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Full Length Research Paper

Effect of particle size of selected composite spices on storability of fried meat

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Meat is a good source of many nutrients and protein in the human diet. In this study, strips of marinated beef were deep fried in oil, packaged and stored under three storage conditions. The study was carried out to determine the proximate composition, oxidative and microbial stability of meat sample marinated with spices of different particle sizes (250, 300 and 500 µm). The untreated sample was compared with marinated samples and stored under ambient, refrigeration and freezing temperatures. The results showed that spice particle size affected protein, fat and ash content of the meat during storage at $p<0.01$ while the effect on moisture content and carbohydrate was at $p<0.001$. Samples marinated with 250 µm had the lowest microbial growth (6.0×10^4) and lowest oxidative rancidity value (0.40). Combined spices give a synergistic effect which is more potent in the inhibition of microbial growth and oxidative rancidity.

Key words: Particle size, storage, meat, proximate, microbial, oxidation.

INTRODUCTION

Meat is one of man's most important sources of high quality protein, vitamins, minerals and other nutrients (Heinz and Hautzinger, 2007). The basic composition of meat varies between types and cut. This has direct influence on the quality attributes of the final product after processing. Owing to the spoilage potential of meat, many preservation techniques are being employed in improving its keeping quality and shelf life. Meat is often processed using a range of traditional methods such as salting, drying, cooking, smoking, marinating, or combination of these operations (Collignan et al., 2001).

Spices such as ginger, garlic, pepper, turmeric, cinnamon, cloves, pimento, rosemary are seed, root, fruit, bark, berry, bud or vegetable substances primarily used for flavouring, colouring or preserving food (Feng and Liu, 2011). Apart from these attributes, studies have also shown that they have some medicinal attributes (P'erez-D'iaz and Mcfeeters, 2010). Many spices have been reported to have natural antimicrobial and anti-oxidation attributes and these account for the reason why they are used for the preservation of meat which is particularly susceptible to spoilage (Fenwick and Hanley, 2011). The use of natural spices as a preservative in meat has proved to be a better and safer alternative to the use of chemicals such as nitrites (Santas et al., 2010). Globally, plant extracts are employed for their antimicrobial,

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antifungal and antiviral properties (Mahesh and Satish, 2008). It is known that more than 400,000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine (P'erez-D'iaz and Mcfeeters, 2010).

Frying is one of the oldest and common cooking methods used in preparing food with favourable sensory properties including a typical flavour, oil, colour and texture (Gazmuri and Bouchon, 2009). Through frying, food is cooked more quickly and has a crispness characteristic (Ogunmoyela et al., 2016). There are different frying techniques: stir frying, deep frying, shallow frying, pan frying with varied conditions such as the amount and type of fat required, cooking time, type of cooking vessel required and the manipulation of the food. Out of these techniques, the most widely used and efficient method in terms of speed of cooking, taste of food and texture of food is deep frying (Savage et al., 2002). Deep-fat frying is a method used to produce dried food where an edible fat heated above the boiling of water serves as the heat transfer medium. Fat also migrates into the food providing nutrients and flavour (Tarmizi and Niranjan, 2011). However, surface darkening and many adverse reactions take place during deep-fat frying because of high temperature (Joshi et al., 2016). When the oil is heated, it enables heat transfer due to conduction and convection, the latter being caused by free water boiling at the surface upon immersion of the moist food in hot oil (Seruga and Budzaki, 2005). The moisture vaporizes out, and creates a path known as capillary pore, through which hot oil enters the food (Mellema, 2003). The reaction occurs by the influence of oil uptake, crust formation, shrinkage and swelling, thus inducing macro- and micro-structural changes (Taylor, 2013). This influences the vapour and liquid diffusion, safety assurance, and yields final products with the taste and textural characteristics expected by the consumer. Therefore, frying is one of the methods of processing foods; it impacts flavour, taste, colour and crispness in fried foods. In spite of the increase in the demand for fried foods by consumers all over the world, the danger posed by consuming too much fat is still a challenge (Oladejo et al., 2017).

Curing is any of the food preservation and flavouring processes such as meat, fish, and vegetables, by adding a combination of salt, nitrates, nitrites or sugar (Boyle and Levin, 2008). However, the use of nitrites in food has been controversial and this is due to the potential to produce nitrosamines when nitrates are present in high concentrations and the product is cooked at high temperature (Karl-Otto, 2008). When nitrites are used in meat, it breaks down in the meat into nitric oxide, which then binds to the iron atom in the centre of myoglobin's heme group, reducing oxidation and producing a reddish brown colour (Kleinbongard et al., 2006).

In foods, nitrosamines are produced from nitrites and secondary amines, which usually occur when amino acids are subjected to heat as a result of cooking or under strong acidic condition such as that of the human stomach (Schurgers and Vermeer, 2000). Under high temperature or acidic condition, the nitrite forms nitrous acid (HNO_2), which is protonated and splits into the nitrosonium cation and water. The nitrosonium cation then reacts with an amine to produce nitrosamine (Krasner et al., 2013). These processes lead to the production of significant levels of nitrosamines in many foodstuffs such as meat (Ferlay et al., 2008). The U.S government established limit on the amount of nitrites used in meat products so as to reduce the risk of having cancer in the country (Karl-Otto, 2008). Consumers are very conscious of what they eat due to the carcinogenic effects of most processed foods (USFDA, 2011).

In view of this, demand for naturally processed foods or foods processed with little or no chemical preservatives or additives is constantly increasing. This study was carried out to investigate proximate composition, antimicrobial and antioxidant effect of onion, garlic and ginger at different particle sizes on fried meat stored under refrigeration, freezing and ambient temperatures.

MATERIALS AND METHODS

The meat, salt and spices which were used were purchased from an open market in Ota, Ogun State, Nigeria. Other materials, equipment and utensils were obtained from the Food Technology and Nutrition laboratory of Bells University of Technology, Ota, Ogun State, Nigeria.

Meat preparation

A deboned lean cow meat extracted from the proximal segment of the vertebrate hind was used for this experiment. The meat was trimmed free of fat and excess connective tissue and washed with water. The chunk of the meat was cut into smaller portions of about 150-200 g. Slicing was done along the fibre axis of each portion giving very thin slices of about 2 mm thickness in continuous sheets. The pieces of sliced meat were then marinated in a plastic bowl covered with a polyethylene film for 24 to 48 h in a refrigerator and then fried (Fenwick and Hanley, 2011). A control experiment was carried out following the same method except that the meats were not marinated. The fried meats were stored in an air tight polyethylene bag separately for analysis.

Spice preparation

The fresh onion, garlic and ginger were obtained, sorted and cleaned by removing the peel. They were cut into smaller pieces, dried in an electric oven at a temperature of 50°C for 24 for 48 h to a moisture content of 5- 6%. After the drying process, the spices were pulverized with the aid of an electric blender for few minutes to prevent loss of flavor; they were sieved to 250, 300 and 500 µm particle sizes and then packaged into air tight plastic containers and stored (Oyas et al., 2013).

Preparation of marinated fried meat

The ingredients were weighed into stainless steel bowls and mixed

Table 1. Composition of ingredients used for slurry.

Ingredient	Weight (g)	Blend (g)
Garlic	10	
Onion	50	50
Ginger	40	
Salt		10
Water		500

thoroughly with the aid of a spoon as shown in Table 1. The meat pieces were soaked in the marinade (slurry) inside a bowl and covered with polyethylene film. The bowl was placed in a refrigerator for 24 h so as to facilitate the absorption of the spices into the meat. The meat was parboiled, fried, packaged and stored for analysis. This process was done for 250, 300 and 500 µm spice particle sizes.

The meat samples were produced from different particle size composition of the spices and the samples were stored for 5 weeks under three different storage conditions; freezing, refrigerating and ambient temperature.

Experimental analysis

The analyses that were carried out on the meat include proximate, microbial profile and oxidative stability.

Proximate analysis

Standard methods of AOAC (2005) were used to determine the crude protein, total ash, crude fat and moisture contents of the samples. The total carbohydrate content was calculated by difference in protein, fat, ash and moisture from 100 (Ajatta et al., 2016).

Microbial examination

Total plate count was carried out on the raw and processed meat samples using the method described by Odom et al. (2012). 10 g of the sample was weighed out and homogenized in sterile distilled water. Five folds dilutions of the homogenate were made using sterile McCartney bottles and 1 ml of 10^{-4} and 10^{-5} dilutions of the homogenate were placed on Nutrient Agar media using the pour plate method. The media was left to solidify after which the plates were incubated at 37°C for 24 to 48 h. After incubation, the colony forming units were counted with the aid of a colony counter. The same process was repeated and 0.5 ml of 10^{-4} and 10^{-5} dilutions of the homogenate were placed on Sabouraud dextrose agar to test for mold growth on the meat samples.

Oxidative stability

The oxidative stability analyses carried out on the meat samples are thiobarbituric acid reactive substance (TBARS) determination and free fatty acid (FFA) determination. Thiobarbituric acid analysis was carried out according to Eke et al. (2012). Ten grams of the macerated sample was mixed with 50 ml of distilled water. The mixture was transferred into a distillation flask where 2.5 ml of 4 M HCL acid was added before it was distilled at a rate so that 50 ml of the distillate was collected in 10 min. Five ml of the distillate was pipette into a glass tube and 5 ml of TBA reagent was added and it was properly mixed before it was heated in a water bath for 35 min. A blank sample was similarly prepared using 5 ml of distilled water for 35 min. The sample and the blank tube were cooled and the absorbance of the sample was measured against the blank using a spectrophotometer (Pye Unicam SP9, Cambridge, UK) at 538 nm.

TBA Value (as mg malonaldehyde per kg sample) = 7.8 × A
Where, A = Absorbance of sample versus blank

Free fatty acid (FFA) determination: Two grams of the sample was weighed into a conical flask; 25 ml of diethyl ether and alcohol was added and it was properly mixed. One ml of phenolphthalein indicator was added and it was neutralized with 0.1 M NaOH. Then the mixture was titrated against 0.1 M NaOH until a pink colour which persisted for 3-5 min was observed.

$$\text{Acid value} = \frac{Titre\ value\ (ml)\ X\ 5.61}{Weight\ of\ Sample}$$

$$\text{FFA} = \frac{Acid\ value}{2}$$

Statistical analysis

The data reported in all the tables are the means of triplicate observations at least ($n \geq 3$). Spice particle sizes (250, 300 and 500 µm) and control experiment were considered as attribute while storage conditions were considered as treatment. Degree of influence of treatment on attribute was determined by analysis of variance (ANOVA) using SAS version 8e software (SAS Institute Inc., Cary, NC, USA) at $p < 0.05$. Means were separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Proximate composition of meat and spices

The proximate composition of onion powder revealed 5.78% moisture, 13.2% protein, 0.22% fat, 3.10% ash and 77.73% carbohydrate content as presented in Table 2. There is slight difference compared to composition of onion reported by (Santas et al., 2010); 11.53% protein, 0.97% fat and 78.36% carbohydrate content. This could be as a result of different variety of fresh onion used and environmental factor. In ginger and garlic; 5.18% moisture, 10.54% protein, 0.13% fat, 4.05% ash and

Table 2. Proximate composition of spices used for marinade.

Parameter	Onion powder	Ginger powder	Garlic powder
Moisture (%)	5.78 ± 0.04	5.18 ±0.10	6.43 ± 0.04
Protein (%)	13.2 ±0.01	10.54 ± 0.02	36.34 ± 0.08
Fat (%)	0.22 ±0.02	0.13 ± 0.01	0.73 ± 0.03
Ash (%)	3.10 ±0.05	4.05 ± 0.08	0.59 ± 0.01
CHO (%)	77.73 ±0.12	89.11 ± 0.21	55.91 ±0.20

Mean ± Standard deviation of samples (n=3).

Table 3. Proximate composition of raw meat and fried treated meat at zero time.

Parameter	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	CHO (%)
Raw meat	76.00 ± 0.00	20.80± 0.05	2.54 ± 0.04	0.61 ± 0.10	0.35 ± 0.01
A	20.63 ± 0.01	50.58 ± 0.01	25.56 ± 0.03	3.08 ± 0.04	0.15 ± 0.10
B	20.48 ± 0.02	50.30 ± 0.10	24.78 ± 0.01	3.04 ± 0.03	1.42 ± 0.20
C	17.57 ± 0.04	49.75 ± 0.02	22.95 ± 0.02	2.88 ± 0.04	6.54 ± 0.40
D	14.47 ± 0.01	48.14 ± 0.01	20.21 ± 0.03	1.64 ± 0.02	15.59 ± 0.01

Mean ± standard deviation of samples (n=3). A. Meat sample + 250 µm particle size; B- Meat sample + 300 µm particle size; C, Meat sample + 500 µm particle size; D, Control.

89.11% carbohydrate; and 6.43% moisture, 36.34% protein, 0.73% fat, 0.59% ash and 55.91% carbohydrate respectively were determined. These are not compatible with the result reported by Suhaj (2006); where 6.37% moisture, 8.58% protein, 5.35% crude fat, 6.30% ash and 68.15% carbohydrate for ginger while in garlic powder; 4.55% moisture, 15.53% protein, 0.72% fat, 4.08% ash and 73.22% carbohydrate were reported. The low moisture content of all the spices could be as a result of dehydration during drying process. Thus, the shelf life of these spices may be extended and microbial deterioration may be limited. The high crude protein content of garlic could be as a result of the presence of active proteinous metabolites such as allicin and ajoene (Dashak and Nwanegbo, 2000). The low ash content of garlic could be due to low organic mineral content (Oloyede, 2005). The carbohydrate contents of onion, ginger and garlic showed that they are carbohydrate rich spices.

The raw meat contained 76% moisture, 20.80% protein, 2.54% fat, 0.61% ash and 0.35% carbohydrate content as shown in Table 3. This is closely related to fresh lean beef that contained 75% moisture, 22.3% protein, 1.8% fat and 1.2% ash as reported by Bakht et al. (2004). A slight difference in the composition was noticed and this could be due to the size of the meat, maturity and the proportion of lean and fat in the meat. The most important component of the muscle in terms of nutritional value and process suitability is the protein content of the meat as it defines the quality and value of the raw meat material and the finished processed meat product.

Effect of spice particle size on proximate composition of marinated meat samples at zero time

The proximate composition presented in Table 3 indicated that the spices increase the nutritional composition of the treated meat samples. Sample A had significantly higher moisture content (20.63%) compared to samples B (20.48%), C (17.57%) and D (14.47%). The low moisture content in sample D could be as a result of moisture diffuses from the meat surface unlike other samples with absorbed spice particles. Protein (50.58%), fat (25.56%) and ash contents (3.08%) in sample A (sample marinated with 250 µm spice particle size) were significantly higher ($P < 0.05$), followed by sample B (sample marinated with 300 µm spice particle size), and sample C (sample marinated with 500 µm spice particle size). This behaviour could be attributed to the fact that more spice with fine size was absorbed into the meat as a result of contact. The fat content slightly increases as spice size reduces. This is most likely because of the large amount of spice absorbed at reduced size thereby increasing the tendency of the meat to retain fat during frying. The ash content also increases at reduced spice sizes. This is not far from the fact that quantity of spice dissolved in water to form slurry would have increased. Ginger and onion have the highest ash content as reported in Table 2 and with higher composition by weight as reflected in Table 1. There was a significant difference ($p < 0.05$) in the carbohydrate content for all the samples. Low moisture, protein, fat and ash could be attributed to the high carbohydrate content in the control experiment.

Figure 1. Samples stored under refrigeration temperature (°C).

Effect of spice particle size and storage condition on the proximate composition of treated meat samples

The fresh meat analyzed shows 76% moisture content. After deep frying, the moisture content of the fried meat was in the range of 15.73% - 20.98%. This is similar to the result reported by Omojola et al. (2014) who recorded a moisture content of 35.57% in deep fried Muscovy drake meat. The observed difference could be as a result of nutritional composition of the treated meat and duration of frying. Aaslyng et al. (2003) revealed that during cooking, meat losses approximately 20 to 40% of its weight because of induced shrinkage which results in moisture being displaced from the meat. Storage conditions, storage periods, spice sizes and combination of storage conditions and spice sizes have significant difference ($P < 0.001$) on moisture content. The combination of spice sizes and storage periods have no significant difference on moisture content.

There was a significant increase ($P < 0.05$) in the protein, fat, and ash content of the fried meat samples compared to that of the raw meat. This increase could be as a result of the additional nutrients contributed by the spices used for the experiment. The protein, fat and ash content of all the samples reduced significantly as the period advanced and a significant difference ($p < 0.01$) was observed between the samples marinated with the three spice particle sizes (250, 300, and 500 µm). However, storage conditions, storage periods, spice sizes and combination of storage periods and spice sizes have significant difference ($P < 0.001$) on fat content while combination of spice sizes and storage conditions have significant difference ($P < 0.05$) on the fat content. All the variables have significant difference ($P < 0.001$) on ash and carbohydrate.

At the end of the 5 weeks storage period, the samples marinated with 250 µm and stored under freezing temperature (-18 ± 2°C) had the highest protein (50.08%), fat (23.40%), and ash (1.26%) contents while the control stored under ambient temperature (27 ± 2°C) had the least protein (47.37%), fat (17.26%) and ash (0.20%) contents. This showed that spices are good sources of nutritional supplements in diet and the preservation of the nutritional content of a food material during storage is best achieved at freezing temperature. Images of the treated meat samples are shown in Figures 1 to 3.

Effect of spice particle size and storage condition on the oxidation stability of treated meat samples

It was observed from the results presented in Table 4 that the FFA and the TBARS values of all the samples increased significantly ($p<0.001$) among the weeks. This could be attributed by the accelerated oxidative reactions in the oil due to the thermal treatment during deep frying. This is in accordance with the result obtained by Presswood (2012) who observed an increase in free fatty acid value of vacuum fried meat under storage. The natural composition of meat, such as the amounts of antioxidant such as vitamin E may also influence the rate and intensity of oxidation reactions. From the result, it was observed that the addition of spices, spice particle size and storage condition had an effect on the FFA and TBARS values of the meat samples. All marinated treatments resulted in low FFA and TBARS values at the end of the storage period compared to the control. At the end of the 5 weeks storage period, the samples marinated with 250 µm spice particle size had the lowest FFA value (ranged from 0.49 to 0.96%) and TBARS value (ranged from 0.40 to 0.88%) while the control (untreated sample) had the highest FFA value (ranged from 1.43 to 4.54%) and TBARS value (ranged from 1.36

Figure 2. Samples stored under freezing temperature (°C).

Figure 3. Samples stored under ambient temperature (°C).

to 3.53%). This could be as a result of the smaller particle size and surface area of the spices making it easier to be absorbed into the meat being antioxidant in nature. However, storage condition as well as spice sizes together with combination of these and storage periods have significant difference ($P < 0.001$) on FFA and TBARS values.

Effect of spices on the total viable count and fungi (yeast and mould) counts of meat samples

The average total viable count from Wk 1 to Wk 5 ranged from (1.5×10^4 to 1.8×105 CFU/g) as shown in Table 5 while average fungi count (yeasts and moulds) from Wk 1 to Wk 5 ranged from 1.0×10^4 to 1.2×10^5 CFU/g on the meat samples as shown in Table 6. These results are in accordance with the recommended microbial limits for cooked beef, pork and lamb meat product at 22 to 37°C (1×10^5 to 5×10^5 CFU/g) as reported by Smith (1968). The combination of onion, garlic, and ginger resulted in a pronounced decrease in the total viable bacteria and fungi counts of the marinated meat samples compared to the control. It was observed that the rate of microbial growth on the meat sample increased as the particle size of the spices increased and as the storage week progressed. At the end of the storage period, the samples marinated with 250 µm spice particle size had lesser bacteria and fungi counts, followed by 300 and 500 µm spice particle size, respectively. The control sample had the highest average bacteria (1.8×10^5 CFU/g) and average fungi count (1.45×10^5 CFU/g). There was a significant microbial growth between the samples stored under refrigeration, freezing and ambient temperature.

Table 4. Analysis of variance on proximate and oxidative composition of fried treated meat samples.

Source	DF	Anova SS	Mean Square	F Value	P Value
MC					
Attribute (1)	3	760.72	253.57	2730.51	***
Treatment (2)	2	40.09	20.05	215.84	***
Week (3)	4	411.78	102.95	1108.53	***
1 X 2	6	3.25	0.54	5.83	***
1 X 3	12	1.29	0.11	1.16	NS
Fat					
Attribute (1)	3	926.58	308.86	17110.30	***
Treatment (2)	2	2.40	1.20	66.51	***
Week (3)	4	98.63	24.66	1366.01	***
1 X 2	6	0.27	0.04	2.46	*
1 X 3	12	1.92	0.16	8.84	***
Ash					
Attribute (1)	3	28.69	9.56	1686.78	***
Treatment (2)	2	3.56	1.78	313.91	***
Week (3)	4	59.41	14.85	2619.46	***
1 X 2	6	0.24	0.04	6.96	***
1 X 3	12	2.26	0.19	33.17	***
CHO					
Attribute (1)	3	5696.34	1898.78	32768.30	***
Treatment (2)	2	2.63	1.32	22.70	***
Week (3)	4	14.87	3.72	64.16	***
1 X 2	6	3.15	0.52	9.05	***
1 X 3	12	6.63	0.55	9.53	***
CP					
Attribute (1)	3	110.19	36.73	4.10	**
Treatment (2)	2	17.88	8.94	1.00	NS
Week (3)	4	49.38	12.35	1.38	NS
1 X 2	6	54.57	9.09	1.02	NS
1 X 3	12	105.61	8.80	0.98	NS
FFA					
Attribute (1)	3	20.70	6.90	91.89	***
Treatment (2)	2	12.50	6.25	83.27	***
Week (3)	4	29.14	7.28	97.02	***
1 X 2	6	8.46	1.41	18.78	***
1 X 3	12	10.78	0.90	11.97	***
TBA					
Attribute (1)	3	11.95	3.98	91.06	***
Treatment (2)	2	7.13	3.56	81.43	***
Week (3)	4	20.40	5.10	116.59	***
1 X 2	6	3.54	0.59	13.49	***
1 X 3	12	6.29	0.52	11.99	***

MC = Moisture content; CHO = carbohydrate; CP = protein; FFA = free fatty acid; TBA = thiobabituric acid; ***significant at P < 0.001; **significant at P < 0.01; *Significant at P < 0.05;NS = not significant.

Table 5. Total bacteria count (CFU/g) of fried treated meat samples stored under refrigeration, freezing and ambient temperatures among the storage periods (35 days).

WK0	WK 1	WK 2	WK 3	WK 4	WK5
A1 <10	9.7×10^4	9.5×10^4	1.0×10^5	1.1×10^5	1.1×10^5
A2 <10	3.5×10^4	4.5×10^4	5.0×10^4	5.5×10^4	6.0×10^4
A3 1.0×10^4	1.0×10^5	1.1×10^5	1.2×10^5	1.2×10^5	1.3×10^5
B1 <10	5.0×10^4	5.7×10^4	1.1×10^5	1.2×10^5	1.2×10^5
B2 <10	1.5×10^4	2.5×10^4	8.0×10^4	8.5×10^4	9.0×10^4
B3 6.6×10^3	9.2×10^4	1.0×10^5	1.3×10^5	1.4×10^5	1.4×10^5
C1 <10	1.4×10^5	1.3×10^5	1.4×10^5	1.4×10^5	1.5×10^5
C2 <10	1.0×10^5	1.1×10^5	1.1×10^5	1.2×10^5	1.2×10^5
C3 5.7×10^4	1.3×10^5	1.5×10^5	1.6×10^5	1.6×10^5	1.7×10^5
D1 <10	8.1×10^4	1.4×10^5	1.4×10^5	1.5×10^5	1.6×10^5
D2 <10	7.2×10^4	1.1×10^5	1.2×10^5	1.2×10^5	1.3×10^5
D3 5.2×10^4	1.3×10^5	$1..7 \times 10^5$	1.7×10^5	1.8×10^5	1.8×10^5

A, Samples marinated with 250 µm spice particle size; B, samples marinated with 300 µm spice particle size; C, Samples marinated with 500 µm spice particle size; D, Control sample, 1, refrigeration temperature; 2, freezing temperature; 3, ambient temperature.

Table 6. Fungi count (yeasts and moulds) (CFU/g) of fried treated meat samples stored under refrigeration, freezing and ambient temperatures among the storage periods (35 days).

WK0	WK 1	WK 2	WK 3	WK 4	WK5
A1 <10	3.2×10^4	4.0×10^4	4.5×10^4	5.0×10^4	5.5×10^4
A2 <10	2.0×10^4	2.5×10^4	3.0×10^4	3.5×10^4	4.0×10^4
A3 6.6×10^3	9.2×10^4	9.8×10^4	1.0×10^5	1.1×10^5	1.2×10^5
B1 <10	3.7×10^4	5.5×10^4	6.0×10^4	6.5×10^4	7.0×10^4
B2 <10	3.7×10^4	5.0×10^4	5.5×10^4	6.0×10^4	6.5×10^4
B3 2.5×10^4	8.2×10^4	1.1×10^5	1.1×10^5	1.2×10^5	1.2×10^5
C1 <10	5.8×10^4	6.7×10^4	7.2×10^4	7.7×10^4	8.3×10^4
C2 <10	1.0×10^4	5.5×10^4	6.0×10^4	6.5×10^4	7.0×10^4
C3 1.0×10^4	1.0×10^5	1.1×10^5	1.2×10^5	1.2×10^5	1.3×10^5
D1 <10	7.8×10^4	7.8×10^4	8.3×10^4	8.8×10^4	9.3×10^4
D2 <10	5.0×10^4	7.2×10^4	1.2×10^5	8.2×10^4	8.7×10^4
D3 3.5×10^4	1.1×10^5	1.2×10^5	1.3×10^5	1.3×10^5	1.4×10^5

A, Samples marinated with 250 µm spice particle size; B, samples marinated with 300 µm spice particle size; C, Samples marinated with 500 µm spice particle size; D, Control sample, 1, refrigeration temperature; 2, freezing temperature; 3, ambient temperature.

Samples stored under ambient temperature had the highest average bacteria (1.8×10^5 CFU/g) and average fungi count (1.3×10^5 CFU/g) while the samples stored under the freezing condition had the lowest average bacteria (6.0×10^4 CFU/g) and average fungi growth (4.0×10^4 CFU/g). This is in conformity with Kwada and Tella (2009) who investigated that at low temperature, the rate of growth of micro-organisms and subsequent spoilage are retarded.

Conclusion

The use of spices at different particle sizes in meat during storage had effect on the proximate composition, microbial and oxidative stability. The samples treated with 250 µm particle size had the highest protein content as well as the least FFA and TBARS over the period of five weeks. The proximate composition of the samples stored under freezing was retained better than those stored

under refrigeration and ambient temperatures.

Spices have active antimicrobial potential which inhibit the growth of a wide range of micro-organisms. They also inhibit oxidative rancidity and retard the development of off flavours in food. The hurdle effect of the spices gives more potent preservation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Assessment of exposure to staphylococcal enterotoxins genes by consumption of ready to consume milk products in milk shop outlets in Mbeya, Tanzania

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This study assessed the exposure of humans to *Staphylococcus* species expressing the Enterotoxins genes (SEs) through consumption of boiled-milk-served-hot and fermented milk in Mbeya, Tanzania. A survey involving 120 consumers revealed that 67.5% of the respondents were buying raw milk from milk shops for home consumption. About 76% of respondents boiled milk before consumption, 14.8% ferment the milk after boiling and 5.8% consumed fermented milk without boiling. Children (30%) consumed milk more frequently than other members in the family. Among consumers who buy milk from the milk shops, 71% were daily consumers of both boiled milk served hot and fermented milk. Approximately, 1197 L (90% CI, 987-1416) of ready to consume milk was sold per day. Of which 860 L (90% CI, 645-1071) and 337 L (90% CI, 168-530) were boiled-milk-served-hot and fermented milk, respectively. Out of the ready to consume milk, 490 L (90% CI, 464-516) of boiled-milk-served-hot was contaminated with SEs gene compared to 77.5 L (90% CI, 67-88) of fermented milk. Daily 2394 people were consumers of milk and their products. Exposure assessment shows that the probability of consuming boiled-milk-served-hot and fermented milk contaminated with SEs gene at a milk shop was 0.42 (90% CI, 0.071-0.838) and 0.17 (90% CI, 0-0.62), respectively. It was estimated that every day, 363 (90% CI, 341-385) and 58 (90% CI, 49-66) people were likely to consume boiled milk taken hot and fermented milk contaminated with SE gene, respectively. The finding shows that exposure to SEs gene was two times more likely to occur in people who consume boiled-milk-served-hot than in people who consume fermented milk (OR. 2.221 (90% CI, 0.6-6.16). Awareness creation on proper food handling among milk handlers to reduce contamination along the milk value chain is recommended.

Key words: Boiled milk served hot, foodborne disease, public health, fermented milk.

INTRODUCTION

Foodborne disease is an important and growing public health concern in many countries around the globe (WHO, 2002; Le Loir et al., 2003). Animal source foods have been cited as an important cause of foodborne

illness and *Staphylococcus aureus* is one of the pathogenic microorganisms most frequently linked with foodborne diseases (Le Loir et al., 2003). This bacterium is usually found in milk and milk products as a result of poor hygiene practices and animals with clinical or subclinical mastitis (Mdegela et al., 2009). Following the ingestion of staphylococcal enterotoxins (SEs) that are produced by enterotoxigenic strains of *S. aureus,* initial symptoms include nausea, vomiting (in spurts), abdominal pain, diarrhoea, dizziness, shivering and general weakness, sometimes associated with a moderate fever (Hennekinne, 2012).

Even though many people suffer from foodborne illness yearly, the accurate estimate of the incidences of food-borne disease is difficult to obtain in developing countries like Tanzania. People with symptoms like vomiting, diarrhoea and stomach cramps rarely go to the hospital, due to the limited access to the biomedicine and disease understanding especially in rural areas. In Tanzania, statistics show that 60 to 70% of the population seek healthcare from practitioners of traditional medicine (URT, 2000). Despite the presence of conventional medicine, traditional medicine is widely used and rapidly growing health care system in the country (Kayombo et al., 2012); therefore, the cases of foodborne diseases are under-reported.

Thus, ensuring the safety of milk from dairy farmer where animal husbandry practices differ widely presents a big challenge. Over 85% of milk consumed in Tanzania is from informal markets (Kurwijila, 2006). This causes the consumers of milk to be exposed to ingestion of milk containing pathogenic bacteria (*S. aureus* and their toxins) in cases where the milk is consumed without heat treatment or other processing capable of inactivating this enterotoxins which is heat resistant (Argudin et al., 2010). Unlike the producer organism, enterotoxins are remarkably heat resistant; as a result, they may be present in foods even when viable *S. aureus* are absent (Jørgensen et al., 2005). According European Commission for Health and Consumer Protection (ECHCP, 2003) inactivation of crude enterotoxins type A (SEA) in buffer was reduced from 21 to <1 µg/ml after heating at 100°C for 130 min and purified SEA (0.2 mg/ml) was completely inactivated in buffer after heating at 80°C for 3 min or 100°C for 1 min. Previous study showed that boiled hot milk ready to consume harboured pathogenic bacteria (*S. aureus*) with genes (SEs) responsible for toxins production (Gratian, 2012; Massawe et al., 2017). The SEs are resistant to inactivation by gastrointestinal proteases such as pepsin and trypsin and the toxins produced showed thermal stability (Argudin et al., 2010), making their elimination difficult to achieve (Le Loir et al., 2003). Thus, the aim of this study was to assess the consumption behaviour and the risk of exposure to milk with SE genes in Mbeya, Tanzania. The outcomes of this study will provide useful information and serves as a case study for future mitigation strategies to decrease the prevalence of *S. aureus* and SEs in the Mbeya milk value chain.

MATERIALS AND METHODS

The study was carried out in Mbeya and Mbozi district in Mbeya region which have a high population of dairy cattle. Description of the study area can be found in Massawe et al. (2017).

The study was carried out in three steps. In the first step, two questionnaires were administered to firstly milk consumers who were in the milk shop at the time of visits and willing to participate in the study. The information collected was on milk consumption pattern, frequency of consumption, amount consumed, type of milk preferred (boiled or fermented), whether they buy milk for home consumption, type of milk bought, amount, treatment performed before consumption and people in the family who consume milk and secondly milk shop owner to record information on procedure followed when receiving milk, access to training on milk handling, source of milk, amount of milk handled, amount sold, type of consumer, number of consumer, milk treatment conducted in their shops and types of quality check conducted. Personal observation was also used to get information on milk handling, type of serving utensils, cleanliness of the milk shop and personal cleanliness of owner and his/her staff. Data was collected during the wet (April 2015 to June 2015) and the dry season (August 2015 to November 2015). Samples were collected from 36 milk shops in the study area (18 sites from each district).

The second step involved sampling of milk (raw, boiled milk served hot and fermented milk) carried out concurrently with administration of questionnaire. The final step was laboratory analysis, where the isolation of *S. aureus* was performed using standard procedure and finally the detection of SE genes in the milk was determined by multiplex polymerase chain reaction (mPCR) (Rahimi, 2013) with modification of annealing temperature. The multiplex PCR was establish using nine pairs of primers (Table 1) allowing the detection of genes encoding staphylococcal enterotoxins genes *sea, seb, sec, sed, see, seg, sei, seh* and *sej*. The amplifications were performed in 0.2 ml reaction tubes in a final reaction volume of 25 µl. The PCR mixture consisted of 5 mM $MgCl_2$, 200 µM dNTPs, buffer, 2 U of Taq polymerase, and 5 µl of DNA. DNA amplification was performed in a Takara thermal cycler (MJ Research, Inc. Tokyo Japan) using the following conditions :initial denaturation for 5 min at 94°C followed by 40 cycles of denaturation (94°C for 30 s), annealing (90 s at 57°C), initial extension for 72°C at 60 s. A final extension step (72°C for 10 min) was performed after the completion of the cycles. The amplified PCR products were visualized by standard gel electrophoresis in a 2% agarose gel stained by Gel red (5 µg/mL). The gel electrophoresis was run for 60 min at 110 V in order to achieve a visible separation of bands. The gels were photographed under ultraviolet light using the Gel-Doc 2000 system (Bio-Rad, USA). Samples that test positive for a particular gene were counted and their isolation rates calculated.

A stochastic model was developed for the exposure to the SE genes by consumers of boiled milk served hot and fermented milk using the following parameters: the number of milk shops (N), the

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Table 1. Oligonucleotide primers for amplification of genes encoding staphylococcal enterotoxins.

Gene	Primer	Primer sequence 5' to 3'	Amplification size (bp)	Reference
sea	GSEAR-1 GSEAR-2	GGT TAT CAA TGT GCG GGT GG CGG CAC TTT TTT CTC TTC GG	102	Mehrotra et al. (2000)
seb	GSEBR-1 GSEBR-2	GTA TGG TGG TGT AAC TGA GC CCA AAT AGT GAC GAG TTA GG	164	Mehrotra et al. (2000)
sec	GSECR-1 GSECR-2	AGA TGA AGT AGT TGA TGT GTA TGGCAC ACT TTT AGA ATC AAC CG	451	Mehrotra et al. (2000)
sed	GSEDR-1 GSEDR-2	CCA ATA ATA GGA GAA AAT AAA AGATT GGT ATT TTT TTT CGT TC	278	Mehrotra et al. (2000)
see	GSEER-1 GSEER-2	AGG TTT TTT CAC AGG TCA TCC CTT TTT TTT CTT CGG TCA ATC	209	Mehrotra et al. (2000)
seg	SEG-1 SEG-2	TGC TAT CGA CAC ACT ACA ACC CCA GAT TCA AAT GCA GAA CC	704	Mehrotra et al. (2000)
seh	SEH-1 SEH-2	CGA AAG CAG AAG ATT TAC ACG GAC CTT TAC TTA TTT CGC TGT C	495	Mehrotra et al. (2000)
sei	SEI-1 SEI-2	GAC AAC AAA ACT GTC GAA ACT G CCA TAT TCT TTG CCT TTA CCA G	630	Mehrotra et al. (2000)
sej	SEJ-1 SEJ-2	CAT CAG AAC TGT TGT TCC GCT AG CTG AAT TTT ACC ATC AAA GGT AC	142	Mehrotra et al. (2000)

total quantity of milk sold daily in the milk shops (Q), the average daily milk sold ($\bar{X}m$), concentration of pathogens in contaminated milk (C), prevalence of SEs in ready to consume milk (P_{RV}), the quantity of milk contaminated daily (Q_c), the proportion of people consuming boiled hot milk (P_B) and fermented milk (P_F), the number of daily milk consumers (D_c), and the probability of consuming milk containing SEs (P) (Figure 1).

The following formulas were used:

(1) Daily quantity of milk sold in the study area was estimated using Equation 1

$$Qm = N \times Cn \times \bar{X}m \quad (1)$$

where Q_m is the daily quantity of milk sold in the milk shops in the study area, N is the number of milk shops selling ready to consume milk, C_n is the number of milk consumers, and $\bar{X}m$ is the average milk consumption per person.

(2) Average quantity of milk sold per shop was estimated using Equation 2

$$\bar{X} = Qm/N \quad (2)$$

where $\bar{X}$ is the average quantity of ready to consume milk sold in the studied milk shop, Qm is the total quantity of milk sold (by type) in the milk shops in the study area, and N is the number of milk shop surveyed.

(3) The quantity of milk contaminated with SEs was estimated using Equation 3

$$Q_C = P_{RV} \times Qm \quad (3)$$

where Q_C is the quantity of milk (L) contaminated, P_{RV} is the prevalence of SEs in milk (laboratory results), Q is the total quantity of milk sold in the studied milk shops.

(4) Number of people consuming contaminated milk daily was estimated using Equation 4

$$NP = Q_{C\,x}\, P_p / \bar{X}c \quad (4)$$

where NP is the number of people consuming contaminated milk daily, Q_C is the quantity of milk (L) contaminated by type (boiled hot milk and fermented milk), P_p is the average proportion of people who consume milk in the milk shops by type, $\bar{X}c$ is the average quantity of milk by type consumed per person per day.

(5) Probability of consuming milk with SEs gene was estimated using Equation 5

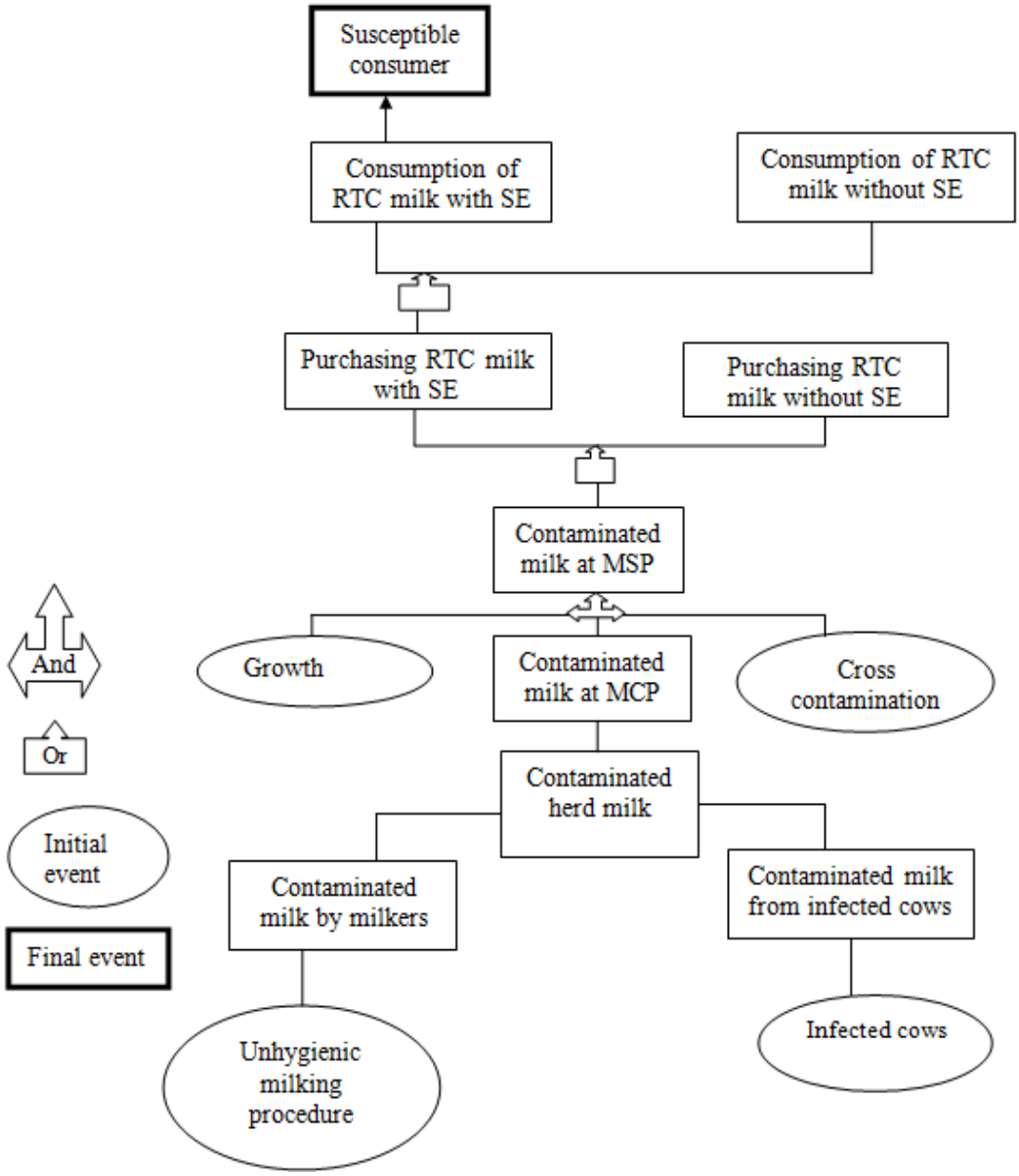

Figure 1. Fault tree for exposure of consumers to SEs. MCP: Milk collection point; MSP: milk shop; RTC: ready to consume.

$$P = P_p \times P_{RV} \quad (5)$$

where P is the probability of consuming milk with SEs genes, P_p is the average proportion of people who consume both milk products in the milk shops, and P_{RV} is the prevalence of SEs in ready to consume milk by type (laboratory results).

$$(P_{RV} = \frac{\text{Total number of Sample with SEs (by milk type)}}{\text{Total number of Sample with } S.aureus \text{ examined (by milk type)}} X\ 100)$$

Statistical analysis

The data was analyzed using SAS (2004). Simple descriptive statistics and frequency distribution were used to explore the variability of the studied parameter involved. Monte Carlo simulation was performed for all the exposure outputs by running 10000 iterations using @Risk 7.5Palisade software.

RESULTS

Milk consumption characteristics

The consumption characteristic of milk in the study area shows that most of the households (80%) consumed milk with other food (Table 2). About 70% of the respondents buy 0.5 to 2 L of milk (amount purchased depend on

Table 2. Milk consumption characteristics in Mbeya and Mbozi the Southern Highlands Zone (N=120).

Parameter	Variable	n (%)
Methods of milk consumption	Consumed with other food	96 (80)
	Consumed alone	24 (20)
Purchase of raw milk for home consumption	Yes	81 (67.5)
	No	39 (32.5)
Frequency of purchase for home consumption	Daily	52 (43.3)
	Twice/week	9 (7.5)
	Thrice/week	31 (25.8)
	Four/week	16 (13.3)
	Once/Week	12 (10.0)
Quantity purchased per day/family	0.5L-2 L	84 (70.0)
	≥3 L	36 (30.0)
Quantity consumed/day/family	0.25-1 L	89 (74.2)
	≥2 L	31 (25.8)
Treatment of milk before consumption	Yes	109 (90.8)
	No	11 (9.2)
Type of treatment	Boil	92 (76.7)
	Ferment(after boiling)	17 (14.1)
	Fermented (without boiling)	7 (5.8)
	No treatment	4 (3.4)
Family member who consume milk	Children and elders	13 (10.8)
	Children only	36 (30.0)
	Elders only	11 (9.2)
	Whole family	60 (50.0)
Frequency of consumption in milk shops	Daily	89 (74.2)
	Occasionally	31 (25.8)

income of the individual) and 74.2% of the respondents consume 0.25 to 1 L per day. Furthermore, 76.7% of the respondent's boiled milk before consumption and 9.2% drink raw milk. Fifty percent of the respondents, whole family members consume the milk (6 people per family), 30% of the respondents only children consume the milk, while in 9.2% of the respondent's only elders consumed milk.

Characteristics of milk shops in the study area

Characteristics and practices conducted in the milk shops are shown in Table 3. Sixty seven percent of the milk shops owners were male and their age ranged from 21 to 73 years old. Fifty eight percent of milk shop owners aged between 21 and 50 years.

Most of the respondents had primary education level (63.9%) and only 11.1% attended food handling training. The utensils used for milk handling were plastic buckets (86.1%) and aluminium cans (2.8%). Most of the milk shops (66.7%) had no cooling facilities. Test for milk quality was common to all milk shops and density in combination with clot on boiling was the most frequently method used by 69.4% of the respondents.

Factors associated with isolation of SEs gene in ready to consume milk

The age of the milk shop owner had influence on the rate of SEs isolation (Table 4). The samples collected from the milk shops owned by younger personnel had greater chance of SEs isolation (OR 1.83 (90% CI, 0.52-6.47)

Table 3. Characteristic of milk shops and milk shop operators in Mbeya and Mbozi, Tanzania (N=36).

Parameter	n (%)
Sex of the respondents	
Female	12 (33.3)
Male	24 (66.7)
Age group	
21-36	7 (19.4)
37-50	14 (38.9)
51-62	9 (25)
≥63	6 (16.7)
Education level	
Primary	23 (63.9)
Secondary	9 (25)
Post-secondary	4 (11.1)
Experience in business (Years)	
1-2	14 (38.9)
3-5	15 (41.7)
≥6	7 (19.4)
Training in food handling	
Yes	4(11.1)
No	32 (88.9)
Personal hygiene	
Clean	33 (91.7)
Dirty	3 (8.3)
Utensil used	
Aluminium	1 (2.8)
Plastic + Aluminium	4 (11.1)
Plastic bucket	24(86.1)
Storage facilities	
Refrigerators	7 (19.4)
Deep freezers	3 (8.3)
Deep + Refrigerator	2 (5.6)
None	24 (66.7)
Washing of utensils	
Hot water and soap	36 (100)
Milk quality measures	
Yes	36 (100)
Types of quality measures	
Density	10 (27.8)
Density + Clot on boiling	25 (69.4)
Acid test + Density	1 (2.8)

Table 4. Social and management factors which might contribute to isolation of SEs gene in ready to consume milk sold in Mbeya, Tanzania.

Parameter	Odd Ratio	90% CI
Age	1.83	0.52-6.47
Education	0.57	0.35-1.84
Experience	1.31	0.46-3.72
Cooling facilities	1.72	0.65-4.48
Washing of utensils with hot water and soap	1.05	0.32-3.51
Use of quality control	0.40	0.10-1.55
Utensil used for milk handling	0.84	0.23-2.97
Quality measures	0.62	0.22-1.83
Training in food handling	0.83	0.33-2.89

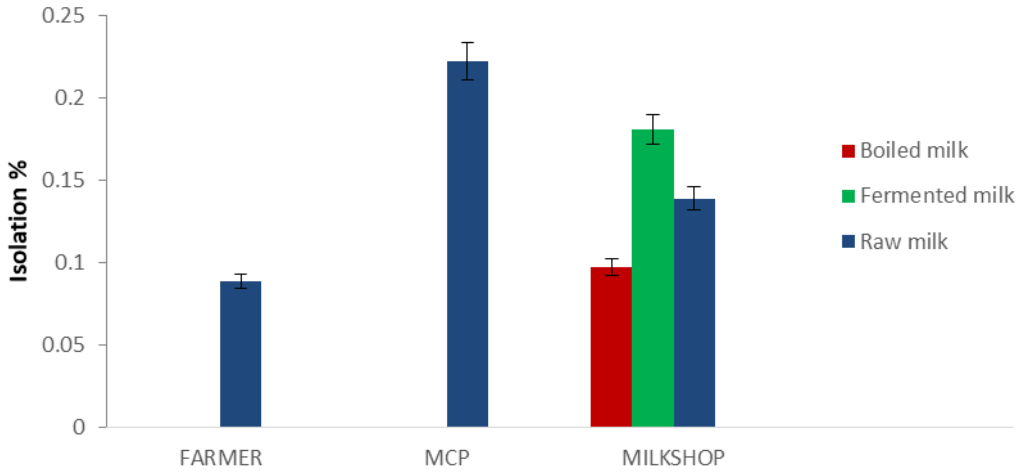

Figure 2. Isolation of *S. aureus* from boiled, fermented (soured) and raw milk in Mbeya, Tanzania.

than those collected from shops of older personnel. Having a post secondary education reduces the odds (OR 0.57 (90% CI, 0.35-1.84) of SEs isolation. In addition, samples from milk shop owned by personnel who had little experience was at relatively higher chance (OR 1.31 (90% CI, 0.46-3.72) of isolating SEs gene. Absences of cooling facilities increase the odds (OR 1.72 (90% CI, 0.65-4.48) of SE isolation. Furthermore, the shops which conducted quality control reduce the odds of SEs isolation by 0.62 (90% CI, 0.22-1.83).

Hazard identification

Analysis of milk samples showed that isolation rates of *S. aureus* from raw milk in farmer, MCP and milk shops were 8.9, 22.2 and 13.9%, respectively (Figure 2). The corresponding percentages for boiled milk served hot and fermented milk were 9.7 and 18.1%, respectively. Among the isolated *S. aureus,* 36.4% had SE coding genes. Thus, SEs is identified as a potential hazard and risk to milk consumers. The SE coding genes were isolated in the ready to consume milk (boiled hot 57.1% and fermented (23.1%) sold in the milk shops in the study area (Figure 3).

Isolation of SEs coding genes at the milk shops were highest in boiled milk (57.1%) followed by raw milk (30%) and fermented milk (23.1%) (Figure 3).

Exposure assessment

In this study, storage time, temperature profiles during harvesting, storage and transportation were not recorded. The quantity of milk contaminated and daily consumption of milk in the study area is shown in Table 5. The quantity of milk sold was estimated to be 1197 L (90% CI, 1109.7 -1316.4) per day. Among this, 860 L (90% CI, 797.3-922.7) was boiled hot and 337 L (90% CI, 312.4-361.6) fermented milk. Approximately, 490 L (90% CI, 464-516) and 77.5 L (90% CI, 67-88) of boiled hot and fermented milk, respectively were contaminated with SE coding

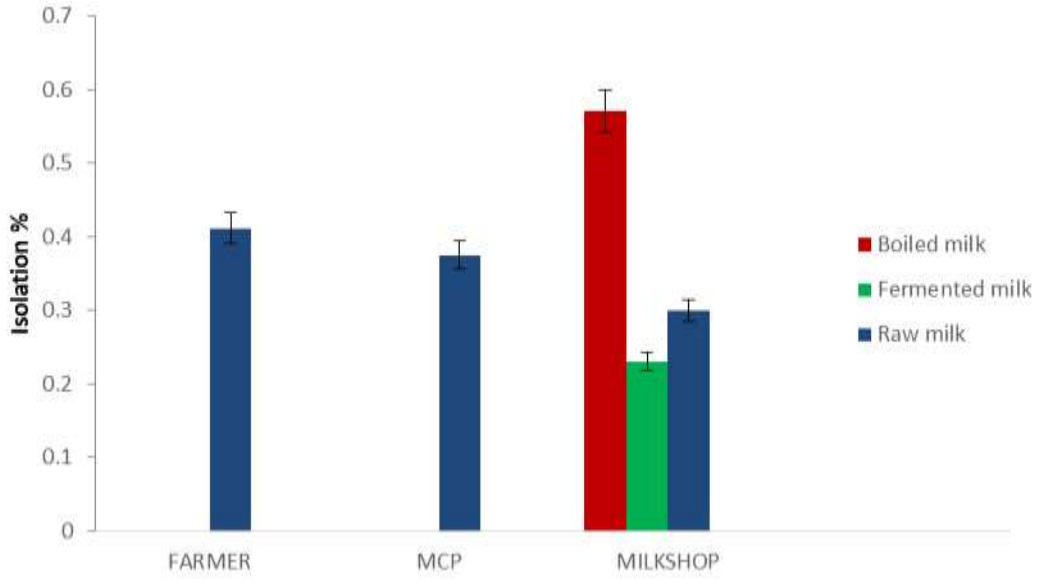

Figure 3. Isolation of SEs from raw, boiled and fermented (soured) milk in Mbeya, Tanzania.

Table 5. Outputs of the model (10000 iterations) for exposure assessment of SEs gene from boiled hot and fermented milk in Mbeya, Tanzania.

Parameter	Mean (90%CI)	Minimum	Maximum
Quantity of boiled hot milk contaminated daily	490 (464-516)	427L	576L
Quantity of soured milk contaminated	77.5 (67-88)	53L	104L
Probability of consuming milk with SE in boiled hot	0.42 (0.071-0.838)	0	0.99
Probability of consuming milk with SE in soured milk	0.17 (0-0.62)	0	0.89
Estimated number of people consuming contaminated boiled hot milk	363 (341-385)	301	414
Estimated number of people consuming contaminated soured milk	58 (49-66)	37	82
Proportion of milk consumers in the milk shops	0.71 (0.464-0.944)	0.13	0.99

gene. Furthermore, 363 (90% CI, 301-414) (Figure 4) and 58 (90% CI, 49-66) persons were estimated to consume contaminated boiled and fermented milk, respectively. The probability of consuming boiled hot milk and fermented milk contaminated with SE gene at a milk shop was 0.42 (90% CI, 0.071- 0.838) (Figure 5) and 0.17 (90% CI, 0-0.62), respectively. Odd ratio analysis showed that the exposure to SE gene was two times more likely to occur in people who consume boiled-milk-served-hot milk ($P < 0.05$) (OR: 2.21 (90% CI, 0.6-6.16) than in people who consume fermented milk.

DISCUSSION

The information on milk consumption in the study area revealed that milk was mostly consumed with other foods. Foods that were mentioned to be consumed with milk include tea, *ugali (*Ugali is made from maize/wheat/fingermillet flours mixed in boiling water and made into a thick porridge*)*, porridge, banana and rice. In the family where keeping of cattle was not practiced, children, elderly and sick individuals were given priority more than other groups. The amount purchased and consumed depends on the economic status of individual/family. Similar findings were reported in Ghana (Aidoo et al., 2009) and Kenya (Njarui et al., 2011) that income of the households head influenced the milk consumption in a family. Boiling of milk before consumption was common practice in the study area. This practice should be encouraged because boiling reduces the microbial load into a level considered to be safe for human consumption, particularly that all pathogens are also destroyed. The finding concurs with Omore et al. (2005) that boiling of milk is a common practice in many households.

In order to safeguard the consumer's health, knowledge on milk safety is very important. Lack of knowledge in milk handling may have a negative impact on consumer's health. In this study, training of the milk

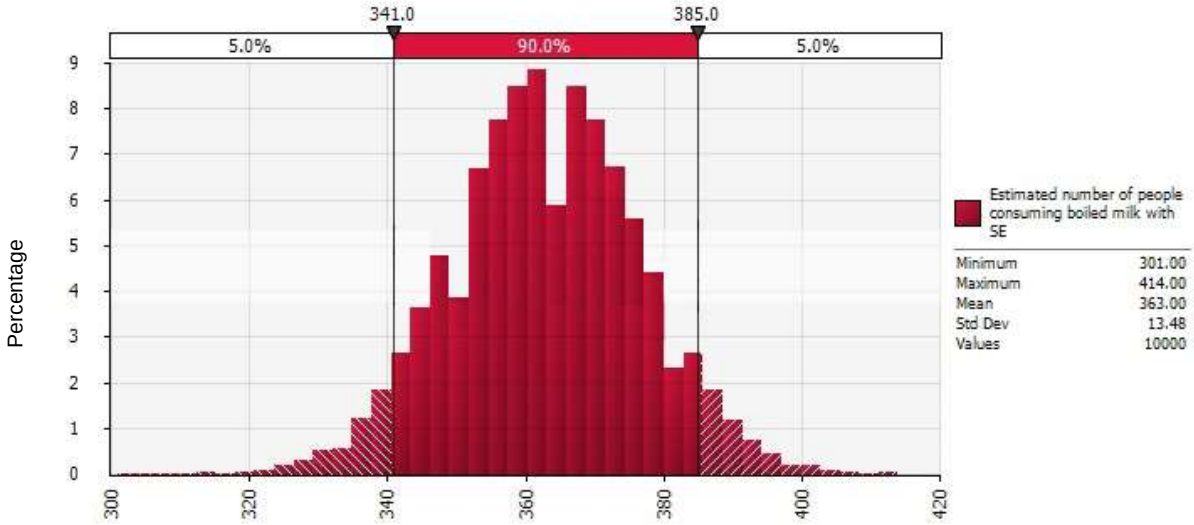

Figure 4. Monte Carlo simulation for the number of people consuming boiled hot milk contaminated by SE gene.

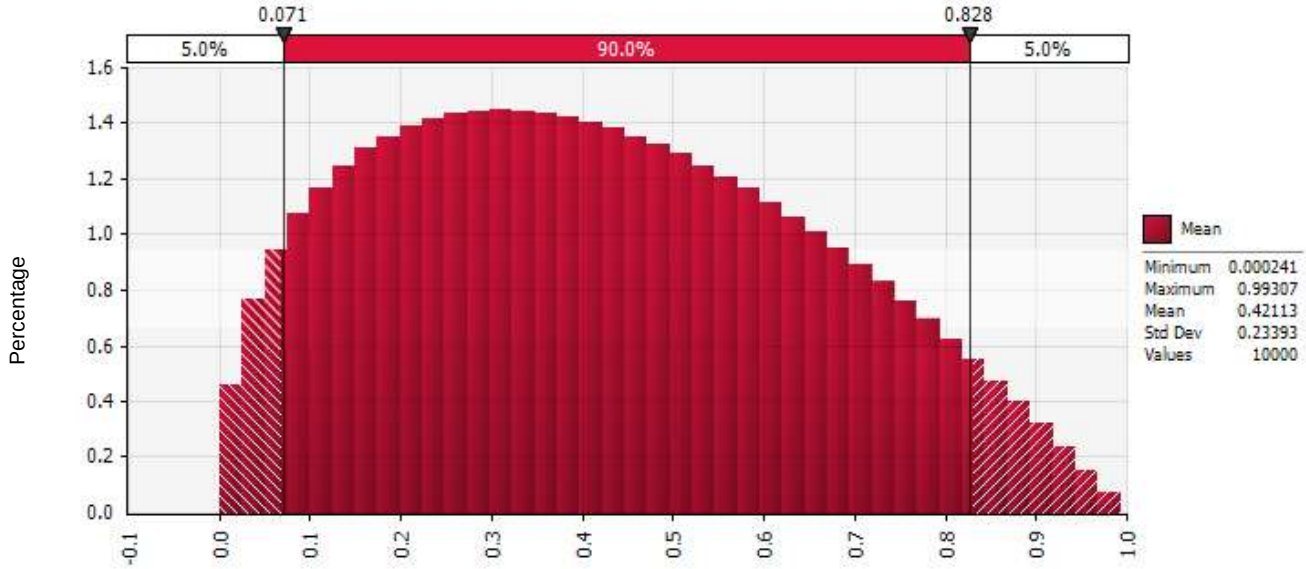

Figure 5. Monte Carlo simulation for the probability consuming boiled hot milk contaminated by SE gene.

shop owner on milk handling had no significant influence on the quality of milk. Though not significant, the milk shop owners who attended training were more likely to sell good quality milk relative to those who lack training. In the present study, training reduces the risk of SEs isolation in milk. The lack of training in milk quality may be a contributing factor to unhygienic milk handling by the informal sector traders (Omore et al., 2005; Kitagwa et al., 2006).

Age of the milk shops owner varied from young to old age. It was observed that a younger age with little experience in milk business related with increased odds of selling milk with SEs in their shops. The finding concurs with the study conducted in India (Singh et al., 2015) which reported that experience in dairying and milk sales had positive and significant correlation with the milk quality. The practice for cold storage in most of milk shops was that the freezer/fridge was operating by day and switched off during the night purposefully for saving the cost of electricity. It was observed that the milk shops that had no cooling facilities were relatively at higher risk of having SEs genes in the milk than the milk shops with cooling facilities. Exposing the milk to ambient temperature creates a good environment for SEs coding

genes to produce toxins (Paulin et al., 2012).

The exposure results show that there is a greater chance for consumers to be exposed to contaminated milk because ready to consume boiled hot milk sold in the milk shops contained SEs coding genes. Despite the fact that boiled milk is considered safe, its consumption could expose the consumers to the risk of consuming SEs coding genes which is heat resistant. This is because more than half of the boiled milk sold in the milk shops in the study area contained SEs coding genes at consumption. Although the study did not estimate the chances of human illness related to consumption of contaminated milk; still, consumption of SE contaminated milk is expected to result into illnesses. Regardless of the quantity of milk contaminated and consumed daily, no outbreaks of bacterial food-borne illness associated with consumption of milk has been reported in the study area. The reason could be that many cases are not reported, which may be due to the limited access to the healthcare system and understanding diseases especially in village areas where the healthcare system is not available and or not well established.

The estimated number of people consuming milk with SE gene is higher especially for boiled-milk-served-hot. This result is alarming because if the isolated genes produce toxins this could affect large number of peoples who consume boiled-milk-served-hot in the milk shops. A study conducted in the Ivory Coast by Sylvie et al. (2012) reported that 652 people were estimated to ingest milk contaminated with *S. aureus* which is higher compared to current study. In their study, only total *S. aureus* was considered, without estimating SE producing strains.

The probability of consuming milk with SEs gene for the people who consume boiled-milk-served-hot and fermented milk in this study indicated that the risk for consumers of boiled-milk-served-hot is higher than consumers of fermented milk. The result shows that the probability of isolating SE gene in boiled-milk-served-hot was more than two times compared to fermented milk. Studies conducted by Gratian (2012) and Sylvie et al. (2012) in Tanzania and Ivory Coast, respectively reported the probability of ingesting milk with *S. aureus* to be 29.9 and 22.7%, without estimating SE producing strains.

The contaminations of milk can occur at any point from the production to selling points if proper hygienic measures are not followed. This was evident by the presence of SEs from production to selling point. The results showed decreasing trend of SEs gene isolation (raw milk) from production through selling point. The higher rate of isolation of SEs from raw milk in production level could be due subclinical mastitis and unhygienic milking procedure. In the milk collection points and milk shops, the effect of dilution and failure of *S. aureus* to compete with other bacteria could be the reason for low isolation rates of SEs. This is because *S. aureus* fails to reach the maximum concentration (>10^5 cfu/ml) for SEs to be detected, thus there is possibility that large number of *S. aureus* isolates with lower concentration of organisms will not reach its growth potential for SEs gene to be detected. It is worth to mention that *S. aureus* bacteria can be destroyed during food processing without destroying SEs; hence, their rate of isolation may differ between food products. Similar result was reported by Noha et al. (2011) that samples collected at farm had higher isolation of SEs followed by street distributors and milk shops. Furthermore, the results showed higher isolation rates of SEs coding gene in boiled milk served hot at consumption which is a potential risk to the consumers. The presence of SEs gene in boiled milk indicates that most of the SEs detected genes could produce toxins responsible for foodborne disease and probably by the time milk was boiled, already milk had heat stable toxins produced by SEs genes. Based on the results of the current study, large numbers of people who consume milk at milk shops could become sick if SEs genes in milk produces toxin. According to Le Loir et al. (2003), the exposure to SEs gene responsible for toxin production exists due to recontamination of food products and difficulties to eliminate SE toxins in the food by normal boiling. Thus, proper milk handling practices along the entire value chain should be a rule of thumb in order to safe guards the health of ready to consume milk in the study area.

CONCLUSIONS AND RECOMMENDATION

(1) Ready to consume milk sold at milk shops contained SE coding gene and pose a potential risk to the health of consumers.
(2) Higher numbers of consumers of boiled-milk-served-hot in the milk shops are exposed to consumption of milk with SE coding genes.
(3) Hygiene training to reduce the contamination of SEs on the ready to consume milk is recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of drying on the nutrient and anti- nutrient composition of *Bombax buonopozense* sepals

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***Bombax buonopozense* is one of the underutilized plants in West African. Most of the parts of this plant are used by some populace in West Africa for medicinal and nutritional purposes while the sepals mostly go waste. The objective of this study is to evaluate the effect of different drying methods on some nutritional and anti- nutritional composition of fresh and dried sepals of *B. buonopozense*. The sepals were oven, solar and sun dried while the fresh sepals served as a control. Proximate, some mineral and anti- nutrient composition of the sepals were determined. The fresh samples were significantly different from all the dried samples ($p<0.05$). Ash content was relatively high with a range of 7.38 to 7.86%; protein, 9.99 to 10.76%; crude fiber, 14.63 to 15.39%; carbohydrate, 56.62 to 63.52%; iron, 1.55 to 3.22 mg/100 g; magnesium, 171.92 to 184.47 mg/100 g for the dried sepals. The various drying methods reduced the content of both oxalate and phytate of the sepals. The present findings show that drying of sepals of *B. buonopozense* tends to retain most of the nutrients as well as reduction in the anti- nutrients. Hence the dried sepals could be used as a potential ingredient in making foods such as soups and sauces.**

Key words: *Bombax buonopozense*, antinutrients, drying methods, nutritional composition.

INTRODUCTION

Plants play a vital role in maintaining the human health through the production of foods which provide nutrients for the body as well as medicinal purposes. Medicinal plants are defined as plants that contain substances useful for therapeutic purposes in one or more of its organs. These plants also serve as precursor for the synthesis of useful drugs (Chisom et al., 2014). Medicinal plants are known to have antioxidants, anti-inflammatory, anti-bacterial and anti- tumor activities etc. The World Health Organization (2010) estimates that 80% of population in Asian and African countries depends on traditional medicine for primary cure. General assumption of dietary constituents contributing to the protective effect of these plants is a secondary metabolite in the form of phytochemicals, vitamins and minerals. In addition to these secondary metabolites, these plants contain other

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Figure 1. *B. buonopozense* sepals.

compounds that moderate the effects of the active ingredients known as Anti- nutrients.

Drying of food materials has advantageous benefit such as; extending the shelf life, inhibiting microbial growth, less transport and storage cost. Moreover, drying causes reduction in the food materials bioactive compounds which may have beneficial health promotion properties such as antioxidants properties (Orphanides et al., 2013). Plants like moringa, dandelion, turkey berry and red silk cotton are mostly consumed for both nutritional and medicinal purposes. Drying of food materials also has side effect depending on the method of drying such as sun, oven, microwave, solara and freeze drying etc.

Red silk cotton plant (*B. buonopozense*) also known as Gold Coast *Bombax* belonging to the family *Bombacaceae* is a perennial plant which is locally called 'Akonkode3 or Akata' and 'Vagba' by the Akans and Dagbani respectively in Ghana. They are mostly found in rain forest zones of West African countries such as Sierra Leone, East Gabon some part of Nigeria (Beentje and Smith, 2001). In Ghana, red silk cotton plant is widely known for its medicinal purpose with a minute contribution to diet. The most utilized parts include the leaves, bark, root, stem, trunk etc. A greater percentage of the populace use it for medicinal purposes such as in the treatment of swellings, fever, convulsion, insanity as well as driving away evil spirits through the burning of its bark. Its seed covered with cotton are mostly harvested and used as stuffing for pillows and dresses. Report by Yi- Fang et al. (2002) indicated that its deep green leaves and deep yellow fruits provide high amount of ascorbic acid, carotene and micro minerals which play a vital role in nutrient metabolism and slowing down of degenerative diseases such as cancer and heart diseases. Its flowers having bright red to pink color attract birds and insects which contribute to pollination of the plant. With emphasis on it flowers some natives dry the sepals of the plant (Figure 1), ground them and add to food to prevent microbial growth. Chisom et al. (2014) discovered that various parts of this plant contain appreciable amount of nutrients such as carbohydrates, proteins calcium, magnesium, zinc as well as anti-nutrients such as oxalates, phytates and cyanide in minute quantity. Over the years, researchers have analyzed the nutrient and anti-nutrients composition of its leaves, stem, and bark, root of Red silk cotton plant; however, there is limited information on these parameters in relations to the sepals and the effects of drying methods on its nutritional and anti-nutrient composition. This research work will provide more information on some nutritional and anti-nutrient compositions of the sepals of *B. buonopozense* and also provide the most effective method of drying these sepals for the retention of higher amount of its nutrients.

MATERIALS AND METHODS

Sources of the sepals and pre-treatment analysis

Flowers of *B. buonopozense* were obtained from Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST). The sepals of the flowers were separated from the petals, sorted out, washed with clean water and allowed to drain. Samples were then divided into four portions together with the control (fresh sepals) by random sampling with equal weight of 200 g. Each of the three portions were solar dried (45 to 55°C) for 72 h, sun dried (25 to 37°C) for 37 h and oven dried (40°C) for 72 h. The dried samples were then pulverized with the blender in order to disintegrate the tissues and stop enzyme action to ensure prolonged storage. The pulverized samples were hermetically sealed in a zip lock bag and kept in refrigerator at 4°C for laboratory analysis.

Moisture content determination

Moisture content was determined using AOAC (1990) and the analysis was done in duplicate. Two grams of the samples were weighed using an analytical balance in clean, dry, weighed and labeled Petri dishes. The Petri dishes with the samples were put into thermostatically controlled oven (Binder heating and drying oven, Tuttingen, Germany) at 105°C for 4 h. The samples were cooled in a desiccator and weighed with the aid of an analytical balance (New classic MF electronic analytical balance, Mettler Toledo, Switzerland). The moisture content of the samples was expressed as;

$$\% \text{ Moisture content (wt/wt)} = \frac{\text{Weight of water in the sample}}{\text{Weight of wet sample}} \times 100$$

Ash content determination

Ash was determined using AOAC (1990). Two grams of the samples were weighed into cleaned, dried, labeled and weighed crucibles which were heated in a muffle furnace for 2 h at 550 to 600°C. After 2 h, the crucible and its contents were cooled in a desiccator and then weighed. The weight of the ash was expressed as a percentage of the initial weight of the sample. The analysis was done in duplicates.

$$\% \text{ Ash content (wt/wt)} = \frac{\textit{weight after ashing}}{\textit{weight before ashing (wet)}} \times 100$$

Crude fat determination

Crude fat was determined based on the soxhlet extraction method of AOAC (1990) using petroleum ether. A 250 mL round bottom soxhlet flask was cleaned, washed and dried in an oven at 103°C for 25 min and allowed to cool at room temperature before it was weighed. Two grams of the sample was weighed into a filter paper, folded and placed in a thimble. The thimble with the samples was placed into the extraction chamber which was attached to a round bottom flask containing 240 mL of petroleum ether. The round bottom flask was mounted on an electric thermal unit while the extraction chamber was connected to the reflux condenser with cold water. The extraction was allowed to run for 6 h after which the petroleum ether was distilled off. The flask containing the extracted fat was dried at 105°C for 1 h in an oven to remove the trapped petroleum ether. The flask and its content were transferred to the desiccator to cool and weighed. The analysis was duplicated. The weight of the fat obtained was expressed as percentage of the initial weight of the sample. Crude fat is calculated as;

$$\% \text{ Crude fat} = \frac{W2}{W1} \times 100$$

Where, W1 = weight of sample and W2 = weight of extract.

Crude fibre determination

Crude fibre was obtained using the method of (AOAC, 1990). The defatted sample which was obtained from crude fat determination was transferred into 750 mL Erlenmeyer flask and approximately 0.5 g of asbestos was added. Two hundred milliliters of boiling 1.25 % H_2SO_4 was added to the flask and immediately the flask was set on hot plate and connected to a condenser. The sample was boiled for 30 min and then filtered using a linen cloth in a funnel and was washed with large volume of boiling water until the residue was no more acidic. The sample was washed back into the flask using 200 mL of boiling 1.25 % NaOH. The flask was connected to the condenser again and boiled for 30 min after which it was filtered through linen cloth and washed thoroughly with boiling water until residue was no more alkaline. The residue was transferred to a crucible and the remaining was washed off using 15 mL ethanol into the crucible. The crucible and its content were dried for 30 min at 105°C and was cooled in a desiccator and weighed. The crucible was ignited in a muffle furnace at 550°C for 30 min, cooled and weighed. The loss in weight was crude fibre content and was expressed as percentage of the initial weight of the sample. The analysis was done in duplicate.

$$\% \text{ Crude fIber} = \frac{W2\text{-}W3}{W1} \times 100$$

Where, W1= weight of defatted samples, W2= weight of Residue and W3 = weight of ash.

Crude protein

The crude protein was determined using the method of AOAC (1990). This process involves digestion, distillation and titration. Two grams of the sample was weighed into a digestion flask and 0.5 g of selenium catalyst was added. A blank sample was prepared alongside; 25 mL of concentrated H_2SO_4 was added and the flask shaken vigorously to mix the contents. The flask was inserted in the digestion chamber for digestion. Digestion was done for 3 h and the sample was prepared in duplicate. The sample was heated until boiling ceased and the resulting solution was clear.

After digestion, the digestion tubes were allowed to cool; contents were diluted with small quantity of distilled water and made up to 100 mL. Twenty-five milliliters of boric acid solution was pipetted into a conical flask and few drops of mixed indicator (bromoeresol green and methyl red) were added to the mixture. Ten milliliters of the digested samples were transferred into the kjedahl flask and 25 mL of 40% NaOH solution was added to the decomposition chamber of the distillation apparatus. The condenser tip of the distillation apparatus was dipped into the boric acid in the conical flask. The ammonia in the sample was distilled into the boric acid until it changes completely to bluish green.

The distillate was titrated with 0.1 N HCl solution until it became colorless. The percentage of total nitrogen and crude protein was calculated using a conversion factor 6.25.

$$\% \text{ Protein content} = \frac{50 \times ()\text{titre value- blank titre}) \times 0.019057 \times 0.01401 \times 100 \times 6.25}{\text{Weight of sample} \times 10}$$

Carbohydrate determination

Carbohydrate content was determined using AOAC (1990), where the value of the crude protein, crude fibre, crude fat, and moisture and ash contents of the samples were added and subtracted from 100.

Carbohydrate % = 100 – (crude protein + crude fat+ crude fiber+ ash content + moisture content)

Mineral determination using atomic absorption spectrometer

a) Chemical procedure
b) Aqua- regia was prepared with mixture of HCl and Nitric acid in the ratio of 3:1 respectively, that is 150 mL of HCl against 50 mL nitric acid.
c) Digestion and reading.

Approximately, 0.5 g of the sample was weighed into different 250 mL Kjeldahl flasks and 20 mL of Aqua –regia was prepared and added to the samples in the kjedahl flasks. The mixture was digested on a digestion block at a very low temperature. The mixture was then allowed to digest till it got to 5 mL. It was then allowed to cool at room temperature. Small amount of distilled water was added, shook and was filtered using Whatman No. 42 filter paper in 100 mL Volumetric flask. The filtrate was diluted to the mark on the volumetric flask with distilled water. The readings were taken using VGP 210 AAS (Atomic Absorption Spectrophotometer) at different wavelengths using each metal lamp. The AAS was auto calibrated to give readings in part per million (ppm). Reading was divided by 100 to convert it to milligram/100 g of the sample.

Anti - nutrient determination

Estimation of oxalates

The oxalates content of the sample was estimated according to the work done by Agbaire (2011) with slight modification. One gram of each sample was weighed and 75 mL of 1.5 N sulphuric acid solution was added; the mixture was carefully stirred intermittently with magnetic stirrer for 1 h and then filtered using whatman No. 1 filter paper. Twenty-five millimeters of the filtrate was collected and titrated hot (80- 90°C) against 0.1 N $KMnO_4$ solution till the end point of a faint pink colour appears that persist for at least 30 min. Then the quantity of Oxalates in each sample was estimated and expressed in mg/g.

Estimation of phytate content

The phytates content was determined using the procedure cited in the work of Aina et al. (2012). Two grams of each samples was weighed into 250 mL conical flask. Each of the samples was soaked in 100 mL of 2% concentrated hydrochloric acid in the conical flask for 3 h and afterwards filtered through a double layer of hardened filter papers. Fifty millimeters of each filtrate was toped up with 107 mL of distilled water and 10 mL of 0.3% ammonium thiocyanate solution was added into each solution as an indicator. This was titrated against with standard iron (II) chloride solution, which contained 0.00195 g iron per ml. The end point was slightly brownish- yellowish and persisted for 5 min. the percentage phytic acid was calculated using the formula.

% Phytic acid = titre value ×0.00195× 1.19×100

Data analysis

All experiments were carried out in duplicates and the results were recorded as mean ± standard deviation. The significant difference among the drying methods and the control (fresh samples) was statistically analyzed using one- way analysis of variance (ANOVA) of SPSS version 20.

RESULTS AND DISCUSSION

Proximate composition

Sepals of *B. buonopozense* are potentially endowed with essential nutrients required for the maintenance of good human health and could be utilized as adaptive technologies for food security. A high degree of browning occurred in all the dried sepals. Rate of enzymatic and non- enzymatic browning reactions increase during drying under favorable conditions which include moisture, temperature and presence of air (Wiriya et al., 2009). These reactions lead to oxidative bleaching through the formation of brown pigments and degradation of original pigments of food material.

The moisture content of the fresh and dried sepals in the present study is shown in Table 1. The results showed that there was significant difference ($p < 0.05$) between the moisture content of the oven dried, solar dried and sun-dried sepals as compared to that of the fresh sepals where the latter recorded the highest moisture content (81.14 % ± 0.35) which was comparable to the moisture content of *Ageratum conyzoides* (83.20 % ± 0.02) reported by Nnamani et al. (2009). Comparing the moisture content of the dried sepals, the sun-dried sepals recorded a higher amount of moisture (9.18 % ± 0.10) with the least recorded for the oven dried sepals (2.81 % ± 0.28). These values of moisture contents for the sun, solar and oven dried sepals were lower than those reported by Chisom et al.(2014) on the leaves (14.34 % ± 2.0), stem (15.26 % ± 1.6) and the roots (17.21 % ± 1.0) of *B. buonopozense* which was an indication that the dried sepals would have a longer shelf life because microbial growth is supported by higher moisture content which would tend to cause the spoilage of the leaves, stem and roots as compared to the dried sepals. Work done by Bassey and Khan (2015) on the leaves of *B. buonopozense* also recorded a higher moisture content of (12.50 % ± 0.01) as compared to that of the dried sepals. These moisture contents were also lower than bitter leaves (10.02%) and Indian spinach (11.57%) with that of sun dried higher than Moringa (6.30%) documented by both Bamishaiye et al. (2011) and Asaolu et al. (2012). High moisture provides greater activity of water-soluble enzymes and co- enzymes needed for metabolic activities of plants (Iheanacho and Udebuani, 2009) as well as a conducive environment for organs to function properly in human bodies (Iroka et al., 2014). Low moisture content of the sepals indicates their stability against microbial attack and potential longer shelf life.

Plants foods that provide more than 12% of their calorific value from protein have shown to be a good source of protein (Ali, 2009). The results of the protein content in Table 1 shows a significant difference ($p < 0.05$) between the fresh sepals and the dried sepals where the least was recorded for the fresh sepals. Comparing the dried sepals, there was no significant differences among these sepals where the highest was recorded for the sun dried (10.76 % ± 0.70) and the least recorded for the solar dried sepals (9.99 % ± 0.80). This indicated that the sepals dried by the sun were able to retain most of their proteins as compared to the oven and solar dried sepals although they were not significantly different. Work done by Chisom et al. (2014) on the leaves, stem and roots of *B. buonopozense* recorded a higher protein content of 13.18% ± 2.0 for the leaves, which is in line with work done by Bassey and Khan (2015) recording 13.85% ± 0.01 on the leaves of *B. buonopozense*. The stem and roots as documented by Chisom et al. (2014) recorded 8.94% ± 1.6 and 6.93% ± 0.5 respectively which were lower than that of the protein content of the sun dried, oven dried and solar dried but higher than the fresh sepal. Chisom et al. (2014) also worked on *Ceiba pentandra* leaves, stem and roots belonging to the same family as *B. buonopozense* and recorded a lower value of protein content in the stem (9.74 % ± 1.6) and roots (6.84% ± 1.58) of the plant. This indicates that the dried sepals would be a good substitute in place of the usage of the stem and roots when inculcated in cooking as it contains a higher amount of protein which is needed for body building, replacement of worn-out tissues, boosting immune system and help in cell division as well as growth (Okeke and Elekwa, 2006).

A documentation by Hanif et al. (2006) indicated that crude fat analysis of vegetables shows the deficiency of vegetables in fats making them good for health and not a source of lipid accumulation. Antia et al. (2006) also emphasized on the fact that lipid accumulation leads to aging as well as arteriosclerosis. From the results in Table 1, there was a significant difference ($p < 0.05$)

Table 1. Proximate composition (%) of fresh and dried sepals of *B. buonopozense* sepals.

Parameter	Fresh sepals	Sun dried sepals	Oven dried sepals	Solar dried sepals
Moisture	81.14 ± 0.35^{d}	9.18 ± 0.10^{c}	2.81 ± 0.28^{a}	5.53 ± 0.19^{b}
Ash	1.29 ± 0.09^{a}	7.86 ± 0.05^{c}	7.46 ± 0.13^{b}	7.38 ± 0.21^{b}
Protein	3.04 ± 0.32^{a}	10.76 ± 0.70^{b}	10.57 ± 0.35^{b}	9.99 ± 0.80^{b}
Crude fat	0.37 ± 0.02^{a}	0.76 ± 0.04^{bc}	1.01 ± 0.15^{c}	0.66 ± 0.12^{b}
Crude fiber	3.21 ± 0.04^{a}	14.83 ± 0.19^{b}	14.63 ± 0.34^{b}	15.39 ± 0.50^{b}
Carbohydrate	11.32 ± 0.09^{a}	56.62 ± 0.99^{b}	63.52 ± 0.69^{c}	61 ± 0.28^{c}

Values represent mean ± SD of duplicates. Means in the same row with the same superscript are not significantly different (p>0.05).

between the crude fiber content of the dried and fresh sepals where the fresh sepals recorded the least (0.37 % ± 0.02). Comparing the dried sepals, the oven dried recorded the highest amount of crude fat (1.01 % ± 0.15), followed by the sun dried with (0.76 % ± 0.04) and the least recorded for solar dried (0.66 ± 0.12) although they were not significantly different (p> 0.05). The oven dried sepals were able to retain most of the fat content. A work documented by Bassey and Khan (2015) recorded 2.50 % ± 0.01 fat for *B. buonopozense* leaves. Also, Chisom et al. (2014) reported fat content of 2.18% ± 1.6, 8.94% ±1.6 and 6.93%± 2.5 for *B. buonopozense* leaves, stem and root respectively which were all higher than that recorded in this study. This shows that the sepals have a low composition of fat as compared to that of the leaves, stem and roots of *B. buonopozense.* Other documentations on fat content of medicinal plants which also have nutritional benefits such as; bitter leaves (9.05%) and Moringa (2.50%) were reported by Asalou et al. (2012) and Bamishaiye et al. (2011) respectively. This indicates that the *B. buonopozense* sepals had a higher fat content as compared to the spinach but lower than that of the bitter leaves and Moringa. Fats and oil help in the production of energy as well as the regulating of blood pressure of vital organs in the body (Iroka et al., 2014).

Carbohydrate content was the highest parameter among the dried sepals. Carbohydrates are hydrolyzed in the body to yield glucose which can be utilized immediately by the body or stored as glycogen in the muscle or liver. The least carbohydrate content was recorded for the fresh sample (11.32 % ± 0.09) where the highest was recorded for oven dried sepals (63.52 % ± 0.69). The fresh and dried sepals were significantly different ($p < 0.05$). Comparing the dried sepals, the carbohydrates content of the sun dried, oven dried and the solar dried (Table 1) were not significantly different from each other ($p > 0.05$). The oven dried sepals recorded the highest with least being the sun-dried sepals (56.62 ± 0.99). These values were higher than that recorded for *B. buonopozense* leaves by Bassey and Khan (2015) with an amount of 47.65% ± 0.01. Chisom et al. (2014) recorded 38.05% ± 0.9, 32.79% ± 2.55 and 31.42% ± 0.71 carbohydrate content for the leaves, stem and roots of *B. buonopozense* respectively. This indicates that the sepals are good source of carbohydrates which has primary usage of providing energy to the body especially the nervous system and brain due to the high levels as compared to the root, stem and leaves. Breakdown of carbohydrate leads to the production of glucose which has a pronounced effect on blood sugar level than fats and proteins whereas increase in carbohydrate intake leads to obesity (Bassey and Khan, 2015).

Ishida et al. (2000) stated that sufficient intake of dietary fiber can lower the serum cholesterol risk of coronary heart diseases, hypertension, and constipation, diabetes, colon and breast cancer. The crude fiber content of *B. buonopozense* sepals was highest in the solar dried sepals (15.39 % ± 0.50). The sun-dried sepals and oven dried sepals recorded 14.83 % ± 0.19 and 14. 63 % ± 0.34, respectively, which was not significantly different. The fresh samples recorded the least for the crude fiber (3.21 % ± 0.04). The crude fiber contents of the sepals were lower than *B. buonopozense* leaves (17.20 %) documented by Bassey and Khan (2015) as well as documentation by Chisom et al. (2014) on the leaves (16.76 % ±1.0), stem (21.34 % ± 3.2) and root (20.65% ± 3.54). Although the crude fiber of the dried sepals was lower than that of the leaves, stem and the roots of *B. buonopozense*, its crude fiber was higher than spinach (7.83 %) and moringa (10.11 %) as documented by Bamishaiye et al. (2011) and Asaolu et al. (2012) respectively.

Ash content is the inorganic residue remaining after water and organic matter have been removed by heating (Bassey and Khan, 2015). Water and volatile materials are vaporized and organic compounds are burnt in the presence of oxygen in air to CO_2, H_2O and N_2. The ash content of the fresh sepals was 1.29 % ± 0.09 which was significantly different from the dried sepals. Comparing the dried sepals, the sun-dried sepals, oven dried sepals and the solar dried sepals recorded 7.86% ± 0.05, 7.46% ± 0.13 and 7.38% ± 0.21 respectively. The sun-dried sepals recorded the highest with the least recorded for solar dried sepals which is an indication that after the

Table 2. Mineral composition (mg/100 g) of fresh and dried *B. buonopozense* sepals in dry basis.

Parameters	Fresh sepals	Oven-dried sepals	Solar-dried sepals	Sun-dried sepals	RDA
Iron	3.29±0.013^{c}	3.22±0.03^{c}	1.55± 0.03^{a}	2.46± 0.02^{b}	0.27-27
Magnesium	773.01±0.14^{c}	171.92±0.05^{a}	175.97±0.06^{a}	184.47± 0.07^{b}	30-410
Zinc	2.25±0.01^{b}	1.99± 0.01^{bc}	1.52±0.01^{a}	1.49±0.01^{a}	4-40
Calcium	422.80 ±0.18^{a}	945.54±0.25^{b}	914.62±0.13^{b}	1117.64±0.40^{c}	1000-3000
Potassium	356.26±0.05^{b}	104.60±0.27^{a}	105.42± 0.40^{a}	108.19±0.16^{a}	300-4700

Values represent mean ± SD of duplicates. Means in the same row with the same superscript are not significantly different ($p>0.05$).

incineration of the samples the sun-dried sepals had the highest amount of inorganic residue remaining. Ash contents of the sepals were higher than the leaves of *B. buonopozense* (6.30 % ± 0.01) documented by Bassey and Khan, (2015) as well as that reported for the leaves (7.26 % ± 2.6), stem (3.23 % ± 1.6) and root (1.83 % ± 0.7) of *B. buonopozense* by Chisom et al*.* (2014) although his report was not significantly different from the dried sepals. Bamishaiye et al. (2011) reported a higher amount of ash content for Moringa (8.00%) as compared to the sepals with the sepals recording a higher ash content than spinach (5.02%) documented by Asaolu et al. (2012).

Mineral composition

Table 2 Shows the mineral composition of *B. buonopozense* sepals, nutritional significant of minerals when compared with the standard Recommended Dietary Allowance (RDA) per 100 g. The sepals contained an ample amount of nutrient when compared to the standard RDA. The values of Iron and magnesium were comparable to the standard whereas the values for Zinc, Calcium and Potassium were moderately low.

Iron is an essential trace element in the human body. It plays a crucial role of Haemopoiesic control of infection and cell mediated immunity (Bhaskaran, 2001). From Table 2, the fresh sepals recorded the highest for iron (3.29 ± 0.013 mg/100 g). Comparing the dried sepals, oven dried (3.22 ± 0.03 mg/100 g) recorded the highest followed by sun dried (2.46 ± 0.02 mg/100 g) with the least being solar dried sepals (1.55 ± 0.03 mg/100g) although they were not significantly different from each other ($p < 0.05$). Values obtained were all within the range of iron by RDA (0.27 to 27 mg/ 100g). Bawa et al. (2017) recorded (3.12± 0.01) for *B. buonopozense* leaves which were higher than that recorded for the dried sepals but lower than the fresh sepals. Reduction of the iron content in the dried sepals can be attributed to the high intensity of heat used which causes the vaporization of some dissolved iron and amount of water during the drying process. Iron deficiency has been described as the most prevalent nutritional deficiency where anemia is estimated to affect more than one billion people worldwide (Trowbridge and Marlorell, 2002). In a research done by Dioxin et al. (2004), the consequence of iron deficiency causes reduction in the work capacity, impairment in behavior and intellectual performance and decrease in resistance to infections. Therefore, these sepals can be added to food in other to provide an appreciable amount of iron need daily.

Magnesium as an essential mineral plays a vital role in the human body. It is needed for enzymes that utilize adenosine triphosphate which contributes to DNA and RNA synthesis during cell proliferation (Wardlaw et al., 2004). The fresh sepals recorded the highest of (773.01 ± 0.14 mg/ 100g) which was above the range for RDA (30-410 mg/100 g). Comparing the dried sepals, sun dried sepals recorded the highest magnesium content (184.47 ± 0.07 mg/100 g) with the least recorded for oven dried sepals (171.92 ± 0.06 mg/100g); however, they were not significantly different. Values obtained were within the range of RDA (30 - 410 mg/100 g). Work done by Bawa et al*.* (2017) reported magnesium content of 37.51± 0.02 mg/100 g for the leaves of *B. buonopozense* which was lower than that recorded for the sepals of *B. buonopozense*. This is an indication that the sepals studied have a high importance in treating some deficiency of Magnesium such as convulsion, irritability and even death when substituted in diets. Magnesium is important for the release of insulin and insulin action on cells. It also decreases blood pressure by dilating the arteries and preventing abdominal heart rhythms (Wardlaw et al., 2004).

Zinc as an essential micro nutrient is needed for human growth and immune function (Black, 2003). Hotz and Brown (2004) estimated that 20% of the world's population is reported to be at risk of inadequate zinc intake. From Table 2, the fresh sepals recorded the highest of zinc content (2.25 ± 0.01 mg/100 g) which was below the standard RDA (4 to 40 mg/100 g). For the dried sepals, the oven dried sepals were able to retain a high amount of the zinc in the sepals compared to the solar dried and the sun-dried sepals with an amount of (1.99± 0.01 mg/100 g). The solar dried sepals and sun-dried sepals recorded 1.52± 0.01 and 1.49± 0.01 mg/100 g respectively. The amount of zinc recorded was

Table 3. Some anti- nutritional composition (mg/100 g) of fresh and dried sepals of *B. buonopozense.*

Parameters	Fresh sepals	Sun dried sepals	Solar dried sepals	Oven dried sepals
Oxalate	117.83±0.56^{c}	72.19±0.61^{a}	75.92±0.23^{a}	87.38±0.40^{b}
Phytates	10.26 ± 0.23^{c}	1.92±0.08^{b}	1.47±0.12^{a}	2.03±0.14^{b}

Values represent mean ± SD of duplicates. Means in the same row with the same superscript are not significantly different (p>0.05).

moderately lower as compared to the standard RDA (4 to 40 mg/100 g). Bawa et al. (2017) reported an amount of zinc present in the leaves of *B. buonopozense* as 1.87 ± 0.01 mg/100 g which was also lower as compared to the standard but not significantly different from the sepals. Sepals of *B. buonopozense* can therefore be combined with foods containing an appreciable amount of zinc in order to provide the daily RDA needed. Research done by Dioxin et al. (2004) indicated that about 20% of children less than 5 years, 28.1% of mothers and 43.9% of pregnant women in Nigeria are affected by zinc deficiency which include loss of appetite, hair loss, diarrhea, hair and skin lesions.

Presence of calcium, magnesium and potassium is known to reduce hypertension and blood pressure (Wardlaw et al., 2004). From Table 2, the fresh sepals recorded the least for calcium (422.80± 0.18 mg/100 g) which was higher than work done by Bawa et al. (2017) which recorded 87.28 ± 0.01 mg/100 g for *B. buonopozense* leaves; however, was below the standard for calcium by RDA (1000 to 3000 mg/100 g). According to National Academy of Sciences (NAS, 2010), the Tolerable Upper Intake of calcium are; Infants – (1000-1500 mg/day), Children – (2500-3000 mg/day), Adults (18 to 30 years) – (2500 mg/day), 31 to 50 years – (2500 mg/day) and 51+ years (2000 mg/day).

The oven dried, solar dried and sun-dried sepals recorded 945.10 ±0.25, 914.62 ± 0.13 and 1117.64± 0.40 mg/100 g, respectively. The sun drying of the sepals which is mostly used by the populace was able to retain the highest amount of calcium content and was within the range provided for RDA (1000 to 3000 mg/100 g). Oven dried sepals and solar dried sepals were moderately lower than the standard RDA. In improvising for the daily calcium for individuals, sepals of *B. buonopozense* can be combined with other foods which contain an appreciable amount of calcium to provide the recommended dietary allowance need for the body daily. Imbalance of calcium- phosphorus tends to cause Oesteoporosis, pyorrhea, rickets and tooth decay (Asaolu et al., 2012).

Potassium is an extracellular cation which plays an important role in humans. Its function as an electrolyte helps in the maintenance of a healthy balance of fluid in the body (Akpanyung, 2005). It is crucial for heart functioning and plays a vital role in the contraction of skeletal and smooth muscles, making it normal for digestive and muscular function. From Table 2, the fresh sepals recorded the highest potassium content (356.26 ± 0.05 mg/100g) which was within the standard RDA (300 to 4700 mg/100 g). The dried sepals recorded 104.60±0.27 mg/100 g, 105.42± 0.40 mg/100g and 108.19 ±0.16 mg/100 g for oven dried, solar dried and sun dried respectively with the oven dried retaining the highest amount of potassium. Work done by Bawa et al. (2017) reported an amount of 162± 0.01 mg/100 g of potassium present in the leaves of *B. buonopozense* which was higher than the dried sepals but lower than the fresh sepals. Some effects of low potassium intake include palpitation, abdominal cramping, bloating nausea and constipation.

Anti-nutrients

According to the report by Bawa et al. (2017), anti-nutrients reduce the bioavailability of nutrients which causes reduction in the ability of the body to use nutrients when absorbed from diet. The oxalate contents of the solar and sun-dried sepals were statistically similar. The values ranged from 72.19 – 117.83 mg / 100 g. The fresh sepals had the highest amount whereas the sun-dried had reduced amount of the oxalate among the dried sepals (Table 3). Oxalate contents in the dried sepals were higher than that recorded for the leaves of *B. buonopozense* (14.55 ± 0.01 mg/ 100g) reported by Bawa et al. (2017). According to Youssef and Mokhtar (2014), drying methods tend to decrease anti- nutrient levels in food. It was observed that the different drying methods were able to decrease the amount of oxalate present in the sepals due to the high heat intensity which disrupts the cells of the sepals therefore causing the oxalate present to vaporize as compared to the fresh sepals. The physiological tolerance level of oxalate is 2 to 5 g/day (Gafar et al., 2012) which indicated that the oxalate contents in the sepals were within acceptable level. Presence of oxalate in food would tend to bind to minerals in the gastrointestinal tract and result in crystals secreted in urine in minute crystals.

The phytates content of the fresh and dried sepal ranged from 1.47 to 10.26 g/100 g (Table 3). The phytates content of the fresh sepals was higher than that of the oven, sun and solar dried sepals. Phytate is reported to have high binding affinity to minerals such as

calcium, potassium, iron and zinc as well as macro nutrients such as carbohydrate, protein and lipids making them unavailable for digestion (Konietzny and Greiner, 2003). It was observed that the drying methods were able to reduce the Phytate level in the fresh to an appreciable level as stated by Youssef and Mokhar (2014) making them less adverse to human health. The phytate level in the sepals was higher than that in the leaves of *B. buonopozense* (10.86 mg/100 g). Although phytate level in the sepals was high they were within the range stated by Reddy (2002). According to Reddy (2002), daily intake of phytate for humans with vegetarian diet should be within the range of 2000 to 2600 mg while inhabitant of rural areas in a developing country with mixed diet should take 150 to 1400 mg per day. This is because high quantities of phytate in the food can make the nutrients in the food bio-unavailable to the human body. Anti-nutrients in the present study were higher than that of the leaves of *B. buonopozense* therefore further processing such as cooking can be used in reducing the high levels of anti- nutrients as they are affected by heat.

Conclusion

The dried sepals of *B. buonopozense* had considerably high amount of carbohydrate, ash, magnesium and calcium content as compared to the stem, leaves and roots of the plants. High amount of carbohydrate, ash content, magnesium and calcium could help improve the nutritional and health benefits in the body. Drying had a significant effect on the dried sepals especially the color and moisture content of the sepals. The different drying methods were able to retain most of the nutrients however there was less variation on the proximate and mineral composition of the dried sepals comparably to the fresh sepals. Although the fresh sepals had a higher amount of mineral, their higher content of anti- nutrients is an indication that consuming these sepals in the fresh state could hinder the bioavailability of nutrients in the body as the anti- nutrients cause the formation of insoluble compounds therefore drying the sepals is appropriate. Notwithstanding, the, solar drying method was the best in terms of quality, nutritional and cost factor.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluation of nutritional composition, bioactive compounds and antimicrobial activity of *Elaeocarpus serratus* fruit extract

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The species *Elaeocarpus serratus* is widely used as an ornamental tree and their fruits are still little explored as food. Thus, the objectives of this research were to determine the physical, chemical and antimicrobial properties of *E. serratus* fruit in order to evaluate its food potential. Therefore, this study evaluated the physical and chemical composition and the antimicrobial activity of *E. serratus* fruit. The biometry revealed an average pulp yield of 82.16%. The physical and chemical characteristics of the *E. serratus* fruit showed that the pulp has pH 2.84 and has a higher content of moisture and crude fiber. The determination of bioactive compounds showed that *E. serratus* fruit presented a good source of flavonoids, condensed tannins, carotenoids and Vitamin C. In the chromatographic analyses, the presence of β-amirin was observed as the major compound by gas chromatography. Among the main phenolic compounds, the presence of kaempferol and quercetin in the liquid chromatography method was evidenced. Additionally, ethanolic extract from *E. serratus* fruit showed antimicrobial activity against *Bacillus cereus*, *Escherichia coli*, *Salmonella cholaresuis*, *Staphylococcus aureus* and *Xanthomonas campestris.*

Key words: Antimicrobial, ceylon olive, food science, functional food, phytochemistry.

INTRODUCTION

The consumption of fruits has become increasingly important due to their potential beneficial health effects related to their nutrient composition (Albuquerque et al., 2016), such as the presence of vitamins, phenolic,

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anthocyanins, flavonoids, tannins, among others (Dimitrios, 2006). Most of these compounds have the ability to prevent cancer, cardiovascular diseases, diabetes, neurodegenerative diseases and osteoporosis (Scalbert et al., 2005). In this context, studies concerning the evaluation of bioactive compounds, especially from unconventional fruit and vegetables, may provide important data concerning their use as food or medicinal product. Besides that, the evaluation of the content of nutrients and bioactive compounds from unconventional crops may be an alternative to their enhancement, providing information concerning the discovery of significant or high levels for specific nutrients or bioactive compounds that can improve the market demand (Herraiz et al., 2016).

Elaeocarpus serratus (Elaeocarpaceae) is an ornamental tree of Asiatic origin. Furthermore, it is a medium size tree with simple leaves, commonly called by Ceylon-olive, being found at East Africa, subtropical and tropical Asia either tropical Australia (Ghani, 2003). Their fruits are considered as drupes and are used to prepare juices in order to increase the appetite of patients by stimulating secretions from taste buds (Biswas et al., 2012). The main characteristic of *E. serratus* fruit is the astringent taste when consumed *in natura*, which are related with the higher amount of alkaloids and tannins present in its composition (Sharker and Shahid, 2010).

From the literature survey, it was found that many works have been conducted to determine the bioactivity of the *E. serratus* leaves. Extracts of *E. serratus* leaves and stem bark have shown antioxidant, antibacterial, antifungal and insecticide activities (Parvin et al., 2009; Sharker and Shahid, 2010; Indhiramuthu et al., 2014). Geetha et al. (2013) have found thirty substances in the ethanolic extract of leaves of *E. serratus* with potential biological activities, which means that this plant can be considered as a good source of bioactive compounds. However, there are still few studies concerning the chemical characterization of *E. serratus* fruit, as well as their biological activities.

As it is an under-utilized fruit tree, the information about physical, chemical and pharmacological characteristics of the *E. serratus* fruit are important to improve its applicability on the food and pharmaceutical industry, contributing to the development of new products. Thus, this study is aimed to evaluate the physical and chemical composition and the antimicrobial activity of *E. serratus* fruit.

MATERIALS AND METHODS

E. serratus fruit

E. serratus fruit were collected in Dourados, Mato Grosso do Sul, Brazil (Latitude: 22°07'09.04", Longitude: 54°47'55.05"). The fruits were selected to obtain a uniform batch regarding maturity stage, size and absence of injuries, washed with tap water and sanitized with a solution of 0.66% sodium dichloroisocyanurate dihydrate (content of active chlorine 3%).

Fruits biometry and yield

The longitudinal and transversal diameters of 120 fruits were determined with the aid of a digital caliper (Mitutoyo). The mass of the whole fruit, pulp (mesocarp), endocarp and seed was determined in an analytical balance (Shimadzu-AUY220).

Physical and chemical analyses of *E. serratus* pulp

Fresh pulp was characterized as to its chemical composition according to AOAC (1975, 1984, 1997, 2000) methods: moisture content, determined using a gravimetric method in an oven with air circulation, adapted for 70°C and 24 h; total sugars, ash, total lipids, and protein using Micro-Kjedahl method and crude fiber content.

Titratable acidity (TA) was determined according to AOAC (1997), pH by direct reading on a digital pH-meter (L*abmeter*) and water activity (a_w) by direct measurement in a hygrometer Aqualab Series 3.0 (Decagon Devices Inc.). Soluble solids were determined using a manual refractometer (Tecnal).

Phytochemical composition by spectrophotometry

Flavonoids

The flavonoids content was determined in the acetonic extracts of *E. serratus* using the colorimetric method involving the reaction with aluminium chloride (Sigma, St. Louis, USA), as described by Chang et al. (2002). Extracts were prepared with 100 g of sample added in 500 mL of acetone (50% w/v) (Sigma Aldrich, Duque de Caxias, Brazil), and they were kept under constant agitation (150 rpm) for seven days. The sample was filtered and the filtrate was considered the flavonoid extract for analysis.

The extract was reacted with aluminium chloride and the readings were performed in a spectrophotometer (Biochrom – *Libra S60*) adjusted at 415 nm. Quercetin solutions at nine concentrations (0.01 to 0.2 $\mu g.\mu L^{-1}$) were reacted with sodium aluminium chloride in order to construct a standard curve. The results were expressed as milligrams of quercetin equivalent (QE mg.100 g^{-1} sample) using the quercetin (Sigma Aldrich, São Paulo, Brazil) standard curve.

Condensed tannins

The content in tannins was determined in the acetonic extracts of *E. serratus* through the colorimetric method described by Maxson and Rooney (1972). Extracts were prepared with 4 g of sample added in 20 mL of acetone (50% w/v), and they were kept under constant agitation (150 rpm) for seven days. The samples were filtered and the filtrate was considered tannins extract for analysis.

The reaction mixture consisted of 1 mL of the extract with 4 mL of vanillin (Dinâmica, Diadema, Brazil) solution (v/v) (concentrated HCl (Vetec, Duque de Caxias, Brazil)) in methanol (Vetec, Duque de Caxias, Brazil) and 8% vanillin in methanol (4%). The absorbance readings were performed after 20 min at a wavelength of 500 nm, using a spectrophotometer (Biochrom – *Libra S60*). Vanillin solution was used as the blank. The results were expressed as milligrams of catechin equivalent (CE mg.100 g^{-1} of sample) using the catechin (Sigma, St. Louis, USA) standard curve.

Carotenoids content

The fruit samples were weighed (2.5 g), macerated with the aid of Hyflosupercel (0.5 g), and then acetone at 10°C was added until the

extraction of all the pigment; the mixture was vacuum filtered and the extract was collected and transferred to a separating funnel containing 40 mL of petroleum ether (Vetec, Duque de Caxias, Brazil). The mixture was slowly washed with distilled water until complete removal of the acetone. The material was transferred into a volumetric flask and the volume completed with petroleum ether (50 mL). The absorbance readings were performed at 450 nm (Rodriguez-Amaya, 2010) using the conversion factor for β-carotene (2592), the most predominant carotenoid with provitamin A function found in olives. The results are expressed as mg.100 g^{-1} of sample.

Vitamin C content

Vitamin C content was determined by volumetric method with a solution of 2.6-dichlorophenolindophenol (Vetec, Duque de Caxias, Brazil), according to the methodology proposed by AOAC (2000). The results are expressed as mg.100 g^{-1} of sample.

Phytochemical composition chromatography

Preparation of the samples

The samples (2 mg) were fractionated with hexane:ethyl acetate (7:3 v:v) and water in proportion (1:1 v:v). The fraction soluble in hexane:ethyl acetate was analyzed by GC-MS and fraction in water by HPLC.

Gas chromatography analysis (GC-MS)

The GC-MS analysis was performed on a gas chromatograph equipped with a mass spectrometer detector (GC-MS Ultra 2010, Shimadzu Kyoto Japan). The identifications were completed by comparing the mass spectra obtained in the NIST21 and WILEY229 libraries. The compound was confirmed by comparison of standards. Standards of the stigmasterol, β-amyrin, α-amyrin, and β-amyrin acetate (Sigma-Aldrich with purity ≥97%) were prepared in the initial concentration of 500 µg/mL. The concentrations of compounds were determined by extern calibration after dilutions appropriated in the range of 0.1 to 50 µg/mL. The analysis was performed in triplicate.

Liquid chromatography analysis (HPLC)

The HPLC analysis was performed in LC (LC-6AD, Shimadzu, Kyoto, Japan) system with a diode array detector (DAD) monitored at λ = 200-800 nm. Standards of the vanilic acid, ferulic acid, p-coumaric acid, quercetin and kaempferol (Sigma, ≥97%) were prepared in the concentration initial of 1000 µg/mL. The concentrations of compounds were determined by extern calibration after dilutions appropriated in the range of 0.01 to 10 µg/mL. The analysis was performed in triplicate.

Antimicrobial activity

Extract preparation

The ethanolic extract was obtained from mixing 10 g of dried powder material with 500 mL absolute ethanol (LabSynth, Diadema, Brazil), followed by agitation at 200 rpm in shaker at 25°C during 3 h. After this period, the extracts were filtered and the remaining residue of the plant material was extracted as previously. Finally, the residue was washed with 250 mL of ethanol and the filtered extracts combined and evaporated under vacuum at 30°C, resulting in dry extract.

An amount of 40 mg of *E. serratus* dry extract was dissolved in 0.016 g.mL^{-1} ethanolic solution of polyvinylpyrrolidone (PVP) (LabSynth, Diadema, Brazil), according to methodology described by Breda et al. (2016). After dissolution, extract in PVP was evaporated under vacuum at 30°C and an aliquot of 10 mL of Mueller Hinton broth (MHB; HiMedia Laboratories, Mumbai, India) was added to the flask containing dry extract in PVP, resulting in a 4 mg.mL^{-1} solution.

Microorganisms

E. serratus extract was evaluated against the following bacterial strains: *Bacillus cereus* ATCC 10876, *Bacillus subtilis* ATCC6051, *Escherichia coli* ATCC 11775, *Listeria innocua* ATCC 33090, *Pseudomonas aeruginosa* ATCC 13388, *Rhodococcus equi* ATCC6939, *Salmonella cholaresuis* ATCC 10708, *Serratiam arcescens* ATCC 1953, *Staphylococcus aureus* ATCC 6538 and *Xanthomonas campestris* ATCC 13951. All the strains were obtained from the American Type Culture Collection, Manassas, VA, USA and cultivated in Nutrient Agar (NA; HiMedia Laboratories, Mumbai, India) at 37°C for 24 h and maintained at 4°C.

Minimal inhibitory concentration (MIC)

Bacteria strains were grown at 37°C for 24 h in Nutrient Agar (NA) plates. Inoculum for antibacterial assays were prepared by diluting the scraped cell mass in sodium chloride solution 0.9% and adjusted in spectrophotometer Shimadzu UV-Mini 1240 (Shimadzu Co., Kyoto, Japan) to an absorbance between 0.08 and 0.10 at 625 nm (corresponding to 1.5×10^8 UFC.mL^{-1}). These suspensions were diluted to 10^5 UFC.mL^{-1} in MHB.

MICs were performed in tissue test plates (96 wells) containing MHB. Diluted extract was transferred into the first wells (second column) and serial dilutions were performed up to 12th column to obtain concentrations ranging between 1.95 and 2000 µg.mL^{-1}. Then, the bacterial suspensions were transferred into the plates and incubated at 37°C for 24 h. After incubation, 50 µL of 0.5% triphenyltetrazolium chloride (TTC; Vetec, Duque de Caxias, Rio de Janeiro, Brazil) (w/v) solution was added into each well and re-incubated for 2 h. MIC was determined as the lowest concentration of the *E. serratus* extractable to inhibit the development of red color in wells (NCCLS 2003). Chloramphenicol (Sigma-Aldrich, St. Louis, USA) was used as positive control.

Minimal bactericide concentration (MBC)

In order to determine the MBC, an aliquot of 10 µL of each incubated well of MIC and higher concentrations was sub cultured on Petri dishes containing NA and incubated at 37°C for 24 h. MBC was defined as the lowest concentration of extract that allowed no visible growth on the specific solid medium.

To determine the nature of the bactericidal effect of extracts, the MBC: MIC ratio was calculated according to Donlan and Costerton (2002). The extract was considered bactericidal when MBC: MIC ratio was between 1:1 and 2:1 and bacteriostatic when the ratio was higher than 2:1.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by the Tukey test was performed using GraphPad Prism (Version 6.0 – GraphPad Software) in order to evaluate possible differences between groups.

Table 1. Biometry and yields of *E. serratus* fruit.

Evaluated parameter	Mean ± Standard deviation
Longitudinal diameter (mm)	41.55 ± 2.31
Transversal diameter (mm)	27.26 ± 1.47
Whole fruit weight (g)	19.45 ± 2.80
Pulp weight (g)	15.98 ± 0.70
Seed weight (g)	3.48 ± 2.33

RESULTS AND DISCUSSION

Biometry and yield

Biometric features and fruit yield are reported in Table 1. The results showed an average longitudinal diameter of 41.55 mm, average cross-sectional diameter of 27.26 mm and the total weight average 19.45 g, the pulp represent the equivalent of 82.16% of the whole fruit and 17.89% of the weight is a seed. The amount of pulp is an important feature, reflecting in the appreciation of the fruit extractivism. Thus, the higher pulp yield found in this work shows a promising potential for industrial use of *E. serratus* fruits.

Olives fruits produced by *Olea europaea* L. tree are often used for production of oil and canned olives. Their fruits generally show biometric values for longitudinal diameter between 14.17 and 18.15 mm and for transversal diameter between 10.66 and 13.99 mm and an average pulp yield around 83.25% (Nogueira, 2012). Knowledge of biometric values and pulp yield of *E. serratus* allows its use for technological development and use on an industrial scale. According to Machado et al. (2015), biometric characterization enables to evaluate the genetic variation between populations of the same species and their relationships with environmental factors.

Physical and chemical characterization

Physical and chemical evaluations of the olive fruits are presented in Table 2. The physical and chemical evaluation shows acidity value of 1.78 lactic acid.100 g^{-1}, pH of 2.84, water activity of 0.98 and soluble solids with a value of 2.33°Brix. According to Lehkoživová et al. (2009), the found value of pH defines the pulp of *E. serratus* fruit as an acid food (pH < 4.5), which is confirmed by the acidity value.

In chemical assessment (Table 2), the moisture content was found to be 84.62 g.100 g^{-1}, indicating high moisture content characteristic of tropical fruit, which is corroborated by the high value of a_w. The high value of water activity characterizes it as a highly perishable food (Fontana, 1998). The ash content was of 6.1 g.100 g^{-1}, which is consistent with the values of ash of the most land vegetables (5 to 10%, dry weight) (United States Department of Agriculture, 2001). The low total sugar content (3.15 g.100 g^{-1}) was expected, being characteristic of the olive tree fruit, while the lipid constituents (1.10 g.100 g^{-1}) were below those different fruits of the olive tree (International Olive Council, 2015).

The values of protein and crude fiber were 4.92 and 17.50 g.100 g^{-1}, respectively. The protein content is in accordance with other olive species which constituted 2.9 to 5.3 g.100 $g^{-1}$of protein (Nogueira, 2012). Concerning the crude fiber content, *E. serratus* fruit may be considered a good source of fibers. The dietary fiber intake recommendation for adults is >25 g/day (Nishida et al., 2004). In other words, the consumption of 100 g of the pulp from *E. serratus* fruit (approximately 6 fruit) could provide 70% of the necessary fiber's amount for an adult.

Phenolic compounds, carotenoids and Vitamin C

Besides the basic nutrition, the fruit presents in their composition some bioactive compounds that exerts an important role in biological functions for humans, such as chronic diseases prevention and maintenance of immune system (Liu, 2004, 2013). Thus, the quantification of these compounds is of utmost importance and the results of bioactive compounds content found in *E. serratus* fruits are shown in Tables 3, 4 and 5.

According to McClements and Decker (2010), phenolic compounds may be found in plants as simple phenolic, phenolic acids, anthocyanins, cinnamic acid derivatives, flavonoids and tannins, whose structures allows free radicals scavenging activity. In plants, these compounds are believed to be related to protection against phytopathogens or insects (Chen et al., 2013), as well as tannins, which can act as a natural antimicrobial agent, increasing the plant resistance against pathogens (Scalbert, 1991).

As shown in Table 3, *E. serratus* fruits show high amount of flavonoids and condensed tannins, whose values were 120.49 QE mg.100 g^{-1} and 16142.40 CE mg.100 g^{-1}, respectively. This value is in accordance with the values of phenolic compounds found by Machado et al. (2013) in olives cv. Cobrançosa under three irrigation regimes and on three different picking dates.

The carotenoids and vitamin C contents (Table 3) were

Table 2. Physical and chemical characteristics of *E. serratus* pulp.

Evaluated parameter	Mean ± Standard deviation
Titratable acidity (lactic acid.100 g^{-1})	1.78 ± 0.01
pH	2.84 ± 0.01
Water activity	0,98 ± 0.00
Soluble solids (°Brix)	2.33 ± 0.11
Moisture content (g.100g^{-1})	84.62 ± 0.00
Ash (g.100g^{-1})	6.01 ± 1.06
Total Sugars (g.100g^{-1})	3.15 ± 0.05
Lipids (g.100g^{-1})	1.10 ± 0.00
Protein (g.100g^{-1})	4.92 ± 0.17
Crude fiber (g.100g^{-1})	17,50 ±0,65

Table 3. Flavonoids, condensed tannins, carotenoids and vitamin C contents of *E. serratus* pulp.

Evaluated parameter	Mean ± Standard deviation
Flavonoids (mg of QE.100 g^{-1})	120.49 ± 0.01
Condensed tannins (mg of CE.100g^{-1})	16142.40 ± 0.00
Carotenoids (mg.100 g^{-1})	4.97 ± 0.90
Vitamin C (mg.100 g^{-1})	5.93 ± 0.00

Table 4. Compounds identified in *E. serratus* pulp by GC-MS.

Retention time (min)	Compounds	Molecular mass	mg/g ± SD
17.02	Stigmasterol	412	3.6 ± 0.1^{a}
17.93	β-amyrin	426	12.5 ± 0.6^{c}
18.45	α-amyrin	426	4.0 ± 0.2^{b}
19.65	β-amyrin acetate	468	4.3 ± 0.2^{b}

Medium results ± standard deviation. Mean values followed by different letters indicate significant differences ($p < 0.05$).

4.97 mg.100 $g^{-1}$and 5.93 mg.100 g^{-1}, respectively. The presence of carotenoids and vitamin C in fruits is often associated with antioxidant activity. Carotenoids are precursors of vitamin A and can prevent the development of chronic diseases (Singh et al., 2012), whereas the consumption of fruit rich in vitamin C is associated with the prevention of cardiovascular diseases and obesity (González-Molina et al., 2010; Ramful et al., 2011). Thus, the supplement of these nutrients from dietary intake of fruits and vegetables is vital, since the human body is unable to synthesize them (Leong and Oey, 2012).

The gas and liquid chromatography phytochemical composition results are shown in Tables 4 and 5, respectively. The presence of β-amyrin (12.5 mg.g^{-1}), β-amyrin acetate (4.3 mg.g^{-1}), α-amyrin (4.0 mg.g^{-1}) and Stigmasterol (3.6 mg.g^{-1}) were identified and quantified by gas chromatography. The component found in greater quantity was the β-amyrin and α-amyrin. Studies with α-amyrin and β-amyrin indicate its potential as a medicinal agent with hepatoprotective and anti-inflammatory activity (Oliveira et al., 2005; Holanda Pinto et al., 2008).

The high-performance liquid chromatography method in the *E. serratus* pulp extract revealed kaempferol (13.4 mg.g^{-1}) and quercetin (12.9 mg.g^{-1}) as the compounds expressed in greater amounts, followed by vanillic acid, ferulic acid and ρ-coumaric acid. Studies with isolated kaempferol and quercetin demonstrated anti-inflammatory activity in acute and chronic inflammatory processes in experimental models *in vivo*, however, they present lower antimicrobial activity than those of the other phenolics (Guardia et al., 2001; Morikawa et al., 2003; Fattouch et al., 2007).

Antimicrobial activity

Antimicrobial activity of *E. serratus* extract evaluated in the concentration range between 1.95 and 2000 µg.mL^{-1}

Table 5. Compounds identified in *E. serratus* pulp by HPLC.

Retention time (min)	Compounds	Molecular mass	mg/g± SD
7.95	Vanillic acid	168	4.9 ± 0.1^{b}
13.48	p-coumaric acid	164	3.3 ± 0.2^{a}
17.28	Ferulic acid	194	3.6 ± 0.1^{a}
35.33	Quercetin	302	12.9 ± 0.3^{c}
41.43	Kaempferol	286	13.4 ± 0.3^{c}

Medium results ± standard deviation. Mean values followed by different letters indicate significant differences ($p < 0.05$).

Table 6. Antimicrobial activity of ethanolic extract from *E. serratus* pulp.

Microorganism	MIC ($\mu g.mL^{-1}$)	MBC ($\mu g.mL^{-1}$)	MBC:MIC ratio
B. cereus	1600	2000	1,25:1
E. coli	1300	2000	1,5:1
S. cholaresuis	1000	1000	1:1
S. aureus	2000	2000	1:1
X. campestris	500	1000	2:1

was only observed for *B. cereus*, *E. coli*, *S. cholaresuis*, *S. aureus* and *X. campestris*, with MIC values between 500 and 2000 $\mu g.mL^{-1}$ (Table 6). According to Duarte et al. (2005), *E. serratus* pulp extract presented elevated inhibition against *X. campestris*, moderate inhibition for *E. coli* and *S. cholaresuis* and weak inhibition against *B. cereus* and *S. aureus*. Regarding to MBC, the extract showed bactericidal effect for all inhibited microorganisms (Donlan and Costerton, 2002).

The antimicrobial activities of *E. serratus* fruit may be explained by higher content of phenolic compounds, flavonoids and tannins in its composition. Flavonoids are common polyphenolic compounds widely found in edible plants, especially fruit, vegetables, tea and wine and are categorized into several subgroups (Puupponen-Pimiä et al., 2001). According to Ammar et al. (2013), the probable mechanism of flavonoids on antimicrobial activity is due to its properties of complexation with soluble extracellular proteins, resulting in microorganism cell wall break, allowing the inhibition of important enzymatic pathways as P450 oxidases dependents, with specific action in blocking steroid hydroxylases dependents.

Condensed tannins are compounds constituted by oligomeric or polymeric various flavonoid units that consists of two phenolic rings with different vicinities (Çakar et al., 2016). These compounds are responsible for defending plants against insects and pathogens attacks (Haslam, 1988). According to Scalbert (1991), the action mechanism of tannins includes action on membranes, enzyme inhibition, substrate or metal ions deprivation. Some hydrolysable tannins have demonstrated antimicrobial activity.

In conclusion, *E. serratus* fruit has high pulp yield and high moisture content, which justify the feasibility and necessity to obtain a processed product. Moreover, the fruit exhibits in their composition high concentrations of bioactive compounds such as flavonoids, condensed tannins and carotenoids. The chromatographic analysis (GC and HPLC) identified β-amyrin, kaempferol and quercetin as majoritary compounds found in *E. serratus* fruits. Ethanolic extract of *E. serratus* fruit shows antimicrobial activity against microorganisms of importance mainly in food, which is possibly related to the presence of flavonoids, hydrolysable tannins and phenolic compounds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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