water compared to the control. Despite the fact that protein is well-known for its ability to hold oil and water and form a stable emulsion due to the lipophilic and hydrophilic groups in the same polymer chain. Physical and chemical properties, such as size, shape, amino acid composition and sequence, net charges, hydrophobicity to hydrophilicity ratio, structures, molecular flexibility and the ability to interact with other components as starches also affect WHC (Egbert and Payne, 2009; Brewer, 2012).

The imitation sausage incorporated with *Mucuna* flour and proteins concentrates keeps more water. Protein and carbohydrate brought by *Mucuna* flour and protein concentrates might have increased hydration, solubility, emulsification and gelation, which are the most important characteristics needed to qualify the stability and acceptability of end products. This observation is confirmed by the increase of WRC with substitution rates of *Mucuna* flour and protein concentrate in sausages.

The hardness of different sausages is presented in Figure 3. There were a general drop ($p<0.05$) of hardness of sausages with the incorporation of *Mucuna* flour and

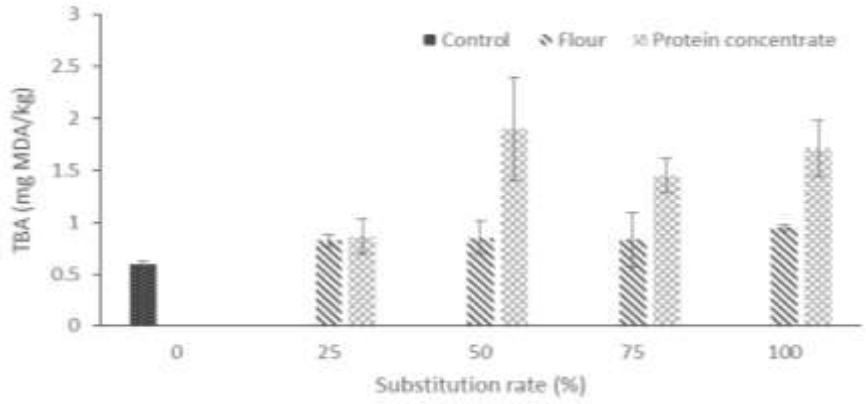

Figure 4. Influence of substitution rate of Lean meat by *Mucuna* flour and *Mucuna* protein concentrate on cooked sausages lipids oxidation

protein concentrate.

The high moisture content and high water binding capacity and even oil absorption capacity of *Mucuna* flour and protein concentrate might be a contributing factor to the lower hardness in sausages. Similar results have been reported by Arun et al. (2008) using soy bean paste in goat meat nuggets and Khalil (2000) using modified corn starch paste in beef patties as fat replacers. Also, the protein content is responsible for the hardness, as rheological parameters are strongly influenced by protein concentration in processed muscle foods such as sausage (Colmenero et al., 1995).

TBA values of sausages are important property, which relates to the quality and shelf life of the product (Figure 4).

TBA values of emulsion sausages incorporated with levels of (25, 50, 75 and 100%) were found to be higher ($p<0.05$). At the same rate of incorporation, sausages containing *Mucuna* protein concentrate are more oxidised ($p<0.05$) than they homologues containing *Mucuna* flour. The presence of minerals such as iron (pro-oxidant) and the type of lipids in *Mucuna* flour and protein concentrate can explain the increase in lipids oxidation of sausage (Zanardi et al., 2004).

Conclusion

Results from this study indicate that the composition of *Mucuna* flour and protein concentrates were different. Also, functional properties and bulk density of *Mucuna* protein concentrate were different from that of *Mucuna* flour. Finally, *Mucuna* flour and protein concentrate exhibited satisfactory functional properties as required in meat products, that is, high fat and water absorption capacity, good emulsion activity and stability. What has been confirmed by increasing of cooking yield with incorporation of *Mucuna* flour and protein concentrate as compared to control. There were also a general drop ($p<0.05$) of hardness of sausages with the incorporation of *Mucuna* flour and protein concentrate.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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