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African Journal of Food Science

Full Length Research Paper

Pesticide residues in locally produced grape wine in Tanzania: a case study of Dodoma urban and Bahi districts

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The aim of this study was to assess the level of pesticide residues in locally produced grape wine in Tanzania. Fifty samples of grape wine from different locations in Dodoma urban and Bahi district were analyzed to determine the presence of 49 pesticides using the quick, easy, cheap, effective, rugged and safe (QuEChERS) multi-residue extraction, followed by gas chromatography-tandem mass spectrometry (GC-MS/MS). Twenty-two pesticides were detected among the 49 pesticides analyzed. The selected 49 pesticides was based on its use in grape cultivation which were reported by grape farmers in the study location which includes fungicides, insecticides and herbicides. The pesticides whose concentrations exceeded the maximum residue levels (MRL) were: Pyroquilon, ethofumasate, chlorobeb, azobenzene and cycloate in 38, 33, 46, 14 and 1 wine samples, respectively. Of the samples analyzed 9 (18%) contained one pesticide, 8 (16%) contained two different pesticides, 23 (46%) contained three different pesticides 8 (16%). The results indicated the occurrence of pesticide residues in grape wine produced in Dodoma urban and Bahi districts, Tanzania, and pointed to an urgent need to develop comprehensive intervention measures to reduce potential health risk to consumers.

Key words: Pesticides residues, grape wine, food safety, maximum residue levels (MRL).

INTRODUCTION

Grapes (*Vitis vinifera*) belong to the *Vitaceae* family and are one of the world's most important economic fruit crops (Kocher and Nikhanj, 2019). This crop has many uses as it can be eaten raw or can be used for the manufacture of wine, jam, juice, jelly, grape seed-extracts, raisins, vinegar and grape-seed oil (Kalimang'asi et al., 2014; Grimalt and Dehouck, 2016). Approximately 4,744 tons of grapes were produced in Tanzania in the year 2018 while the largest producer of grapes in the world for the year 2018 was China with 13,494,811 tons (FAOSTAT, 2020). Grape production is the mainstay for many farmers in the Dodoma region, including Dodoma city, Bahi, Chamwino and Kongwa districts (Kalimang'asi et al., 2014). The excellence of wine depends on the quality of grapes and to obtain high-quality wines, grapes at the correct stage of ripeness and free from parasites must be used (Caboni and Cabras, 2010). In addition, the quality of wine

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depends on the vinification process, the geographical origin of the grapes as well as the varietal composition of the grape wort; therefore, grapes traceability is important in quality control and suppliers' information (Espineira and Santaclara, 2016).

There is increasing interest in health and safety aspects associated with pesticide use and the presence of their residues in processed foods and beverages (Lian et al., 2010). Pesticide use in viticulture is a major issue for grape protection, increased productivity and wine quality (Martins et al., 2011). Wine has beneficial effects on human health, if moderately consumed, including prevention of heart and circulatory diseases, favorable to the fight against obesity, provides greater longevity and quality of life, creates barriers to the development of dementia, the meal accompanied by wine results in a better digestion, anti-infective effect, can prevent blindness, have anti-inflammatory action and can alleviate lung diseases (Wurz, 2019).

However, wine may also contain components, which have detrimental effects on human health such as pesticide residues, as a result of their use in viticulture and those that can remain on grapes after harvest and may be transferred to the wine (Grimalt and Dehouck, 2016; Cepo et al., 2018). Grey mould (*Botrytis cinerea*), powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) are responsible for serious yield loss in the wine sector resulting in significant commercial losses (Weng et al., 2014). In addition to an important crop yield loss, these diseases can also reduce the wine quality by providing an unstable colour, oxidative damages, premature aging, unpleasant flavours, and clarification difficulties (Rodriguez et al., 2020). The correct use of these phytosanitary products has no adverse effects on public or environmental health, but indiscriminate treatment applied without respecting the safety periods or the recommended doses may result in residues being present in the grapes used for winemaking these fungicides may be passed on to the must and then to the wine during fermentation (Vaquero-Fernandez et al., 2012).

Grapes are usually harvested and directly used for follow-up fermentation without washing or other treatment to reduce pesticides residues, this may lead to their presence in wine offered commercially or public consumption, therefore, their determination in grape wine is designed to ensure the safe consumption of the important beverage in the community (Jiang et al., 2009).

In order to ensure food safety for consumers and protect human health, many organizations and countries around the world have established Maximum Residue Limits (MRLs) for pesticides in food commodities (Cepo et al., 2018). The MRL is the maximum level of a pesticide residue (expressed in mg/kg) which is legally permitted in food or animal feed (Jallow et al., 2017). The MRLs which specify the maximum concentration of a pesticide that can exist in certain agricultural commodities were regulated by many nations to promote good agricultural practice (GAP) (Lekei et al., 2016). Furthermore, current agricultural practices are based on the wide use of chemical pesticides that have been associated with negative impacts on human health, wildlife and natural environment (Goulson, 2013; Nicolopoulou-Stamati et al., 2016). This, in turn, raises concerns among consumers and producers due to possible health hazards as well as the impact of these residues on the sensory quality of wines. These facts emphasized the need for continuous monitoring of pesticide residues in wine (Cepo et al., 2018).

The study aims to establish the levels of pesticide residues in locally manufactured wine, particularly in the leading producer region Dodoma, in order to determine its quality and safety. This study will serve as a basis for awareness creation to farmers, consumers, processors and other stakeholders and enable the government to regulate the sector and advocate use of best practices and prevent economic losses.

MATERIALS AND METHODS

Sample collection

Fifty bottled grape wine samples were collected from all 15 wine processors, branded (with label) and unbranded (without label sold in bulk) in Dodoma urban (capital of Tanzania) and Bahi district in Dodoma region, these districts were selected purposively due to high production of grapes. Approximately one liter of each branded and unbranded wine 36 red wines and 14 white wine from small, medium and large scale industries was packed into sterile amber plastic containers, sealed, coded, stored at -2°C and transported to the Tanzania Bureau of Standards laboratory in Dar es Salaam. The samples were stored at -2°C for further analysis.

Sample extraction

Quick, easy, cheap, effective, rugged and safe (QuEChERS) method (Anastassiades et al., 2003; Jiang et al., 2009; Pazzirota et al., 2013; Wang and Telepchak, 2013) with some modifications were used to extract pesticide residues from the samples. 10 ml of sample was transferred into an empty 50 ml centrifuge tube polytetrafluoroethylene (PTFE), followed by 10 ml of acetonitrile (Romil Ltd The Source Convent Drive Waterbeach Cambridge Gb-Cb259QT) and vortexed for 1 min using Stuart auto vortex Mixer (Stuart Scientific Co. Ltd-England). A blank sample was fortified with 10 µl of standard pesticides.

A sachet made up of 4 g of magnesium sulfate anhydrous, 1 g of sodium chloride 1 g of trisodium citrate dihydrate, and 0.5 g of disodium hydrogen citrate sesquihydrate was added (Quercher Extract Pouch, En Method shaked and vortexed for 1 min, then centrifuged for 5 min at 4,000 rpm using Centrifuge 5810R (Eppendorf Ag Co. Ltd Harmburg, Germany).

Sample clean up

Exactly 7.5 ml of the supernatant (upper layer) was transferred

into 15 ml PTFE, then 750 mg of $MgSO_4$ (Surechem Products Ltd Suffolk, England) and 150 Primary Secondary Amine (PSA) was added into PTFE tube. The mixture was then vortexed for one minute then centrifuged at 4,000 rpm for 5 min. Then 5 ml of extracted solution was added into a test tube and evaporated to near dryness under nitrogen at the temperature below 42°C. Then reconstituted to 1 ml with toluene and homogenized for 5 s and transferred into autosampler vials.

Blank sample preparation

A blank sample was prepared using distilled water which was prepared using Evoqua Water Technologies PTE LTD Farrernberg-Germany) and also wine from South Africa which was free from pesticide residues used as black and the same procedures for extraction was followed.

Standard preparation

Individual pesticides solution was prepared by 10 mg of each standard pesticides purchased from (Sigma- Aldrich Co. Ltd. Steinheim am Albuch - Germany) were dissolved in 10 ml acetone and stored at -20°C, then intermediate Standard solution was prepared by 100 µl stock standard solution which was brought to 10 ml volumetric flask mixed with acetone and stored at 2°C. Working solution was prepared by 10 µl which was dissolved to 10 ml acetone and six levels of an intermediate standard solution of each pesticide were prepared to maintain the same matrix concentration for the preparation of calibration curve and stored at 2°C.

Gas chromatography-tandem mass spectrometry (GC-MS-MS) conditions

An Agilent 7010 GC/MS Triple Quad (Agilent Technologies Co. Ltd. Waldbronn-Germany) with a 7693 Autosampler with a capacity of 150 samples at a time was used for the analysis of pesticide. GC-Column –J&W HP-5MS UI (15 m × 0.25 mm i.d. × 0.25 µm film thickness) was used for GC separation, with helium (99.99%) as the carrier gas at a constant flow rate of 1.5 ml/min. The column inlet temperature was initially at 60°C (hold for 1 min), increased to 120°C at rate of 40°C/min, then to 310°C at a rate of 5°C/min, the total holding time was 40.50 min. Quantification and results calculation were done by mass hunter software using the following formula.

Concentration of each analyte (mg/L) = Concentration from curve × dilution factor

Where by, concentration from curve = Peak area of the analyte/Peak area of internal standard.

Linearity was studied in the range 0.5 - 100 mg/L with five calibration points (0.5, 10, 25, 50, 75 and 100 mg/L) by matrix-matched standard calibration which were spiked with the corresponding volume of the working solution. Linear calibration graphs were constructed by least-squares regression of concentration versus relative peak area (analyte/IS) of the calibration standards. Linearity values, calculated as determination correlation coefficients (R^2), were in the range 0.986-0.999 (Table 1).

Pesticides analysis

Wine samples were analyzed at the Tanzania Bureau of Standards (TBS) quality control laboratory, Dar es Salaam. The samples which were stored at -2°C were equilibrated to room temperature (25°C) before analysis.

Statistical analysis

Data were analyzed using R- version 3.5.0 (2018). Analysis of variance (ANOVA) was used to test significant differences on the pesticide residues concentration (mg/L) amongst the brand, color of wine and among the categories of processors (Large, Medium and Small Scale). Statistical Package for Social Sciences (IBM SPSS® Version 20) was used to test compliance of pesticide residues detected to European Union Database MRL.

Method validation

Percentage recovery of pesticides

Recovery studies were performed to examine the efficacy of extraction and clean up. Grape wine samples were spiked with known concentration of the pure pesticides standard solution and extraction and clean-up were performed as described earlier in the process of sample extraction.

$$\%\ \text{Recovery} = \frac{Spiked - Unspiked\ Conc}{expected\ Conc} \times 100\%$$

The sensitivity of the method was obtained by determining the percent recovery of pesticides by calculating the percentage recovery of pesticides spiked samples and unspiked samples. Accuracy was evaluated in terms of recovery, and the satisfactory recoveries were from 70 to 110%) indicating the suitability and good performance of GC-MSMS). To test the performance of an analytical method, the following criteria have to be considered: pesticide recoveries should be in the range 70-120%. The precision of the method was studied; intra- and inter-day variations were estimated and expressed as relative standard deviation (RSD) of the signals or peak areas for each analyte following an analysis of 0.1 mg/L standard working solution injected five times consecutively on the same day and injected five times over four consecutive days. The results in Table 1 showed that inter-day variation of peak areas for 49 pesticides were in the range of 1.26- 10.24%, and intra-day variations of 1.68-14.22% (Vaquero-Fernandez et al., 2012; Machado et al., 2016).

Limit of detection and quantification

The qualitative pesticides were based on the retention time of peak and abundance ratios of the selected ions for each pesticides. Limit of detection (LOD) and limit of quatification (LOQ) was determined based on the signal to noise ratio of quantifier transition of all analyte. The retention time, precusor and product ion, LOD, LOQ of the analyzed pesticides on the samples are presented in Table 1.

RESULTS AND DISCUSSION

Pesticide residues in grape wine

Results indicated that, twenty-two pesticides were detected among the 49 pesticides analyzed (Figure 1).

Table 1. Parameters for the simultaneous determination of 49 pesticide residues in grape wine by GC-MS/MS.

Pesticides	LOD (mg/L)	LOQ (mg/L)	Retention time (min)	Precursor ion	Production	R^2	Precision RSD (%)		Recovery
							Intra day	Inter day	
Azobenzene	0.002	0.008	13.864	77.0	51.0	0.997	2.36	4.32	78.30
Butafenacil	0.004	0.01	33.762	331.0	180.0	0.998	6.72	7.38	82.46
Chlormephos	0.004	0.01	9.719	121.0	65.0	0.998	4.75	4.46	88.72
Chloroneb	0.003	0.009	11.426	206.0	191.1	0.999	6.78	8.22	90.20
Chlorothalonil	0.004	0.009	17.862	263.8	168.0	0.987	14.22	10.24	89.24
Chloropropylate	0.003	0.008	25.856	139.1	111.0	0.988	5.38	5.04	75.32
Chlorpyrifos	0.004	0.009	21.094	197.0	169.0	0.997	3.68	7.54	94.42
Cloquintocet-mexyl	0.003	0.009	29.987	192.0	190.0	0.987	9.25	7.86	74.27
Cyanofenphos	0.002	0.008	27.263	169.0	141.1	0.989	8.23	8.78	80.06
Cycloate	0.004	0.009	13.828	83.0	55.1	0.996	9.84	10.13	96.58
Cyfluthrin I	0.003	0.009	34.401	162.9	90.9	0.987	1.68	1.26	77.82
Cypermethrin III-beta	0.003	0.008	34.458	163.0	91.0	0.986	7.56	9.62	92.16
Cyphenothrin II	0.004	0.008	31.401	181.0	152.1	0.995	2.35	4.79	86.48
Cyproconazole	0.003	0.01	25.820	139.0	111.0	0.986	6.37	7.24	110.46
o,p'-DDT	0.004	0.008	24.771	235.0	165.2	0.997	9.14	9.84	84.82
DEET	0.002	0.009	11.383	119.1	91.0	0.997	4.78	6.82	82.63
Demeton-S- Methyl-sulfon	0.003	0.008	19.400	169.1	125.1	0.987	7.82	9.46	72.78
Deltamethrin	0.004	0.009	37.861	252.9	93.0	0.996	10.22	9.88	92.78
Diclobutrazole	0.002	0.009	25.270	269.8	158.9	0.991	5.68	5.32	86.40
Dichloroaniline	0.003	0.008	9.595	161.0	90.0	0.986	4.74	7.66	74.86
Diphenamid	0.004	0.008	21.788	167.1	165.1	0.986	8.45	8.25	91.76
Diphenylamine	0.004	0.01	13.803	168.0	167.2	0.987	7.69	9.64	75.34
Endosulfan I	0.003	0.008	22.094	197.0	169.0	0.995	6.65	4.76	82.74
Esfenvalerate	0.004	0.009	36.644	167.0	125.1	0.999	9.36	9.88	76.90
Etaconazole	0.003	0.01	24.757	173.0	145.0	0.991	2.58	4.74	78.26
Ethofumesate	0.003	0.01	19.669	161.0	105.1	0.986	7.44	9.42	82.23
Ethoxyquin	0.002	0.008	14.978	202.1	145.1	0.996	9.16	10.22	86.43
Flamprop-isopropyl	0.004	0.008	26.278	105.0	77.1	0.988	7.06	8.96	78.96
Fenvalerate II	0.005	0.009	36.644	167.0	125.1	0.999	5.67	7.09	85.82
Hexaconazole	0.002	0.008	24.269	231.0	175.0	0.986	9.12	9.84	90.56
Leptophos	0.004	0.008	30.432	171.0	77.1	0.987	5.71	9.04	78.09
Malaoxon	0.003	0.01	19.343	126.0	99.0	0.998	8.25	7.78	86.75
Metalaxyl	0.002	0.008	19.766	234	146.1	0.996	6.54	6.47	112.26
Mevinphos	0.003	0.009	9.976	125.0	109.0	0.991	9.34	7.46	76.54
Metazachlor	0.003	0.008	20.943	133.1	132.1	0.987	3.68	5.44	87.66

Table 1. Contd.

Methoxychlor-p,p’	0.004	0.01	29.819	227.0	141.1	0.996	7.44	6.72	88.75
Nitrapyrin	0.003	0.009	10.367	194.0	133.0	0.997	9.18	10.06	91.64
Permethrin II- trans	0.004	0.01	33.107	182.9	168.1	0.994	2.34	2.78	71.06
Pentachlorobenzene	0.004	0.009	11.751	249.9	215.0	0.997	4.06	8.56	74.88
Phorate –sulfoxide	0.003	0.008	18.777	96.9	64.9	0.987	11.08	9.74	92.84
Phosmet	0.003	0.01	29.317	160.0	77.1	0.986	6.76	8.94	78.96
Piperophos	0.004	0.009	29.618	320.0	122.0	0.987	9.16	8.26	82.44
Pyrazophos	0.005	0.009	30.029	221.0	193.1	0.988	7.66	8.48	76.98
Pyroquilon	0.003	0.008	16.048	129.9	77.0	0.986	2.66	4.36	80.98
Sulfotep	0.004	0.008	15.290	201.8	145.9	0.999	7.68	9.12	72.86
Tefluthrin cis	0.004	0.009	17.858	177.1	127.1	0.999	9.84	8.36	91.64
Tetramethrin I	0.002	0.009	29.427	164.0	77.1	0.996	4.76	9.14	88.72
Thiometon	0.003	0.008	13.935	125.0	79.0	0.987	3.62	7.98	95.84
Tebuconazole	0.004	0.01	27.514	125.0	89.0	0.986	8.48	8.22	78.66

These included: mevinphos, thiometon, diethyl-m-toluene (DEET), chloroneb, dipnenylamine, pyroquilon, metalaxyl, Chlorpyrifos, endosulfan 1, o, p´-DDT, cryproconazole, tebuconazole, deltamethrin, cypermethrin-beta, cyanofenophos, ethofumesate, metazachlor, azobenzene, cycloate, phosmet, chloropropylate and chlormephos. Among the detected pesticides, there were five pesticides that exceeds the MRL according to the European Union pesticides specification of grape wine. These pesticides included pyroquilon whose concentation exceeded MRL in 38 wine samples, ethofumesate exceeded MRL in 33 samples, chloroneb exceeded MRL in 46 sample, azobenzene exceeded MRL in 14 samples and cycloate exceeded MRL in 1 sample.

Among the 50 wine samples analysed 9 (18%) contained one pesticide, 8 (16%) contained two different pesticides, 23(46%) contained three different pesticides 8 (16%) contained four diffent pesticides among those exceeds MRL (pyroquilon, ethofumesate, chloroneb, azobenzene and cycloate). The incidence of having multiple pesticide residues in grape wine sample was reported in different studies. The study conducted by Cus et al. (2010) on pesticide residues and microbiological quality of bottled wine indicated that, among 25 wines, two wines did not contain residues of pesticides analyzed, eight wine samples contained residues of one pesticide, four wine samples contained the residues of two pesticides, seven wine samples contained the residues of three, and four wine samples contained the residues of four pesticides.

Also the study conducted by European Food Safety Authority in the year 2018, on pesticide residues in food, it was reported that, 1,317 samples of wine (red or white) made from grapes were analyzed, where 768 samples (58.3%), had no quantifiable pesticide residues, while 549 samples (41.7%) contained one or several pesticides in quantified concentrations and multiple residues were reported in 263 samples (20%); up to 10 different pesticides were reported in an individual wine sample (EFSA, 2018). The incidence of having multiple pesticide in grape wine were also reported by (Duca et al., 2012; Esteve-Turrillas et al., 2016).

Presence of multiple pesticides in grape wine might be due to unfavourable weather conditions most commonly occurring in geographical area which favours the development of pests and diseases in the vineyards, so more intense application of different types of pesticides are generally required in order to safeguard grape quality, which eventually may result in greater fungicide residues in wines (Esteve-Turrillas et al., 2016; Jallow et al., 2017).

All pesticides that were not detected were removed during data analysis therefore twenty-two pesticides was used for further statistical analysis such as ANOVA. The study revealed that there was significant difference at ($p<0.05$) on pesticide concentration when comparing

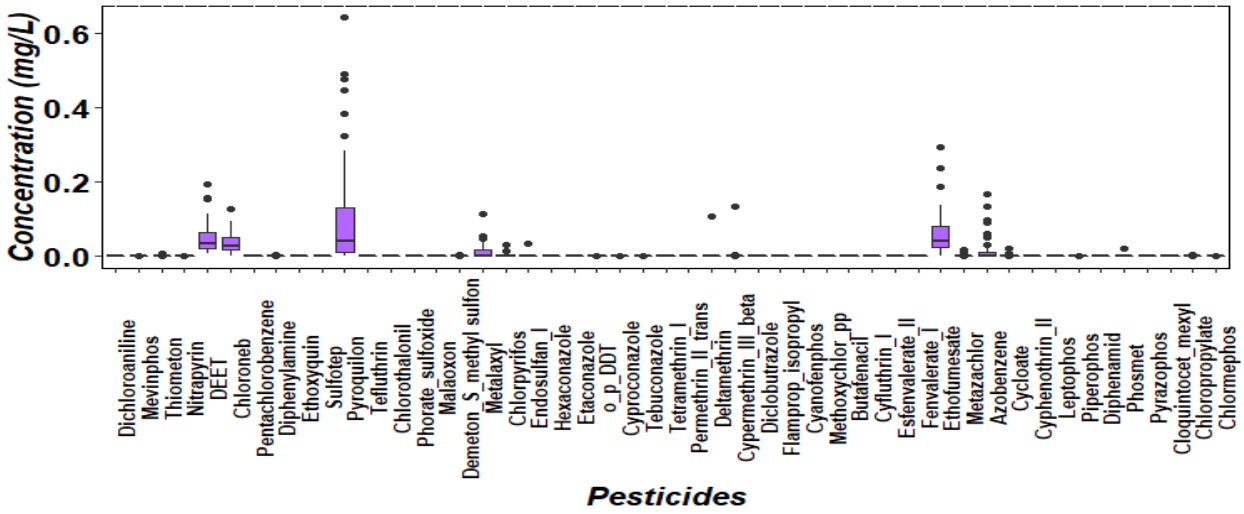

Figure 1. Box plot showing all 49 analyzed pesticides.

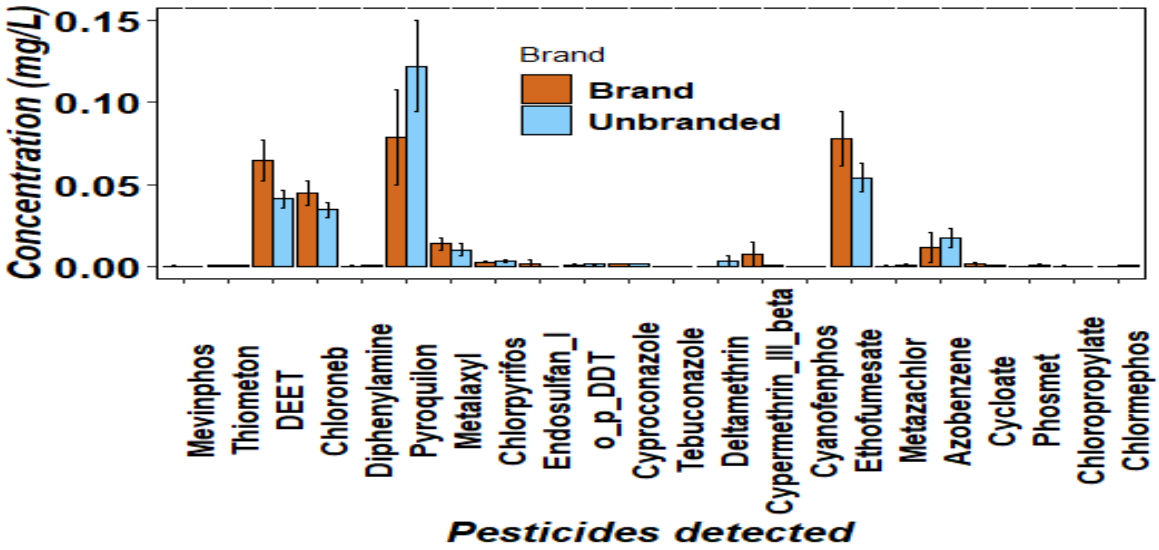

Figure 2. Bar plot showing concentration of pesticides according to brand and unbranded.

according to branded and unbranded grape wine (Figure 2). There was high concentration in unbranded wine compared to branded wine especially for Pyroquilon, Chlorpyrifos, o,p'-DDT, Deltamethrin and Azobenzene. According to the survey conducted to wine processors with the branded wine (labeled) they have high production capacity and they undergoes wine clarification and fermentation take long time compared to unbranded wine processors which help to reduce pesticide residues in grape wine. Wine processors of unbranded wine they usually depend on spontaneous fermentation by using natural fermenting yeasts present in grapes and they usually sell their products in bulk without clarification stage. Wine clarification help to reduce the level of pesticides residues. Among the clarifying substances commonly used in wine (bentonite, charcoal, gelatin, polyvinylpolypyrrolidone, potassium caseinate, and colloidal silicon dioxide), charcoal allowed the complete elimination of most pesticides, especially at low levels, whereas the other clarifying substances were ineffective (Cabras and Angioni, 2000). Also during the fermentative process, yeasts can cause the disappearance of pesticide residues by degradation or absorption at the end of the fermentation when yeasts are deposited as lees (Caboni and Cabras, 2010). It was also observed that

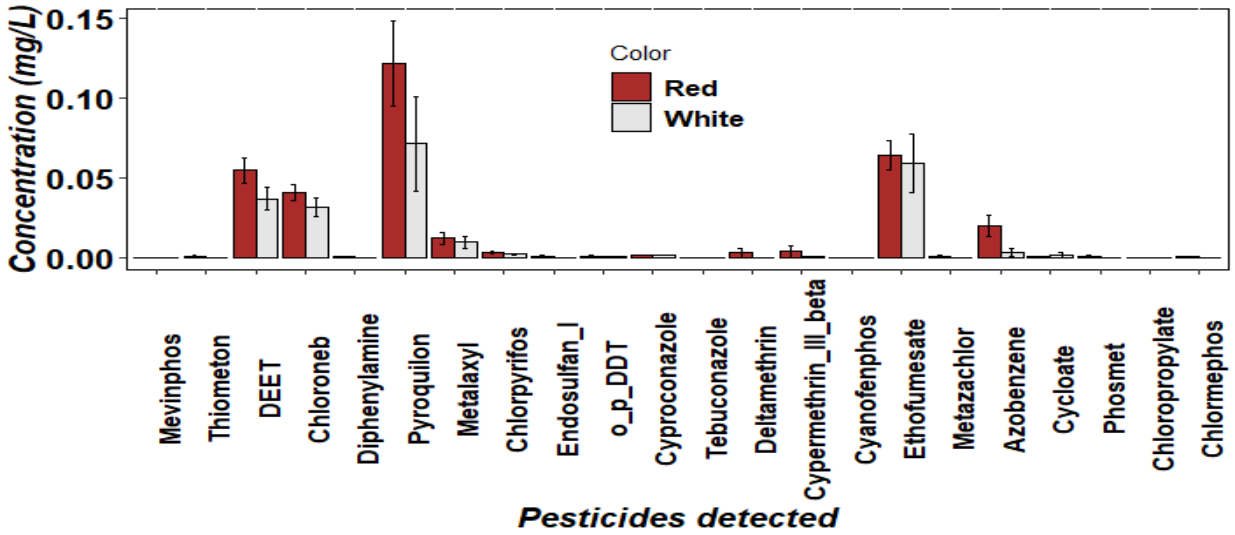

Figure 3. Box plot showing pesticides concentration according to color of wine sample.

deltametrin, permethrin and fenvalerate were completely degraded after fermentation with *Saccharomyces cerevisiae* that can be attributable to the yeast activity, while the fungicides benalaxyl, folpet, furalaxyl, metalaxyl, iprodione, procymidone, and ofurace remained unaffected (Regueiro et al., 2014). Malolactic fermentation using *Oenococcus oeni* resulted in significant reduction in chlorpyrifos and dicofol concentrations which were reduced by 70 and 30%, respectively, where as the concentrations of chlorothalonil and procymidone diminished only slightly (Bajwa and Sandhu, 2011).

There was a significance difference ($p<0.05$) in pesticides concentration with colour of grape wine (Figure 3). The study showed that red wine has high concentration of pesticide residues compared to white wine this suggest the idea that there might be a correlation between applied winemaking technology, red or white the skin maceration could contribute to higher degree of pesticide residues transferred to wine. Fermentation on the skins, as carried out in red wine production, is likely to lead to higher residue levels in raw wine (Pazzirota et al., 2013). This was also observed in another study which was conducted in Moldovan wine products originated from traditional agriculture which showed that red wine had higher levels of pesticide residues than white wine due to grape skin maceration (Duca et al., 2012; Regueiro et al., 2014). The study revealed that there was no significance difference ($p<0.05$) on pesticides concentration when comparing to scales of grape wine producers (Figure 4). It was expected that there would be a large amount of pesticides in the wine produced by small producers due to the poor production system. But it was different because nothing was done to reduce the amount of pesticides before they started production, this was observed during survey conducted to different scale of wine producers.

Conclusion

This study investigated the levels of pesticide residues in grape wine produced in Dodoma urban and Bahi districts (Tanzania). The results indicated that, majority of grape wine samples were contaminated with pesticide residues some of which had their concentrations above the MRL. According to the public health perspective, the observed levels of pesticide residues would pose potential health risks to the public. In order to reduce health and environmental problems there is a need for sensitization to grape farmers, on better pesticide safety use and handling practices and the need for continuous pesticide residues through regular monitoring.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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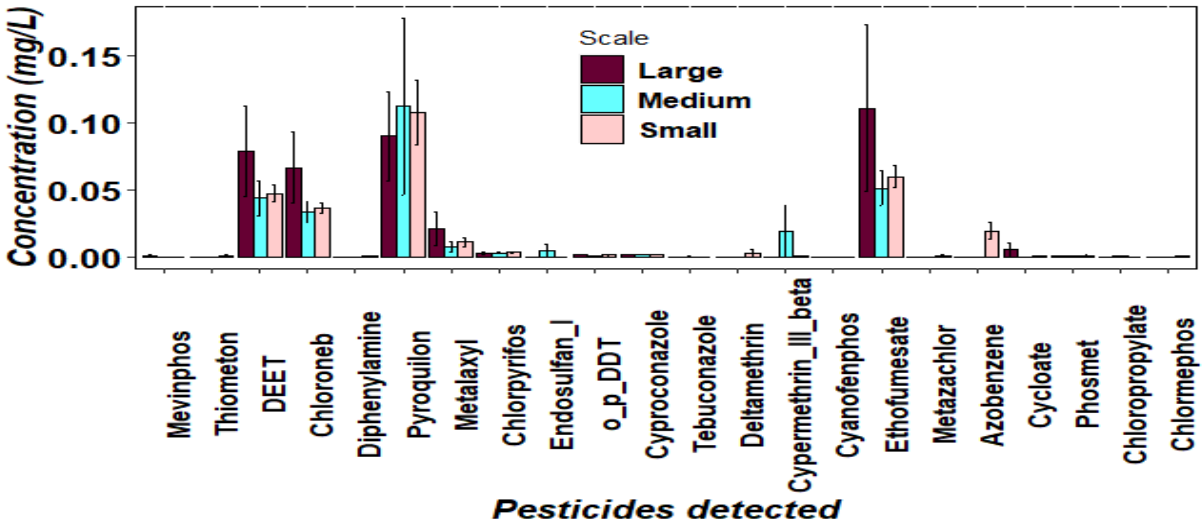

Figure 4. Bar plot showing pesticides concentration according to the scale of produce.

pesticides residues.

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Biochemical composition of *Saba senegalensis* fruits from Burkina Faso

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The aim of this study was to determine the biochemical characteristics of the fresh pulp and the dried hulls of *Saba senegalensis* fruits collected in seven localities of Burkina Faso. The biochemical composition of the samples was determined using standards methods. Results showed that the pulp presented mean values of 83.23%, 18.74%, 2.83 and 4.48 for moisture, Brix, pH and titratable acidity, respectively. Based on dry mater (DM), the pulp contained 5.9 g/kg of ashes, 144.2 g/kg of total carbohydrates, 11.5 g/kg of fat and 5.9 g/kg of proteins. The pulp contents (mg/kg) in minerals such as Fe, Zn, Mg, Ca and K were 25.4, 2.2, 1070.1, 1302.2 and 2535.7, respectively. The pulp has a potential energetic value of 704.2 kcal/kg for human consumption. *S. senegalensis* hulls presented higher mean values of proteins (30.8 g/kg), fat (150.6 g/kg), ashes (54.6 g/kg) and carbohydrates (637.8 g/kg). Hulls also displayed high contents in mineral elements such as Mg, K, Fe and Zn. This study demonstrated that pulps as well as hulls of *S. senegalensis* fruits from Burkina Faso have interesting nutritional potential which could be used to improve the diet of population.

Key words: *Saba senegalensis*, characterization, pulp, hull, nutrients, Burkina Faso.

INTRODUCTION

Saba senegalensis is an indigenous climbing plant belonging to the Apocynaceae family. It is found as wild plant in many African countries such as Burkina Faso, Côte d'Ivoire, Gambia, Guinea, Guinea-Bissau, Ghana, Mali, Niger, Senegal, and Tanzania (Diabagaté et al., 2019). It is one of the ten most important non-timber forest products in Burkina Faso with 79 454 tons of fruits produced in 2012 according to the Agency for the Promotion of Non-Timber Forest Products (PNSAN, 2013). Fruits of *S. senegalensis* are called *zaban* (in Mali), *malombo* (in the Congo Basin), *maad* (in Senegal), *wèda* (in Burkina Faso) and *côcôta* (in Côte d'Ivoire). The fruit consists of a globular hull coating seeds with very soft and juicy yellow pulp (Kini et al., 2008;

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Figure 1. Map of Burkina Faso showing the seven sampling areas. Green circles indicate the localities and their names.

Kouakoua et al., 2019). Ripe fruit is tasty, sweet-sour with yellow pulp (Boamponsem et al., 2013) and is generally consumed fresh without any processing. However, the fruits could be processed into products such as juice, nectar or jam. It could also be used in other preparations such as cakes (Kouakoua et al., 2019). The fruit is rich in vitamin A, vitamin C, dietary fiber and contains minerals such as potassium, magnesium and calcium (Omale et al., 2010; Diabagaté et al., 2019). The presence of active compounds in *Saba* fruit could play an important role in the prevention and treatment of certain vitamin deficiencies and metabolic diseases (Kini et al., 2008). Despite its intrinsic qualities and its economic contribution, the valorization of the *S. senegalensis* fruit as most of climacteric fruits in Burkina Faso is limited by its seasonality and its high perishability. Indeed, the fruit harvesting period is three months (May to August) and once at maturity, the fruit storage time is very short and usually does not exceed three days (Lamien et al., 2010). In addition, the lack of methods of processing as well as methods of preserving the fruit results in its non-availability throughout the year. The objective of this study was to determine the biochemical composition of the pulp and the hull of *S. senegalensis* fruit for a better valorization in Burkina Faso.

MATERIAL AND METHODS

Sampling

Fruits of *S. senegalensis* were collected from seven production areas in Burkina Faso (Figure 1): Boromo (Brm), Pô (Pô), Saponé (Spn), Houndé (Hnd), Nouna (Nna), Ouahigouya (Ohg) and Ouagadougou (Ogd). In each locality, 200 to 230 fruits (Figure 2a) were directly picked from randomly selected trees, pooled to form a composite sample of 30 to 35 kg. Each composite sample was divided into three equal parts. Three samples were then obtained per locality and a total of 21 samples was collected and transported to the pilot plant of Département Technologie Alimentaire (DTA/IRSAT/CNRST) for treatment before analyses.

Samples treatment

Samples from each locality were first sorted and fruits of good quality and maturity were selected. The fruits were then washed with soap, disinfected with sodium hypochlorite (0.2%), rinsed twice and cutted into half with stainless steel knife. The seeds containing the pulp (Figure 2b) were then removed with stainless spoon. The pulp was extracted from the pulpy seeds without adding water using a mixer of mark SAYONA (NO: SZJ-LH 923B/AC 220V 50/60 Hz 500W) and a stainless sieve according to the diagram in Figure 3. The pulp obtained (Figure 2c) was collected in sterile freezer bags and stored at -18°C for the various analyses. The hulls of each sample were dried following the steps described in Figure 3. The

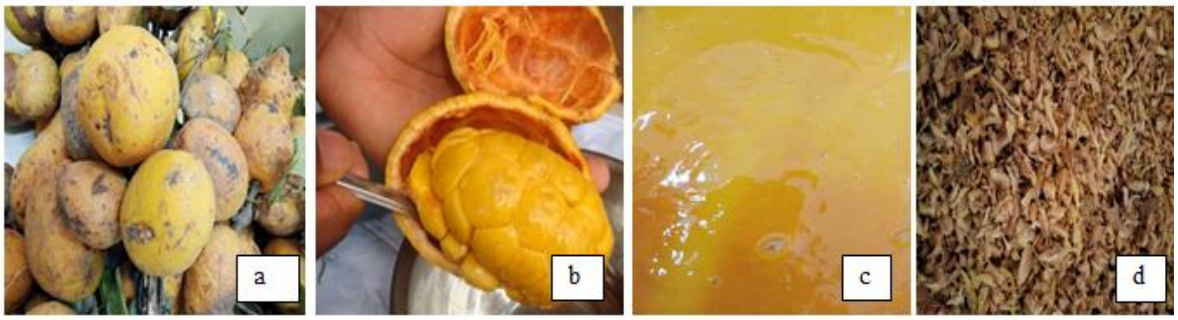

Figure 2. Ripe fruit of *S. senegalensis.* (a) Whole fruit; (b) open fruit, (c) pulp, (d) dried hulls.

obtained dried hulls (Figure 2d) were crushed using a porcelain mortar, then packaged in plastic boxes and kept at room temperature for analyses. After treatment, 21 samples of pulp and 21 samples of hull were obtained for the different analyses.

Biochemical analysis

The pH and the titratable acidity were determined according to the AFNOR methods (1986) applicable to fruits and vegetables and derived products. Samples (5 g) were homogenized with 25 ml of distilled water. The pH of the homogenate was determined using a digital pH meter (Hanna, France) calibrated with standard buffer solutions pH 4.0 and 7.0. The solution used for measuring the pH was centrifuged at 3 500 rpm. The supernatant was collected for titrimetric assay.

The percentage of soluble dry matter or Brix level was determined using a digital refractometer (Abbe, France). The moisture content was determined by drying the sample at 105 ± 2°C for 12 h according to the standard ISO 712 (2009). Ash content was determined by incineration at 550°C for 4 h according to the standard IS0 2171 (2007). Proteins content was determined by the Kjeldahl method after acid digestion according to the standard AFNOR NF V03 50 (1970). Fat content was determined with Soxhlet apparatus using n-hexane according to the standard ISO 659, (1998). Total carbohydrates content was estimated by the difference method according to the formula: % Total carbohydrates = 100% - (% moisture + % proteins + % fat + % ash). The energy value was calculated according to the Atwater coefficients. The determination of mineral elements was carried out by flame atomic absorption spectrometry (Perkin-Elmer model 303) according to AOAC (2005).

Statistical analysis

The physico-chemical analyses were carried out in triplicate. Data were processed using Microsoft Excel 2013 for the calculation of averages and standard deviation.

RESULTS AND DISCUSSION

Biochemical composition of *S. senegalensis* pulp

The biochemical composition of the pulp of *S. senegalensis* fruit on a dry matter (DM) basis is different among samples (Table 1). The moisture content of the pulp ranged from 80.94 ± 0.04% (Ogdp) to 88.03 ± 0.02% (Nnap) with a mean value of 83.23 ± 2.39%. This mean is higher than the value reported by Paget (2004) which was 80%. The moisture content is an important criterion for the stability of food. Fruits that contain a large amount of water are subject to rapid deterioration due to mold growth and insect damage (Boamposem et al., 2013). The variation in the moisture content of the different pulps could be explained by their ecological origin. Indeed, previous studies have reported that the humidity is not a varietal characteristic, but depends much more on soil and climatic conditions and therefore can be influenced by the effect of environment (Derrardji, 2014).

The pH of the pulps ranged from 2.7 ± 0.0 (Spnp) to 2.94 ± 0.01 (Brmp). The mean pH value for all samples was 2.83 ± 0.08, showing that *S. senegalensis* is an acidic fruit. This acidity could allow extending the shelf life of fresh fruit and inhibiting the multiplication of pathogenic microorganisms (Mudambi and Rajagopal, 2006). The mean pH of the *S. senegalensis* pulps from Burkina Faso was closed to those obtained by Kouakoua et al. (2019) from Côte d'Ivoire (pH 2.85) and Sarr et al. (2018b) from Senegal (pH 2.77). In contrast, Boamponsen et al. (2013) reported a lower pH for the pulp harvested in Ghana. This difference could be justified by the differences in soil, climate and the level of the maturity of the fruit (Kouakoua et al., 2019).

Like pH, titratable acidity is also very important for the determination of the fruit pulp quality. Titratable acidity is related to the sour taste of food and expresses the quantity of organic acids (Diabagaté et al., 2019). In this study, the content of titratable acidity varied from 3.53 ± 0.12% (Hndp) to 5.29 ± 0.49% (Spnp) with an average of 4.48 ± 0.57%. This average is higher than that (2.20%) reported by Kouakoua et al. (2019). The fruit being a climacteric fruit, this difference could be explained by ripeness and varietal differences among fruits. Indeed, during the ripening metabolism the reducing sugar contents increase and the sugar/acid ratio is consequently high. Therefore, the level of acidity is considered as an indicator of ripeness (Boamponsem et al., 2013).

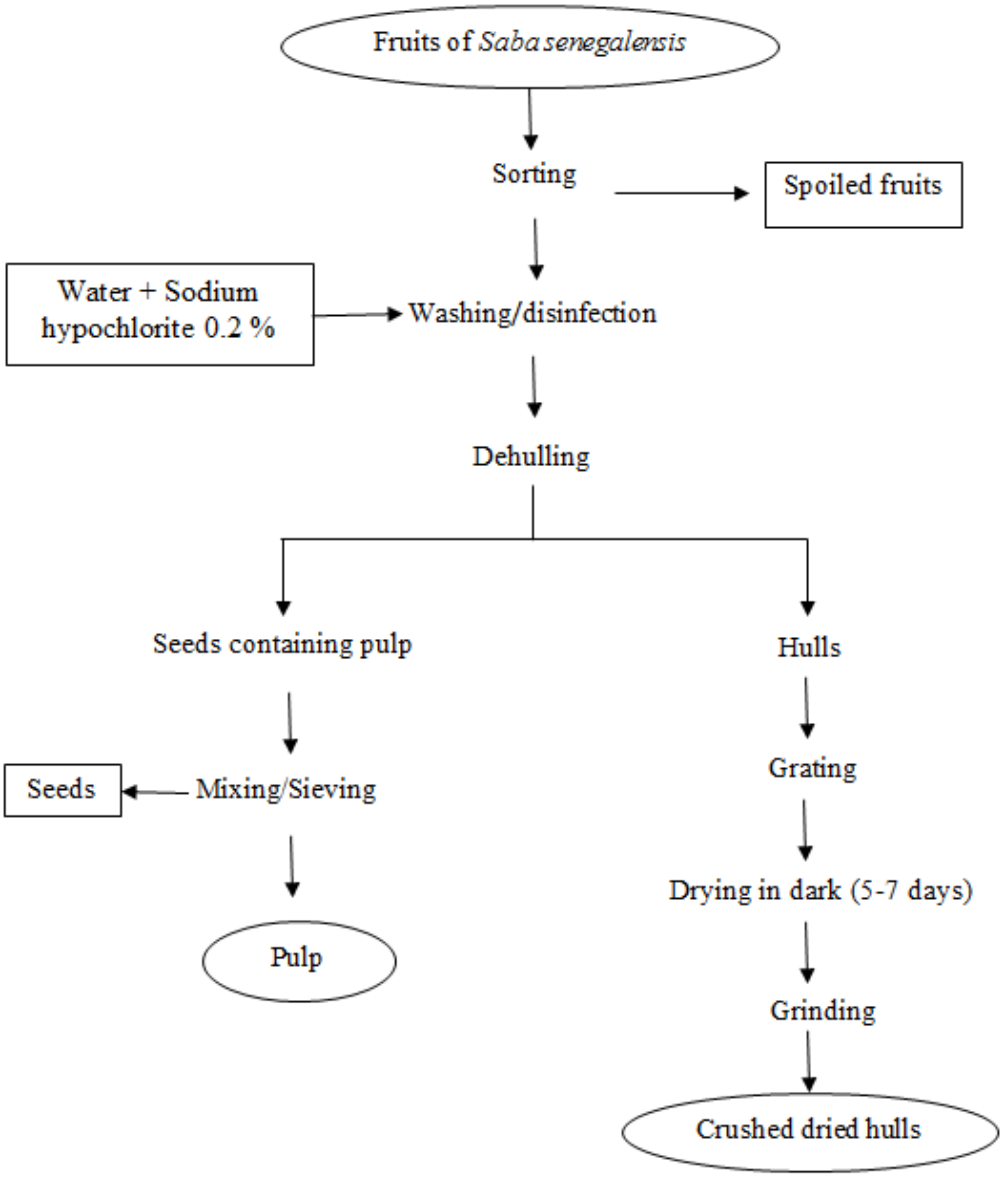

Figure 3. Diagram of extraction of the pulp and drying of the hulls of *S. senegalensis* fruits.

Samples Brix values ranged from 13.4 ± 0.00% (Nnap) to 22.1 ± 0.00% (Ogdp), with a mean value of 18.74 ± 0.00%. The Brix level is one of the basic criteria used for the definition of fruit juice (Boamposem et al., 2013). Theoretically, it is well known that the Brix value indicates the percentage of water-soluble dry matter in fruit juice. This soluble dry matter is mostly made up of sugar and minerals. Testing the brix level of fruits gives an idea of the fruit quality; high brix means better nutritional quality as well as better flavor (Boamposem et al., 2013). It can depend on many factors including the variety of the fruit, the locality, the level of ripeness, etc. (Derrardji, 2014). This could explain the difference between that found from the present study and those from Boamponsem et al. (2013) in Ghana, Sarr et al. (2018b) in Senegal and Kouakoua et al. (2019) in Côte d'Ivoire which were 14.10, 16.50 and 19.60%, respectively.

Ash contents of the pulps varied from 2.5 ± 0.1 (Nnap) to 10.4 ± 1.18 g/kg (Hndp) with an average of 5.9 ± 3.1 g/kg. The average ash contents of *S. senegalensis* pulp from Burkina Faso is closed to that (4.6 g/kg DM) obtained by Diabagaté et al. (2019) for pulp from Côte d'Ivoire. However, Boamponsem et al. (2013) reported higher ash contents (28 g/kg DM) for *S. senegalensis* pulp from Ghana. The difference in ash contents may be due to the variety, the nature of the climate and the property of the soil on which the plant grows.

Protein contents ranged from 3.7 ± 0.0 (Nnap) to 7.9 ±

Table 1. Biochemical composition of the pulp of *S. senegalensis* fruit.

Sample	Moisture (%)	Ashes (g/kg DM)	Fat (g/kg DM)	Total Carbohydrates (g/kg DM)	Proteins (g/kg DM)	pH	Titratable acidity (%)	Brix value (%)	Energy (kcal/kg DM)
Brmp	84.55±0.23	4.2±0.2	11.5±0.2	132.8±1.1	5.7±0.2	2.94±0.01	4.29±0.42	16.1±0.00	657.5
Hndp	82.09±0.17	10.4±1.18	12.5±0.8	148.1±1.0	7.9±1.5	2.83±0.02	3.53±0.12	19.9±0.00	736.5
Nnap	88.03±0.02	2.5±0.1	8.8±0.5	104.5±0.7	3.7±0.0	2.83±0.00	3.98±0.10	13.4±0.00	512
Ogdp	80.94±0.04	10.2±0.61	11.7±0.6	162.3±5.9	6.3±0.3	2.78±0.03	5.14±0.31	22.1±0.00	779.7
Ohgp	82.45±0.01	5.2±0.4	13.1±0.3	150.9±9	6.0±0.0	2.82±0.01	4.19±1.31	19.4±0.00	745.5
Pôp	81.76±0.20	5.8±0.1	11.7±0.3	158.8±1.5	5.8±0.4	2.89±0.00	4.02±0.45	20.4±0.00	763.7
Spnp	82.75±0.03	3.1±0.16	11.6±0.2	152.0±1.9	5.6±0.2	2.70±0.00	5.29±0.46	19.9±0.00	734.8
Mean	83.23±2.39	5.9±3.1	11.5±0.2	144.2±4.7	5.9±1.2	2.83±0.08	4.48±0.57	18.74±0.00	704.2

Brmp: Pulp from Boromo, Hndp: pulp from Houndé, Nnap: pulp from Nouna, Ogdp: pulp from Ouagadougou, Ohgp: pulp from Ouahigouya, Pôp: pulp from Pô, Spnp: pulp from Saponé.

1.5 g/kg (Hndp) with an average value of 5.9 ± 1.2 g/kg. Thus, as for most fresh fruits, the pulps of *S. senegalensis* fruits from Burkina Faso were poor in proteins. The mean value of protein contents of the pulps is in agreement with the value (5 g/kg DM) reported by Boamponsem et al. (2013) but lower than the value reported by Diabagaté et al. (2019) which was 29.1 g/kg.

Regarding total carbohydrates content, the pulps of *S. senegalensis* fruit displayed a mean value of 144.2 ± 4.7 g/kg. In previous studies, higher carbohydrates content (185 g/kg DM) of *S. senegalensis* pulp from Burkina Faso was reported by Zerbo et al. (2007) and PADEC (2018). The content of total carbohydrates in *S. senegalensis* pulp reported by other authors also varied (Kini et al., 2008; Nafan et al., 2013; Sarr et al., 2018a; Diabagaté et al., 2019). This variation could be explained by various conditions such as geographical localization, genetic constitution, pedological and climatic conditions, etc. (Diabagaté et al., 2019).

Fat content is known to provide high energy content and can be reservoir of fat-soluble vitamins. Pulps showed fat contents ranging from 8.8 ± 0.5 g/kg (Nnap) to 13.1 ± 0.3 g/kg (Ohgp) with a mean value of 11.5 ± 0.2 g/kg. This average is in line with the fat content (14.8 g/kg) of *S. senegalensis* pulps from Côte d'Ivoire (Diabagaté et al., 2019). Food with low fat content may be interesting for obese people (Ayessou et al., 2009). The mean fat content of all the samples are nevertheless lower than that found by Boamponsem et al. (2013) which was 89.3 g/kg DM.

The potential calorific value of *S. senegalensis* pulp ranged from 512 kcal/kg (Nnap) to 779.7 kcal/kg (Ogdp) with an average of 704.2 kcal/kg. This low energy can be explained by the low contents of macronutrients in *S. senegalensis* pulps. However, the average potential nutritive value obtained for the pulp in this study is much lower than that reported by Boamponsem et al. (2013) which was 3793.2 kcal/kg. The high calorific content reported by these authors was due to the high fat content (89.2 g/kg DM) found in their fruits compared to the fruits from Burkina Faso. Indeed, the calorific value is associated with the fat content. A high fat content necessarily influences the energy value because fat has a high calorific coefficient. Considering the average low energy value, *S. senegalensis* fruits collected in the seven localities of Burkina Faso could be recommended to the obese and diabetic patients or in overweight.

The composition in mineral elements expressed in mg/kg of dry matter (DM) of the pulps of *S. senegalensis* fruits from the 7 localities of Burkina is presented in Table 2. Results showed that the iron content ranged from 3.7 ± 0.0 (Ogdp) to 81 ± 0.4 (Brmp) with an average of 25.4 ± 0.3 for all the samples. A weak content in zinc (Zn) was observed with an average of 2.2 ± 0.0. The lowest content (1.7 ± 0.0) was reported for the fruits of Boromo (Brmp) while the highest content (2.7 ± 0.0) was noted for the fruits of Saponé (Spnp). However, high percentage of magnesium, potassium, and calcium was found. For magnesium, the highest content (1622.5 ± 6.6) was obtained with Pô samples (Pop) and the lowest content (850 ± 1.6) with Houndé samples (Hndp). The mean value of Mg for all samples was 1070.1 ± 4.3. A variation of 674.8 ± 7.4 (Nnap) to 11062.4 ± 72.7 (Hndp) was observed for K and the average found was 2535.7 ± 27. Concerning Ca the content ranged from 249.4 ± 20.0 (Pop) to 2848.7 ± 59.1 (Ogdp). The mean value obtained for Ca was 1302.2 ± 25.1. Found data were comparable with those of Diabagaté et al. (2019) who showed that K (1169.6 ± 20.6 mg/kg DM) and Ca (366.1 ± 27.9 mg/kg DM) are the most abundant minerals in the pulp of *S. senegalensis* fruits from Côte d'Ivoire. Most pulps from the seven localities of Burkina Faso also presented higher content in Ca than that reported for the orange fruits (255 ± 19.0 mg/kg DM) by Dipak and Ranajit (2004).

Data showed that the pulp of *S. senegalensis* fruits from Burkina Faso were rich in minerals compared to other consumed fruits. The consumption of *S.*

Table 2. Mineral composition of the pulp of *S. senegalensis* fruit (mg/kg DM).

Sample	Fe	Zn	Mg	Ca	K
Brmp	81.0±0.4	1.7±0.0	892.9±2.5	384.8±12.5	955.3±25.7
Hndp	06.3±0.3	2.2±0.0	850.0±1.6	465.2±41.2	11062.4±72.7
Nnap	10.1±0.0	1.8±0.0	907.1±3.7	2160.2±4.9	674.8±8.7
Ogdp	03.7±0.0	1.8±0.0	1018.5±2.1	2848.7±59.1	1569.7±8.4
Ohgp	37.2±0.3	2.4±0.0	1143.3±3.7	4525±18.8	957.5±13.2
Pôp	27.1±0.3	2.6±0.0	1622.5±6.6	249.4±20.0	1591.2±46.5
Spnp	12.1±0.0	2.7±0.0	1056.2±1.1	2549.9±38.1	937.8±07.4
Mean	25.4±0.3	2.2±0.0	1070.1±4.3	1302.2±25.1	2535.7±27

Brmp: pulp from Boromo, Hndp: pulp from Houndé, Nnap: pulp from Nouna, Ogdp: pulp from Ouagadougou, Ohgp: pulp from Ouahigouya, Pôp: pulp from Pô, Spnp: pulp from Saponé.

Table 3. Biochemical composition of the hulls of *S. senegalensis* fruit.

Sample	Moisture (%)	Ashes (g/kg DM)	Total carbohydrates (g/kg DM)	Fat (g/kg DM)	Proteins (g/kg DM)	Titratable acidity (%)	pH	Energy (kcal/kg DM)
Brmh	10.82±0.12	52.1±1.1	673.8±4.9	137.3±5.7	28.4±1.7	3.66±0.33	3.69±0.23	4044.5
Hndh	13.89±0.13	62.7±5.2	597.4±2.5	172.3±2.5	28.6±0.6	4.06±0.41	3.40±0.01	4054.7
Nnah	13.69±0.03	49.0±3.	619.5±5.1	165.8±1.0	28.6±1.0	2.61±1.25	3.55±0.03	4084.6
Ogdh	16.60±0.10	51.8±1.2	652.4±29.5	95.1±31.2	34.5±0.4	0.58±0.13	4.87±0.09	3603.5
Ohgh	13.35±0.07	55.7±1.9	667.7±92.2	108.7±94.2	34.3±0.2	0.30±0.20	5.01±0.23	3786.3
Poh	13.40±0.07	63.3±1.7	598.6±5.8	169.6±04.5[a]	34.3±0.2	4.19±0.42	3.44±0.00	4058
Spnh	11.91±0.01	47.3±0.8	655.2±2.0	151.3±0.8[a]	26.8±0.6	2.64±0.51	3.62±0.00	4089.7
Mean	13.38±1.80	54.6±1.5	637.8± 21.2	150.6±27.3	30.8±3.3	2.58±1.58	3.94±0.69	3960.1

Brmh: hulls from Boromo, Hndh: hulls from Houndé, Nnah: hulls from Nouna, Ogdh: hulls from Ouagadougou, Ohgh: hulls from Ouahigouya, Pôh: hulls from Pô, Spnh: hulls from Saponé.

senegalensis fruit could therefore contribute to cover the body's needs in minerals (Paget, 2004). Indeed, the mineral elements are involved in a wide range of functions in the organism such as mineralization, ion balance control, enzyme and hormonal systems, muscular system, nervous and immune systems. For example, there is no hemoglobin synthesis without Fe and no muscle contraction without Ca, K and Mg (Ciqual, 2013).

Biochemical composition of *S. senegalensis* hulls

From the results, the moisture content of the hulls samples from the 7 localities ranged from 10.81 ± 0.12% (Brmh) to 16.60 ± 0.10% (Ogdh) with an average of 13.38 ± 1.80% (Table 3). The mean value of moisture content of the hulls corroborates the limit set by the Codex Alimentarius Commission (1991) for dried food which is 15%. The pH of the hulls ranged from 3.40 ± 0.01 (Hndh) to 5.01 ± 0.23 (Ohgh) with a mean value of 3.94 ± 0.69.

Concerning the titratable acidity, it ranged from 0.12 ± 0.20% (Ogdh) to 4.19 ± 0.42% (Poh). The mean value obtained for titratable acidity was 2.58 ± 1.58%. The biochemical composition of the hulls was different from those of the pulps. Ash contents varied from 47.3 ± 0.8 (Spnh) to 63.3 ± 1.7 g/kg (Poh) with an average of 54.6 ± 1.5 g/kg. It appears that the hulls contain higher percentage of ashes than the pulps. Hulls could therefore constitute a significant source of mineral elements, important for human consumption.

For the protein contents, they ranged from 26.8 ± 0.6 (Spnh) to 34.5 ± 0.4 g/kg (Ogdh) with an average of 30.8 ± 3.3 g/kg. *Saba senegalensis* fruit hull samples contain a high average protein content compared to pulp samples (5.9 ± 1.2 g/kg). The main function of proteins for the body is the building of new tissues and maintaining and repair of those already built. Synthesis of regulatory and protective substances such as enzymes, hormones and antibodies is also a function of food proteins.

Regarding the fat contents, they ranged from 95.1 ± 31.2 (Ogdh) to 172.3 ± 2.5 g/kg (Hndh). The mean content obtained was 150.6 ± 27.3 g/kg. This average is higher than that obtained for the pulp which was 11.5 ±

Table 4. Mineral composition of the hulls of *S. senegalensis* fruit (mg/kg DM).

Sample	Fe	Zn	Mg	Ca	K
Brmh	86.4±1.8	16.6±0.0	1870.5±4.5	3335.9±24.2	14703.3±65.9
Hndh	85.4±0.3	11.1±0.0	1154.3±1.6	161.3±31.7	857.5±10.8
Nnah	28.0±0.3	5.5±0.5	1882.2±5.2	80.6±7.7	9544.4±89.9
Ogdh	46.8±0.3	3.8±0.0	1920.1±6.3	161.6±10.9	14306.6±60
Ohgh	35.6±0.3	3.5±0.0	1387.2±3.2	453.1±18.4	12894±9.3
Poh	28.6±0.3	9.3±0.0	1082.8±2.9	157.3±0.7	8389.9±26.2
Spnh	79.7±0.9	11.4±0.0	1093±3.3	231.5±14.6	10024.2±8.9
Mean	56.1±1.2	8.8±0.1	1484.3±4.7	654.5±16.5	10103.1±38.8

Brmh: Hulls from Boromo, Hndh: hulls from Houndé, Nnah: hulls from Nouna, Ogdh: hulls from Ouagadougou, Ohgh: hulls from Ouahigouya, Pôh: hulls from Pô, Spnh: hulls from Saponé.

0.2 g/kg, meaning that *S. senegalensis* hulls is a good source of fat. Fats are concentrated sources of energy, carriers of fat, soluble vitamins and a source of essential fatty acids (Mudambi and Rajagopal, 2006).

The total carbohydrate contents of the hulls ranged from 597.4 ± 2.5 (Hndh) to 673.8 ± 4.9 g/kg (Brmh) with an average of 637.8 ± 21.2 g/kg. It appeared that the hulls had higher mean value of total carbohydrates than the pulps (144.2 ± 4.7 g/kg). The chief function of carbohydrates is to provide energy needed by our body.

The hulls samples presented energetic value ranging between 3603.5 and 4089.7 kcal/kg with an average of 3960.1 kcal/kg. Hulls therefore have very good nutritive value which is attributed to the high carbohydrates and fats content.

The mineral content (expressed in mg/kg of DM) of the dried hulls is shown in Table 4. The highest Fe value was obtained with Boromo fruits hulls (86.4 ± 1.8) and the lowest value with Nouna fruits hulls (28 ± 0.3) with an average of 56.1 ± 1.2. The content of Mg ranged from 1082.8 ± 2.9 (Pôh) to 1920.1 ± 6 (Ogdh) with a mean value of 1484.3 ± 4.7. Regarding the Zn content, the highest content was found in Brmh samples (16.6 ± 0.0) and the lowest content in Ohgh sample (3.5 ± 0.0) with a mean value of 8.8 ± 0.1. Concerning Ca, the content ranged from 80.6 ± 7.7 (Nnah) to 3335.9 ± 24.2 (Brmh) with an average of 654.5 ± 16.5. The content of K ranged from 857.5 ± 10.8 (Hndh) to 14703.3 ± 65.9 (Brmh). The mean K concentration obtained for all the hull samples was 10103.1 ± 38.8. Except for the Ca, the contents of all the determined mineral elements in the hulls are higher than those found in the pulps.

In summary, the results showed that the hull samples contain interesting contents of macronutrients (carbohydrates, fats, ashes) and micronutrients (Fe, Ca, Mg, K) which highlight the nutritional potential of the hulls of *S. senegalensis* fruit analyzed. In Burkina Faso, the dried hulls are generally ground, mixed with water, sieved and used as acidifier in the preparation of certain foods. With regards to their nutritional potential, the consumption of *S. senegalensis* hulls could be encouraged as a food supplement to improve the diet of population.

Conclusion

This study highlighted the biochemical composition and nutritional potential of *S. senegalensis* fruits (pulp and hull) from Burkina Faso. The results showed that the pulp is a nutritionally rich food for human consumption. The results also demonstrated that the contents in macronutrients and minerals of the hulls were much higher than those of the pulp. The hulls of *S. senegalensis* may therefore be considered as cheap source of nutrients for particularly rural consumers. Further studies will assess the influence of geographical localization on the biochemical composition of *S. senegalensis* fruit. In addition, identifying appropriate technologies for preserving and processing the *S. senegalensis* fruit will make this resource available out of season and add its value.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Changes in physicochemical properties and bioactive compounds of tomato pulp submitted to different processing techniques

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Tomato is a vegetable/fruit highly consumed all other the world. This study was to assess the effect of some postharvest processing such as thermal treatment, microwave, ultrasound and ultrasound combined with heat treatment on some physicochemical characteristics as well as nutrients content of tomato pulp during storage at room temperature. Results showed that the pulp samples submitted to ultrasonic and microwave treatments gave an increase in water content (95.65 to 96.75%) and total acidity (0.70 to 1.16% citric acid equivalent) and a decrease in pH (4.02 to 3.59) and brix degree (4.93 to 4.02% Brix) during the first 15 days of storage. Ultrasound treatment associated with heat treatment did not affect the physicochemical characteristics of tomato pulp and the β-carotene, but slightly reduced the total phenolic content during the first 15 days of storage (723.98 to 659.66 mg GAE/100 g DM). A significant increase in the total phenolic content (647.33 to 832.78 mg GAE/100 g DM) and β-carotene (10.77 to 12.90 mg GAE/100 g DM) was observed during storage of pulp samples treated with ultrasound and microwaves. This study showed that the ultrasound treatment associated with heat treatment can be recommended to processors for nutrients preservation during storage.

Key words: Tomato pulp, ß-carotene, total phenolic, physicochemical characteristics, ultrasound, heat.

INTRODUCTION

Tomato (*Lycopersicum esculentum* Mill.) is a vegetable grown all over the world for its fruits, which are consumed especially for their organoleptic and nutritional qualities. In Burkina Faso, the annual tomato production was

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estimated to 167400 tons in 2018 (DGESS/MAAH, 2020). Tomato is a climacteric fruit, known as an excellent source of bioactive molecules such as lycopene, carotenoids, phenolic compounds, vitamins and other nutraceutics (Toor and Savage, 2005; Tudor-Radu et al., 2016). She is rich in a plethora of natural antioxidant compounds (Shatta et al., 2017). Epidemiological studies have shown that regular consumption of tomatoes or tomato products are associated with a decreased risk of chronic diseases such as cardiovascular diseases, prostate, gastrointestinal and epithelial cell cancers (Donkor et al., 2015; Pinela et al., 2016; Przybylska, 2020). Tomatoes do not lose their health benefits as they are processed or cooked. In fact, lycopene in cooked and processed tomatoes (sauce, paste, salsa, canned tomatoes) is more easily absorbed than fresh tomatoes (Shatta et al., 2017).

Tomato after harvesting, is generally subject to culinary practices, processing and preservation. Processed tomato products included pulp, paste, powder, juice, sauce, jam, etc. During processes, several treatments such as thermal and non-thermal ones can be applied. Storage and processing may be led to changes in levels of phytochemicals, impacting the quality of the end-products (Lavelli and Giovanelli, 2003; Capanoglu et al., 2008; Chanforan, 2010). Studies on the impact of treatment methods on carotenoids, vitamins and total phenolic in processed products have shown controversial results (Abushita et al., 2000; Dewanto et al., 2002; Seybold et al., 2004; Capanoglu et al., 2008; Shatta et al., 2017). Thermal techniques are the most commonly used conventional methods for preservation. These techniques allowed the inactivation of microorganisms and enzymes in food, but have some impacts on organoleptic and nutritional qualities (Stratakos et al., 2016). Nowadays, consumers tend to require processed products that have a good taste and which maintain their nutritional qualities. Previous studies reported that combined microwave and ultrasound treatments can improve the microbiological quality of food while having a lesser impact on taste and nutritional qualities (Montemurro et al., 2014; Stratakos et al., 2016; Lagnika et al., 2017). However, the effect of the treatments on the stability of antioxidant molecules of processed tomato during storage is not well documented.

This study was to assess the effect of some of thermal and non-thermal techniques on the stability of physicochemical parameters and bioactive compounds in tomato pulp during storage at ambient temperature.

MATERIALS AND METHODS

Plant material and sample preparation

Fresh and firm tomatoes of the Mongal F1 variety were bought at a market in the city of Ouagadougou (Burkina Faso). Tomatoes were sorted, washed in water bath, disinfected for 10 min with 0.24% sodium hypochlorite and then rinsed with water. Afterwards, pulps were separated using stainless steel pulp extractor. Obtained pulps were weighed and packaged into 72 glass jars of 300 ± 5 ml and divided into six equal parts.

Processing treatment

The treatment techniques were previously described by Lagnika et al. (2017) with a slight modification as follows. The six groups have been submitted to the following treatments: a first group not having undergone any treatment, it was used as a control (C); a second group was subjected to a heat treatment at 65°C for 15 min in a water bath (WB65); a third group was subjected to a heat treatment at 85°C for 15 min in a water bath (WB85); a fourth group was sonicated (Bioblock Scientific, Vibra-Cell 88169, Germany) at a power of 286-312 W and a frequency of 35 kHz; a fifth group was subjected both to heat treatment and sonication (USWB). The temperature was kept below 65°C using an ice-bath around the reactor. The temperature of the juice was monitored using a thermometer so that the temperature remained below 65°C; the last group of pulp was treated in microwave with a power of 630 W for 1 min at a temperature of 72°C (MW). Each treatment was done in triplicate.

The different tomato pulp samples were stored at room temperature (29 ± 02°C) and analyzed over time at 15 days intervals for 45 days (t0= 0, t1= 15, t2=30, t3= 45 days).

Analytical methods

Determination of water content, pH, acidity and brix degree

The water content was determined by drying in an oven at 105°C overnight (NF-V03-707, 2000). Acidity and pH were determined according to standardized method (AFNOR, 1986). Briefly, sample (5 g) is mixed with in 25 ml of distilled water, with magnetic stirrer, the pH was measured.

For the acidity, the solution is centrifuged at 5000 g for 5 min; the collected supernatant was then titrated with 0.1 M NaOH in the presence of a few drops of phenolphthalein and the acidity was calculated as citric acid equivalent. Brix degree was measured directly with refractometer (B+S RFM712, United Kingdom) (Norme Francaise-VO5-109, 1970).

Determination of β-carotene

The β-carotene content was quantitated by High Performance Liquid Chromatography (HPLC, AGILENT 1100, Germany). Analyses were carried out under yellow light and the sample containers were protected from light using aluminium foil. For the preparation of standard curve, 0.15 mg of β-carotene (standard, Sigma BCVV2933) was dissolved in 3 ml of hexane. Dilutions 1/10, 1/100, 1/1000 of this solution were made. The optical densities (OD) were read at 450 nm. The sample solutions with OD between 0.1 and 0.9 were chosen. Their concentration was then calculated according to the formula: $C = (OD / \varepsilon_{\lambda}) \times 10^{-3}$ (µg ml^{-1}). Where "OD" is the optical density and ε_{λ} is the molar extinction coefficient at 450 nm.

For the extraction, a sample (1 g) of paste was put in a tube. The β-carotene was extracted by vortexing with 2 × 2 ml of hexane in the presence of echinenone (internal standard) at a concentration of 0.6 pmol $µl^{-1}$. After vigorous stirring, the mixture was centrifuged at 3000 rpm, for 5 min at -5°C. The supernatants were combined and evaporated under a stream of nitrogen. The resulting residue was combined with 800 µl of acetonitrile containing 15 pmol/20 µl of the internal standard. After filtration, the sample was injected in the HPLC column (Kinetex Phénomenex) using a loop of 20 µl. After

injection of the calibration mixture, of defined concentration, and including the internal standard, for each peak, a relative calibration factor was calculated according to Somé et al. (2004).

Determination of total phenolic

The total phenolic content of pulp tomato was determined by spectrophotometry according to Singleton et al. (1999) with slight modifications. For extraction, 1% methanol-HCl solvent was used for extraction. Tomato pulp (5 g) was mixed with 100 ml of the solvent and ground for 10 min in a homogenizer and then transferred to conical flask. The ground samples were extracted using the maceration technique by soaking the samples in the solvents for 24 h, 4°C; followed by filtration using Whatman No. 1. The filtered extract was used to determine the total phenolic content. For the assay, 0.250 ml of each sample was introduced into test tubes and mixed with 1.25 ml of a 2 N Folin-Ciocalteu reagent. After 5 min of incubation, 1 ml of 7.5% sodium carbonate was added to generate the phenolate ions. The tubes were covered with aluminium foil and placed in a water bath at 65°C for 20 min before the absorbance was read at 760 nm using spectrophotometer (JENWAY, Bibby Scientific Ltd., United Kingdom). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The results were expressed in mg of gallic acid equivalent (GAE) per 100 g of dry matter.

Statistical analysis

All experiments were performed in triplicate. The data were submitted for analysis of variance (ANOVA) using the XLSTAT software (Ver.2014.5.03, Addinsoft). The differences between the means were evaluated by the Duncan test. Significance was defined at $P < 0.05$.

RESULTS AND DISCUSSION

There was significant difference in water content, total acidity, brix degree and pH of the tomato pulp subjected to different processing treatments during 45 days of storage (Table 1).

The water content of the control, US and MW samples increased (95.64 ± 0.024 to 96.84 ± 0.25%) during the first 15 days (t_0 to t_1) of storage with a significant difference ($p < 0.05$). On the other hand, for the WB65, WB85 and USWB samples, the water content remained stable (95.60 ± 0.063 to 95.75 ± 0.02%) during storage.

The pH values changed from 3.46 ± 0.01 to 4.02 ± 0.03 in the control sample (C). In the other samples, a decrease, stability or increase was observed (Table 1).

The total acidity changed inversely compared to pH during the first 15 days of storage. The total acidity of the control, US and MW samples increased significantly ($p < 0.05$) from 0.67 ± 0.03 to 1.19 ± 0.14% during the first 15 days. However, the total acidity remained stable for the BW65, BW85 and USBW samples during storage.

The Brix degree also changed according to treatments (Table 1). It can be particularly noticed that the Brix degree of control, US, and MW samples significantly decreased during the first 15 days of storage. For the BW65, BW85 and USBW samples, it remained stable (4.91 ± 0.00 to 4.80 ± 0.05) during storage.

In general, the treatment at the water-bath (65 and 85°C) and the ultrasonic treatment combined with a heat treatment have maintained stable the water content, pH, total acidity and brix degree of the tomato pulp samples during storage. In contrast, ultrasonic and microwave treatments varied the water content, pH, total acidity and brix degree from t0 to t1 before stabilizing from t_1 to t_3. A study by Lagnika et al. (2017) on pineapple juice treated with ultrasound, water bath and ultrasound combined with mild heat pasteurization had found similar results on the evolution of pH, total acidity and degree of brix during storage. The change in physicochemical parameters of treated tomato pulp samples may depend on treatment time and the used method. The variation of total acidity, pH and brix degree during the first 15 days of storage (t_0 to t_1) of the US and MW tomato pulp samples could be due to chemical reactions such as hydrolysis, oxidation, fermentation and the decomposition (Lagnika et al., 2017). The decrease in pH may be due to the production of organic acid (citric acid) and the hydrolysis of endogenous polysaccharides during storage (Bhardwaj and Pandey, 2011).

The total phenolic contents of the US and MW samples increased from 647.33 to 832.78 mg GAE/100 g DM during the first 15 days (t0 to t1) of storage (Figure 1) with a significant difference ($p < 0.05$).

The total phenolic content of BW65, BW85 and USBW samples decreased from 492.10 to 514.68 mg GAE/100 g DW during the first 15 days (t_0 to t_1) with a significant difference ($p < 0, 05$) and then stabilized (t_1 to t_3). The decrease in phenolic compounds was small for samples treated with ultrasound combined with heat treatment. This is in line with the findings of Lavelli and Giovanelli (2003) and Garcia-Alonso et al. (2009) that showed that when preserving tomatoes, the content of phenolic compounds can remain stable or increase.

The increase in the total polyphenol content of the ultrasonic and microwave samples during storage could be justified by an improvement in the extraction of these compounds in the tomato pulp initiated during the treatments. The increase can be explained by diffusion, during cooking, of the phenolic compounds previously linked to the cellular constituents (Kebe, 2014). This evolution may be related to the stimulation of the activity of the enzymes involved in the biosynthesis of polyphenols during storage.

The β-carotene increased significantly ($p < 0.05$) as those of total phenolic in tomato pulp samples treated with ultrasound and microwaves during storage (Figure 2).

For BW65, BW85 and USBW samples, the level remained stable. Lavelli and Giovanelli (2003) working on tomato products (pulp, puree, paste) stored at 40°C for three months also observed a decrease in β-carotene content. However, Ordóñez-Santos et al. (2009) have

Table 1. Changes of the moisture content, pH, total acidity and brix degree of the tomato pulp subjected to different processing treatment during 45 days of storage.

Parameter	Treatments	Time of storage (days)			
		t_0 (0)	t_1 (15)	t_2 (30)	t_3 (45)
Water content (%)	C	95.53 ± 0.02^{c}	97.07±0.45^{ab}	96.94±0.13^{ab}	97.39±0.65^{a}
	WB65	95.60 ±0.13^{c}	95.73±0.03^{c}	95.66±0.04^{c}	95.90±0.0^{c}
	WB85	95.66 ± 0.06^{c}	95.63±0.03^{c}	95.51±0.14^{c}	95.63±0.19^{c}
	US	95.65 ±0.04^{c}	96.75±0.18^{b}	96.80±0.03^{b}	96.78±0.01^{b}
	USWB	95.55 ± 0.00^{c}	95.67±0.03^{c}	95.59±0.14^{c}	95.71±0.05^{c}
	MW	95.74±0.08^{c}	96.71±0.12^{b}	96.74±0.07^{b}	96.91±0.65^{ab}
pH	C	4.02±0.01^{ab}	3.56±0.10^{cd}	3.46±0.1^{c}	3.54±0.03^{cd}
	WB65	4.01±0.00^{b}	4.05±0.01^{ab}	4.08±0.02^{ab}	4.14±0.02^{a}
	WB85	4.02±0.00^{ab}	4.06±0.01^{ab}	4.09±0.00^{ab}	4.12±0.00^{ab}
	US	4.03±0.00^{ab}	3.59±0.00^{c}	3.59±0.07^{cc}	3.58±0.03^{c}
	USWB	4.02±0.00^{ab}	4.05±0.01^{ab}	4.09±0.00^{ab}	4.13±0.02^{a}
	MW	4,02±0.01^{ab}	3.57±0.09^{cd}	3.53±0.12^{cd}	3.54±0.14^{cd}
total acidity (% citric acid equivalent)	C	0.60±0.05^{b}	1.19±0.01^{a}	1.25±0.00^{a}	1.24±0.10^{a}
	WB65	0.63±0.09^{b}	0.63±0.00^{b}	0.64±0.04^{b}	0.60±0.02^{b}
	WB85	0.69±0.01^{b}	0.70±0.12^{b}	0.68±0.10^{b}	0.70±0.10^{b}
	US	0.71±0.01^{b}	1.16±0.20^{a}	1.19±0.09^{a}	1.23±0.12^{a}
	USWB	0.73±0.06^{b}	0.69±0.14^{b}	0.75±0.16^{b}	0.75±0.00^{b}
	MW	0.70±0.03^{b}	1.14±0.28^{a}	1.13±0.27^{a}	1.25±0.018^{a}
brix degree (% Brix)	C	4.90±0.00^{a}	3.82±0.07^{cd}	3.85±0.07^{cd}	3.60±0.00^{de}
	WB65	4.90±0.00^{a}	4.90±0.00^{a}	4.95±0.07^{a}	4.60±0.00^{b}
	WB85	4.95±0.07^{a}	4.95±0.07^{a}	4.90±0.00^{a}	4.85±0.07^{ab}
	US	4.93±0.05^{a}	4.02±0.12^{c}	4.00±0.00^{c}	3.75±0.07^{cd}
	USWB	4.93±0.00^{a}	4.97±0.05^{a}	5.05±0.07^{a}	4.95±0.07^{a}
	MW	4.95±0.00^{a}	3.88±0.05^{cd}	3.95±0.07^{c}	3.40±0.07^{e}

Values are mean ± standard deviation of triplicates. Data in same column with different letters are significantly different ($p < 0.05$). C: Control: tomato pulp without any treatment; WB65: tomato pulp subjected to a heat treatment at 65°C for 15 min in a water bath; WB85: tomato pulp subjected to a heat treatment at 85°C for 15 min in a water bath; US: tomato pulp sonicated; USWB: ultrasound combined with mild heat pasteurization; MW: tomato pulp treated in microwaves bath.

observed a significant increase of β-carotene content in pulp stored for 6 months at 20°C. The increase of β-carotene content of tomato pulp from the US and MW samples during storage could be due to their better extractability. The increase could also be linked to an improvement in the availability of ß-carotene by softening or breaking of cell walls (Asami et al., 2003; Bernhardt and Schlich, 2006).

The ultrasound treatment, microwave and ultrasound combined with heat treatment have been retained polyphenols and β-carotene during storage compared to water bath. The decrease of β-carotene during storage can be related by an isomerization initiated during heat treatment. In fact, while Marx et al. (2003) showed that moderate heat treatment had a low rate on the trans-cis isomerization of ß-carotene, Mordi (1992) has shown that ß-carotene could be degraded by transient isomerization followed by the formation of a singlet diradical.

Conclusion

This study showed that ultrasound treatment combined with heat treatment slightly affected the levels of total phenolic content but maintained stable the β-carotene and the physicochemical characteristics of tomato pulp during storage. Compared to the other treatments, ultrasound treatment associated with a thermal treatment is the best processing method for tomato pulp treatment regarding to its bioactive compounds preservation during storage. This processing may be applied along with the respect good manufacturer practices of the products

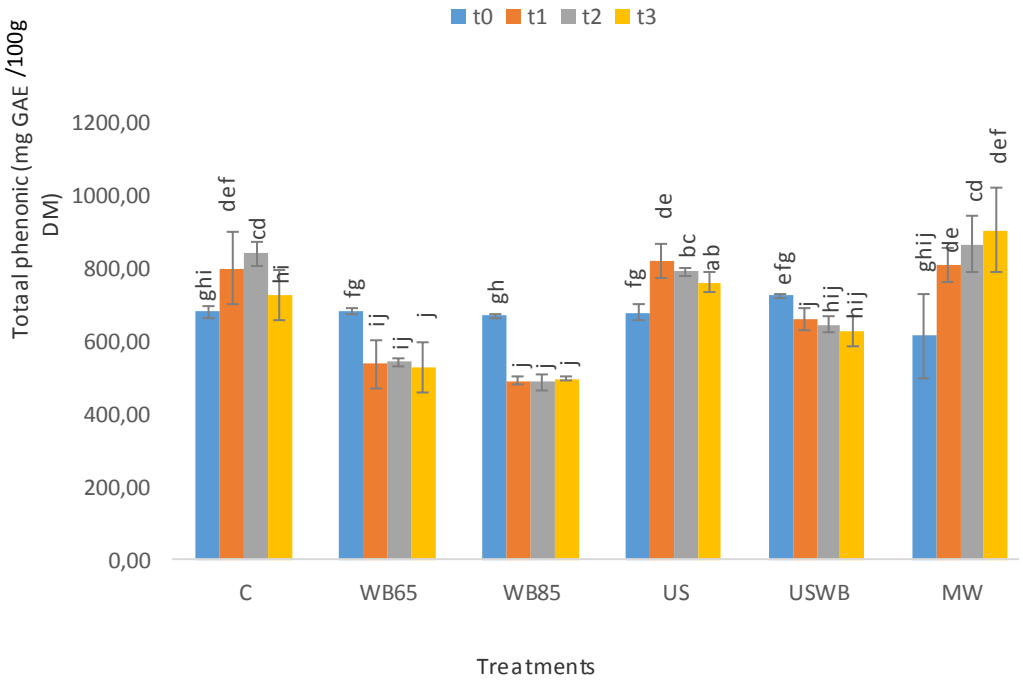

Figure 1. Variation of the total phenolic content of the tomato pulp of different treatments stoked for 45 days and a half at room temperature. Error bars indicated one standard deviation. Data points marked with the same letter are not significantly different ($p < 0.05$). t0: 0, t1: 15 days, t2: 30 days, t3: 45 days of storage. C: Control: tomato pulp without any treatment; WB65: tomato pulp subjected to a heat treatment at 65°C for 15 min in a water bath; WB85: tomato pulp subjected to a heat treatment at 85°C for 15 min in a water bath; US: tomato pulp sonicated; USWB: ultrasound combined with mild heat pasteurization; MW: tomato pulp treated in microwaves bath.

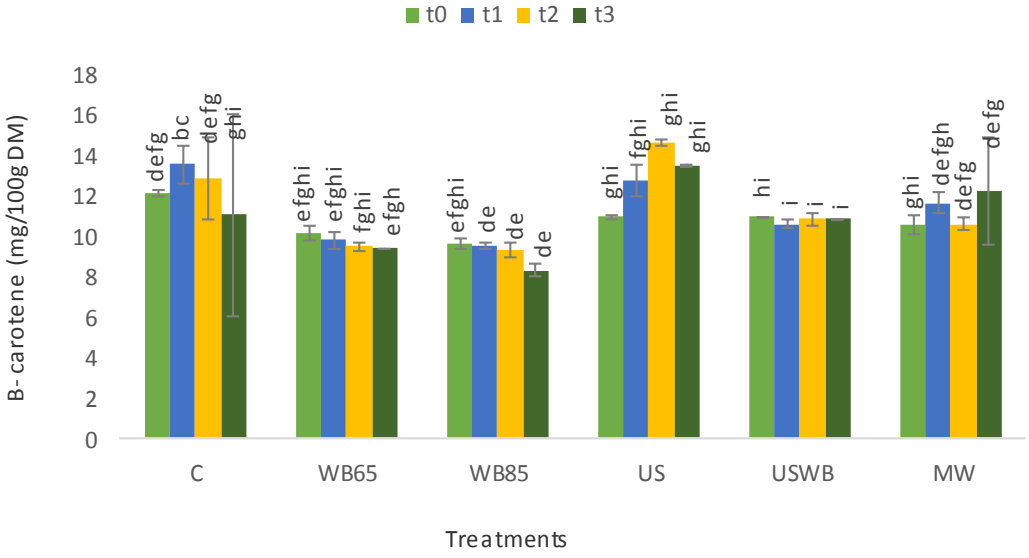

Figure 2. Variation of the β-carotene content of the tomato pulp of different treatments stoked for 45 days and a half at room temperature. Error bars indicated one standard deviation. Error bars indicate one standard deviation. Data points marked with the same letter are not significantly different ($p < 0.05$). t0: 0, t1: 15 days, t2: 30 days, t3: 45 days of storage. C: Control: tomato pulp without any treatment; WB65: tomato pulp subjected to a heat treatment at 65°C for 15 min in a water bath; WB85: tomato pulp subjected to a heat treatment at 85°C for 15 min in a water bath; US: tomato pulp sonicated; USWB: ultrasound combined with mild heat pasteurization; MW: tomato pulp treated in microwaves bath.

during storage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Conceptualization and designing of a beef quality determination framework based on beef supply chain in Tanzania

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A framework for determining the quality of beef was developed based on the assessment of Beef Supply Chain (BSC) between the slaughter and retail points. In this respect, slaughtering, distribution, retailing and consumer preferences models were developed for assessing the beef processing designed features (BPDFs) across the BSC specifically in Slaughtering Facilities (SFs), Beef Distributing Facilities (BDFs), and Beef Retailing Premises (BRPs) in Dar es Salaam and Mbeya cities. The organized ideas, rules and beliefs to a set known as a framework for determining the quality of beef was one of the outcomes of this study. This study suggests that the claim that beef-quality is a complex phenomenon and cannot be objectively determined is not always correct. Applicability of the designed framework is useful as it avoids mistakes and misunderstanding resulting from the desire to measure the quality of object. The study proposed the development of other frameworks for beef quality determination across other processing units (breeding, grazing, cattle transportation and post retailing activities for its preparation at household levels).

Key words: Beef quality determination, slaughtering facilities, beef distribution facilities, beef retailing premises, compliance level.

INTRODUCTION

The current means of determining the quality of retailed beef depends on mass inspection specifically on grading systems like Meat Standards Australia (MSA) and United States Department of Agriculture (USDA) of the processed products at the end of production (Tatum et al., 2011). The quality of beef like any other products is also determined based on factors like price in such a way that the higher the quality of the product, the better the price, and vice versa (Becker, 2002). In this respect, other studies have shown that there is lack of global implementable framework for determining the quality of beef in trading (Becker, 2000; Grunert et al., 2004; Robles et al., 2011; Bevilacqua et al., 2013).This has led to the difficulty in making decision on the suitability of

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beef at purchasing point based on the available quality attributes (Becker, 2000; Grunert et al., 2004; Robles et al., 2011; Bevilacqua et al., 2013). In this respect, service quality (the comparison of the expectations with performance) based frameworks that comprehensively characterize various factors across the Beef Supply Chain (BSC) were developed for overwhelming the multi-faceted nature of the term 'quality' in business strategy (Seth et al., 2005; Dhanalaksmi et al., 2010; Gupta and Singh, 2012; Bevilacqua et al., 2013). The named frameworks were developed based on different factors of their respective areas of suitability, such as geographical, demographic, social and economic factors.

Quality cues based frameworks for measuring the suitability of beef were widely formalized and used by a set of indicators, ranging from small numbers to a dozen by carcass/meat classification systems. For, example, degree of marbling (dispersion of fat within lean) has been used for a long time in many developed countries as the main assessment index in the systematic methods and standards for evaluating beef quality (Polkinghorne and Thomson, 2010; Cheng et al., 2015; Gagaoua et al., 2017). Although various grading systems were developed and extensively implemented in meat trade, two main draw backs were not yet addressed. The first one is related to the complexity and heterogeneity of beef carcass that is too high to be evaluated by a single classification system. Another drawback is connected with different interest, views and interpretations among the beef processing stakeholders (breeders, slaughtering personnel, retailers and consumers) along the beef supply chain on the meaning of the term quality.

Different from other studies on the framework for quality determination, this study went deep in quality measurement by designing the infrastructural beef processing designed features (BPDFs). In connection to this, beef supply units, that is, Slaughtering Facilities (SFs), Beef Distributing Facilities (BDFs) and Beef Retailing Premises (BRPs) were assessed based on both their availability and compliance of BPDFs across the BSC. Therefore, this study was designed to work as a benchmarking model for conceptualization and designing of frameworks for quality measurement of various products across the processing chain.

Beef quality concept

Beef quality is conceptually described by using a flowchart that divides the scholars into multi-disciplinary and requirements-based schools of thought as shown in Figure 1. In multi-disciplinary based group, beef quality is classified into four categories: economic, marketing, operational and management and transcendent approach of philosophy (Bevan, 1995; Beker, 2002; Fields et al., 2014). Economic discipline, in this case, dwells on profit maximization and market equilibrium of product between produces and the respective users. Marketing approach, on the other hand, refers to determinants of buying behavior and customer satisfaction through value, cost and comparison of disparate objects and experience variables. In addition to that, operation management category focuses on engineering practices and manufacturing controls (Bevan, 1995; Becker, 2002; Fields et al., 2014). A transcendent approach of philosophy, as other discipline in this regard, details the quality as an innate excellence, absolute and universally recognizable, and unanalyzable (experience based recognizable) property.

In the requirements-based studies, beef quality can be described as a multi-faceted issue due to the involvement of BQTRs like FAO based guidelines with respect to nutritional value, absence of alteration, genuineness, safety and traceability (Mullen, 2002; Moody and Shanks, 2003; Bevilacqua et al., 2013). This is extended to an argument that, quality is described based on the direct observation of the accessible indicators through human interpretation based on the given BQTRs (Bevan, 1995; Henson and Loider, 2001; Schröder and McEachern, 2002; Harvey, 2006). In connection to this, the quality of any product can be deconstructed into a set of qualities on a sense that the more complex the product the longer and more multidimensional is the list of qualities (Schröder and McEachern, 2002; Harvey, 2006). This is described by Cheng et al. (2015), during which beef quality is characteristically described by color, tenderness, intramuscular fat, moisture and ultimate pH. In the requirement-based approach, quality is distinguished from qualities, which are the explicit expression of attributes rather than an essential embodiment of character (Schröder and McEachern, 2002; Harvey, 2006). Qualities include characteristics, attributes or properties that describe quality of product in terms of values that are inferred from indicators (Harvey, 2006). This information indicates that quality cannot be defined by a list of qualities rather it is a concept that evokes the essence of the product (Becker, 2002; Mitra and Golder, 2006; Harvey, 2006). The mentioned essence of the product is described based on the type of information (facts, estimates, predictions and relationships) and specific constructs (concepts or ideas about an object, attribute or phenomena that are worthy of measurement) that are relevant for decision making under the BQTRs (Schröder and McEachern, 2002; Harvey, 2006).

Despite the connectivity of the term 'quality' with a given product, it is disregarded as a product's attribute, as the attributes required for quality depend on how the product is used (Bevan, 1995). In this regard, the term'quality' is mentioned as being complicated in two

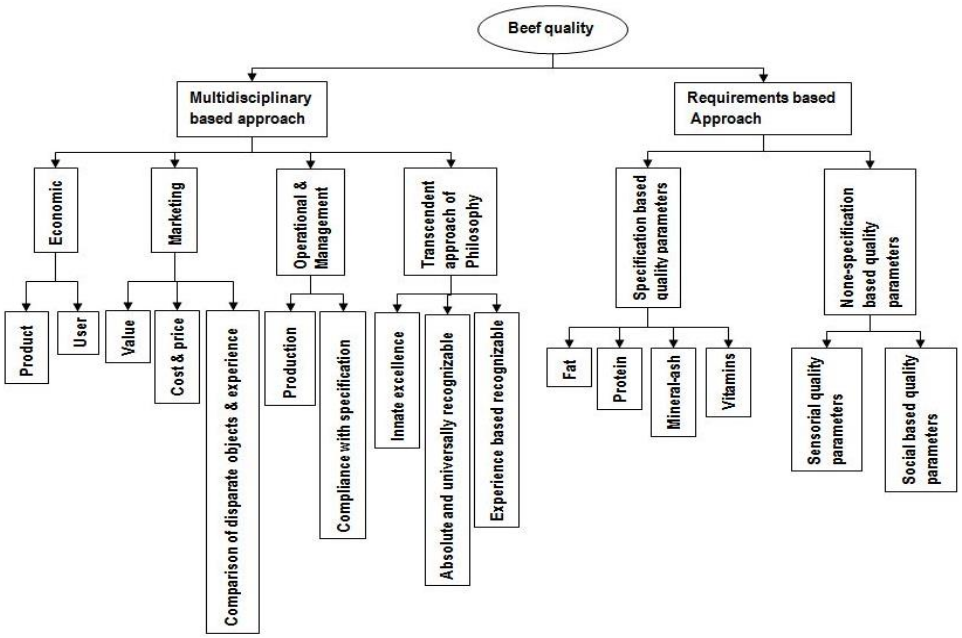

Figure 1. Flowchart for describing beef quality concept.
Source: Reeves and Bedner (1994); Acebrøn and Dopico (2000); Becker (2002); Mullen (2002); Costa et al., (2004); Troy and Kerry, 2010).

scenarios. The first one is related to the description of quality against the given standards (Harvey, 2006). The other scenario is on the description of quality of product that is based on inherent characteristics of the product (Reeves and Bedner, 1994; Acebrøn and Dopico, 2000; Becker, 2002; Mullen, 2002; Costa et al., 2004; Troy and Kerry, 2010). Based on this fact, it is concluded that, it is not possible to have a single definition for the term quality (Reeves and Bedner, 1994; Miliauskaité, 2012; Fields et al., 2014). The concept has wide variety of phenomena and depends on the context and even the time period in which it is examined (Miliauskaité, 2012). Conflicts among the identified scholars are inevitable because each defines quality from different point of views. Besides the strengths and weaknesses of each definition, no one is better than others in every situation (Bevan, 1995; Miliauskaité, 2012). Philosophically, these definitions were formulated based on the consumer demands that are relevant to the perceived quality decisions. This is therefore, highlighted by other studies that, when a given technical product specifications are responsible for objective quality, the goal has been to improve the physical food product, based on the demands of the consumers (Acebrón and Dopico, 2000).

Quality of the given product can be determined by using standards on either being specific or measurable outcome for comparative purposes (Schröder and McEachern, 2002; Harvey, 2006). Standard is one of the complicated terms due to its existence as both fixed criterion (against which outcome can be matched) and a measure of attaining a given level. In quality determination, the emphasis is on the use of the term standard as a fixed criterion and not level of attainment (Schröder and McEachern, 2002). Standard or specification" is a document comprising a list of requirements in connection to the quality cues of the interested product (Schröder and McEachern, 2002). In this case, there are two types of standards, that is, statutory standards and commercial standards (Schröder and McEachern, 2002). Statutory standards are compulsory requirement in public quality schemes (Becker, 2002) but are not necessarily helpful to consumers (Schröder and McEachern, 2002). Commercial standards are generally communicated through labeling information, but the actual underlying specifications are rarely accessible (Schröder and McEachern, 2002). When a beef is taken as an example to address this situation, nutritional quality parameters, that is, fat, protein, mineral ash, and moisture have specifications; whereas there is no report of specifications in sensorial and social quality parameters.

Quality determination

Determination of the quality of the product is also

connected to the seeking of consumers' optimum needs and motivations based on economic and deeper levels involving emotions, cultural norms and values, and group affiliations (Devine et al., 2004). It was also highlighted that the term optimum in product quality should not be considered as a single unchangeable state, but it depends on the end use of the product and even the way the product is prepared (Becker, 2002). This is subjective based quality determination and may be defined as fitness for use, fitness for certain goals, or as the composite of product attributes which yield consumer satisfaction (Becker, 2002; Grunert et al., 2004). Studies have linked the quality determination concept to qualitative grading-based analysis through synthetic based measurement (de Vicente Lama et al., 2010; Kukula and Bogocz, 2014; Egbunike et al., 2018). In this respect, studies have shown that quality as a complex phenomenon is explained by *r* requirements, that is, x_1, x_2,..,x_r, whereas, each of the requirements is described by *n* designed features (Kukula and Bogocz, 2014; Grzegorz, 2015). In addition to that, information of the BPDFs forms a two dimensional matrix as shown in Equation 1, where x_{ij}, represents values of variable *X* in the object Wi.

$$X = [x_{ij}] = \begin{bmatrix} x_{1(1)} & x_{1(2)} \cdots & x_{1(71)} \\ x_{2(1)} & x_{2(2)} \cdots & x_{2(18)} \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ x_{r(1)} & x_{r(2)\ldots} & x_{r(n)} \end{bmatrix} \quad (1)$$

Every requirement that contributes in the complexity of the quality in the measured product is characterized by a vector of BPDFs as shown in Equation 2, whereas, *I* = *1…, r*.

$$X_i = [x_{i1} \quad x_{i2}, \quad . \quad ., \quad x_{in}] \quad (2)$$

According to the method of zero unitarization there is a constant reference point, which is the range of normalized variables that is expressed by Equation 3. This parameter is used in the normalization of both stimulant (*X* Є *S*) and de-stimulant (*X* Є *D*) based BPDFs at range [0, 1] as shown in Equations 4 and 5, respectively.

$$R(X_j) = \max x_{ij} - \min x_{ij} \quad (3)$$

$$z_{ij} = \frac{x_{ij} - \min x_{ij}}{\max x_{ij} - \min x_{ij}} \quad (4)$$

$$z_{ij} = \frac{\max x_{ij} - x_{ij}}{\max x_{ij} - \min x_{ij}} \quad (5)$$

It was noted that the normalized stimulant and de-stimulant based BPDFs are subjected to a linear transformation according to zero unitilization method (ZUM) and form the matrix as shown in Equation 6.

$$Z=[z_{ij}]=\begin{pmatrix} z_{11} & z_{12} & . & . & . & z_{1n} \\ z_{21} & x_{22} & . & . & . & z_{2n} \\ . & . & . & . & . & . \\ . & . & . & . & . & . \\ . & . & . & . & . & . \\ z_{r1} & z_{r2} & . & . & . & z_{rn} \end{pmatrix}_{rxn} \quad (6)$$

Thus, the matrix (X) with dimensions (*r* × *n*) crosses through the matrix (Z) with the same dimensions. In this regard, each of the requirements is described by a vector of normalized BPDFs as shown in Equation 7.

$$[Z_i] = [z_{i1}, z_{i2}, \ldots, z_{in}] \quad (7)$$

In order to assess the quality of a given complex product, a number of normalized variables of their respective BPDFs should be assessed by setting and summing up by using Equation 8. In this regard, *n* is the total number of normalized BPDFs for appropriately designed object, q_i is the sum of complied normalized BPDFs and z_i is the i-th variable (Kukula and Bogocz, 2014). This is followed by assessing the synthetic variable Q_i that characterizes the *i-th* normalized requirement as shown in Equation 9. In addition to that function aggregating the partial measurements normalized features in relative scale is used in quality determination as shown in Equation 10.

$$q_i = \sum_{j=1}^{n} z_{ij} \quad (8)$$

$$Q_i = \frac{1}{n}\sum_{j=1}^{n} q_i \quad (9)$$

$$CI_j = 100x\frac{1}{n}\sum_{i=1}^{n} q_i \quad (10)$$

The assessed opinions with respect to the requirements of the level of quality of a given object can be divided into different groups: the range $R(Q_i)$ and groups (N_g) group classifying parameter, k_i, as shown in Equations 11 and 12, respectively.

$$R(Q_j) = \max Q_i - \min Q_i \quad (11)$$

$$k_i = \frac{R(Q_i)}{N_g} \quad (12)$$

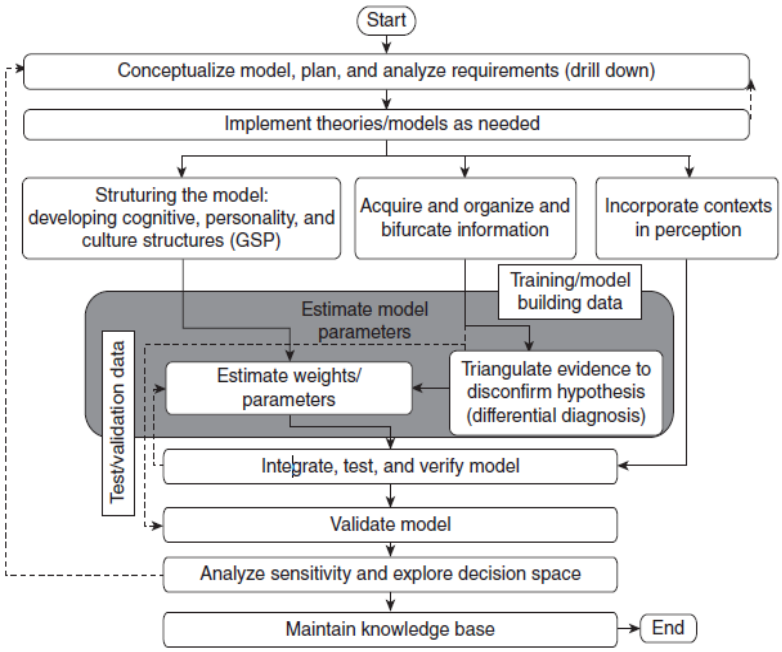

Figure 2. Framework construction processes.
Source: Silverman et al. (2010).

Frameworks for quality determination

The beef quality determination frameworks are currently depending on mass inspection of the processed beef by grading systems (Tatum et al., 2011).

This determination involves the use of standards that were published on reacting on the raised complaints as carcasses were traded unseen by buyer (Polkinghorn and Thompson, 1999). Some of these standards include MSA, USDA grading system, Canadian grading system, Japanese Meat Grading Association (JMGA) grading and Europe classification.

The knowledge engineering-based model building process (KEMBP) as shown in other studies is a model for frameworks designing (Moody and Shanks, 2003; Silverman et al, 2010). The KEMBP is required for framework designing as it addresses the following key functional requirements: systematically transforming empirical evidence, tacit knowledge, and expert knowledge into data for modeling; reduce human errors and cognitive biases (e.g., confirmation bias); verify and validate the model as a whole; and maintain the knowledge base over time (Silverman et al, 2010). Studies have detailed the following stages for framework building process as shown in Figure 2: model conceptualization, planning, and analysis requirements; theories/model review/implementation; model structuring; information acquiring, organization and bifurcation; model parameters estimation; context in perception incorporation; model integration, testing and verification; model validation; sensitivity analysis; and knowledge based maintaining (Moody and Shanks, 2003; Silverman et al, 2010).

Model conceptualization, planning, and analysis requirements as the first stage involve the characterization of modeling problem based on specific objective and nature of the domain. In general, the modeling problem, along with the context, provides what needs to be accomplished and serves to define the method to go about. At the end of this stage, the following were expected: clarified objectives; understanding the contexts surrounding the model; appropriate information from other studies; and the conception of the modeling problem. In the next stage, the basic theories necessary to describe the social systems are implemented in the designed framework. Quality measurement was reported as one of the critical parameters in the highly competitive market (Kennerley and Neely, 2002; Grzegorz, 2015). Techniques involved in quality determination were classified into quality measurement methods and quality determination conceptual models (Seth et al., 2005; Grzegorz, 2015).

The former technique was expressed by a six sigma, that is, a set of management techniques and tools for process improvement (Klefsjö et al., 2001; Goh, 2012). However, this technique was criticized as it was noted as

Table 1. Summary of the models based on service quality.

S/N	Model	Key findings	Selected weakness
1	Technical and functional quality model (Seth et al., 2005; Dhanalakshmi et al., 2010)	Service quality depends on technical quality, functional quality and corporate image of the organization in consideration	Silent on the means of measuring technical and functional quality
2	GAP model (Parasuraman et al., 1994; Seth et al., 2005; Dhanalakshmi et al., 2010; Gupta and Singh, 2012)	Used as analytical tool as it is helpful in systematic identification of service quality gaps between a numbers of variables affecting the quality of offering	The model does not explain the clear measurement procedure for the measurement of gaps at different levels
3	Model of perceived quality and satisfaction (Spreng and Mackoy, 1996; Seth et al., 2005;Gupta and Singh, 2012)	A key determinant of service quality and customer satisfaction is meeting customer desire	Does not highlight how service quality is achieved and operationalized
4	Retail service quality and perceived value (Seth et al., 2005;Gupta and Singh, 2012)	The technical service quality is an important contributor to product quality and value perceptions and hence influences willingness to buy	The model considers only one value construct, that is, value for money
5	Framework of Quality Measurement (Grzegorz, 2015)	Quality measurement may be conducted in analytical and synthetic levels	Aggregate function describing the quality was not clearly defined.

too narrow designed to both fix the existing processes and allowing little room for new ideas (Klefsjö et al., 2001; Goh, 2012; Grzegorz, 2015). The latter technique, on the other hand, was highlighted in a list of some of the frameworks that were reviewed in developing this study as shown in Table 1. The main gap in the first five frameworks was that what is measured in connection to quality determination cannot be expressed in numbers. Although this gap was addressed in the sixth item, there was a gap on describing the quality as the framework depends on a literature-based quality weighting factor. In this respect, this work is developed for addressing this gap by using Likert based information for quality determination.

MATERIALS AND METHODS

Framework conceptualization-features and governance

Figure 3 shows a flow chart that is used for characterizing the process of beef quality determination. Slaughtering, distribution, retailing and consumer preferences models were developed and tested based on information from other studies and Likert based information (Mwashiuya et al., 2018a, b). These models were used in the assessment of beef processing features across the supply chain in Dar es Salaam and Mbeya cities. According to the 2002 population and housing census, as the former city with a total area of 1,800 km2 has a population of 4.36 million, the latter one with 19,098 km2 has a population of 0.69 million (United Republic of Tanzania (URT), 2013). As indicated in other studies, Dar es Salaam city was purposively selected based on researcher experience and judgment (Guarte and Barrios, 2006; Etikan et al., 2016). This is one of the non-probabilities sampling technique that a researcher uses to choose a sample of subjects/units from a population (Guarte and Barrios, 2006; Triola et al., 2006; Etikan et al., 2016). Although this sampling approach is too subjective to have good representative sample from the population, it is useful especially when randomization is impossible like when the population has unique characteristics (Etikan et al., 2016). In this regard, Dar es Salaam was selected because of its unusually high population due to its wider metropolitan characteristics which attract many people looking for employment in the industries. Mbeya city, on the other hand, was conveniently selected among other three cities as shown in other Literature (FAO, 1997). The convenience in this case was described in terms of more availability and accessibility of SFs, BDFs and BRPs in Mbeya as compared to other cities.

Result associated with this assessment was discussed based on information from other studies in seven steps for designing a framework for quality determination. The designed framework was then used for measuring of the quality of beef in Dar es Salaam and Mbeya cities.

Requirement quality cue based theories on consumer behaviour towards beef quality determination was implemented in the designed framework. In this case, BQDF was designed through internal consultation and analysis of existing cattle slaughtering, beef distribution and retailing chain. In this respect, BQDF was designed under four main steps through assessment of the current beef supply chain as shown in Table 2. In this regard, the Likert scale based BQDFs in both analytical and synthetic levels was designed based on the results of the infrastructural assessment of the current slaughtering, distribution and retailing chain as shown in

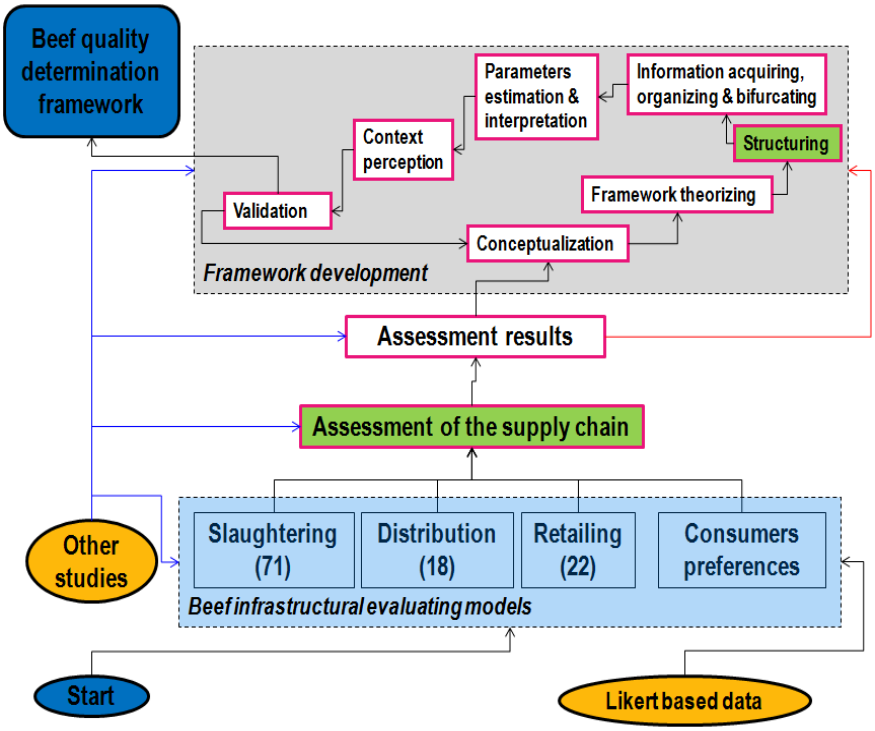

Figure 3. Conceptualized flow chart that shows designing steps for beef quality determination framework.

Table 2. Infrastructural characteristics-based designing steps and governance of beef quality determination framework as customized from other studies (Das et al., 2006).

Designing step	Outcome
Concept selection	Image and symbolic presentation of an abstract idea, that is, SFs, BDFs and BRPs
Interrelationship identification	The link between central and local authorities against all actors in beef trading
Formulation of definition	Conceptual and operation definitions
Formulation of theoretical rationale	Knowledge and confirmation of the identified theoretical connections between variables

other studies (Grzergorz, 2015).

Framework structuring

The framework for beef quality measurement on analytical level was structured through the four steps: first, the requirements for beef quality production at fixed time between slaughtering and retailing points were identified; then, type and number of quality cues describing the identified requirements were found; next, the unit and accuracy of measurement was established for each quality cue; and finally, for each of the listed quality cues, overall score in percentage was provided by using Equations 13, 14, and 15 for the assessed slaughtering, distribution and retailing practices, respectively.

$$TS_i = 100x\frac{1}{N_{ts}}\sum_{i=1}^{n_{df}} ts \quad (13)$$

$$OV_i = 100x\frac{\sum_{i=1}^{N_v} Vc_i}{N_v} \quad (14)$$

$$OS_i(\%) = \left[\frac{\sum_{i=1}^{N_B} Bc_i}{N_B}\right] x\ 100 \quad (15)$$

The framework on analytical level was presented as shown in other studies by a set of three vectors δ_3, where δ_3 constitutes three-dimensional vectors, that is, slaughtering (δ_1,γ_1), distribution (δ_2,γ_2)

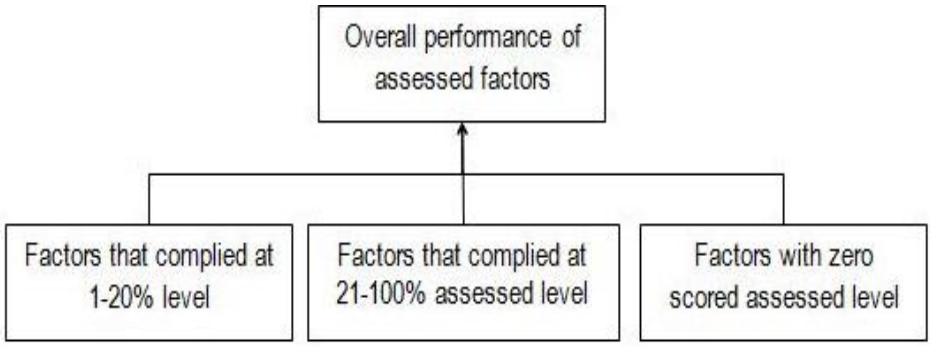

Figure 4. Transformation of the assessed features to the overall framework data.

and retailing (δ_3,γ_3) (Grzergorz, 2014). When a vector (δ_i,γ_i) is taken for detailing this case: δi for i=1,2,3 was described as the i-th requirement formulated by one of the three identified blocks in relation to the processed beef; and γ_i means importance of the i-th requirement on the interval scale $0…m$. In this framework, the level of compliance was described based on algebraic Equation that includes the assessed factors that were categorized with respect to levels of compliance as shown in Figure 4. In this regard, the overall performance of the assessed factors was used as a main quality determination subject for BQIF at 1-20% level, 21-100% level; and zero scored level.

The grouping of the requirements in the assessed beef processing components (SFs, BDFs and BRPs) was adopted from other studies into the following steps: First, the range of the complied features was sought by using Equation 16. This is followed by estimation of a three groups dividing parameter, k by using Equation 17. This parameter was used to divide the entire set of the requirements into the following groups as shown in Equations 18, 19 and 20: first, the highest complied features; the second group was composed of the average conformed features; and the last group with low level of compliance.

$$\text{Range } (Q_i) = \text{Maximum } (Q_i) - \text{Minimum } (Q_i) \quad (16)$$

$$k = \frac{Range\ (Q_i)}{3} \quad (17)$$

$$\text{Group.1: } Q_i \in [\text{Maximum } x_{ij}\text{-}k, \text{Maximum } x_{ij}) \quad (18)$$

$$\text{Group.2: } Q_i \in [\text{Maximum } x_{ij}\text{-}2k, \text{Maximum } x_{ij}\text{-}k) \quad (19)$$

$$\text{Group.2: } Q_i \in [\text{Maximum } x_{ij}\text{-}3k, \text{Maximum } x_{ij}\text{-}2k \quad (20)$$

After having the conceptual framework, its respective data requirements were determined from other studies. In this respect, strategies for determining appropriate SFs, BDFs and BRPs as the requirements for beef consumers were developed. This was implemented by aligning the development actions with the quality assurance system in which not only concrete measures, that is, quality documentation (pre-registration reports), quality assessment (inspection and surveillance) and quality reviews were defined, but also the decision of achieving the respective measures were delineated.

After eliciting the expert input from the reviewed studies, critical pieces of information were verified by pitting against other sources of information like empirical evidence from database and event data. In this respect evidence based Equation and flowcharts were designed by organizing the empirical evidence or expert input by breaking statements into simpler units. Any specific reliable information was used to identify and tag for further investigation and sensitivity analysis.

Incorporation of context in perception

The argument of consumer's characteristics of changing preference on beef as reported in other studies (Becker, 2000, 2002) was used as an important parameter for gathering information from stakeholders across the surveyed SFs, BDFs and BRPs. In this regard, an extensive survey was designed to elicit knowledge for beef quality from all stakeholders across these infrastructural based characteristics in the targeted format.

RESULTS AND DISCUSSION

Features and governance in the framework conceptualization were presented with respect to the flow chart as shown in Figure 5. In this respect, the beef quality determination was identified as a problem in this development. This was followed by establishment of specific objective and nature of domain as the problem characterizing criteria. The former criterion categorizes the modelling problem into beef processing (system type) and compliance assessment (system purpose); whereas BQIF as information and designed BPDFs, that is, processing units (SFs, BDFs and BRPs) were identified as categories of the latter dividing factor. Finally, objectives in connection to the need to be accomplished and definition of the quality measurement method were developed from the established categories.

The conceptualized framework for measuring the quality of beef across the slaughtering, distribution and retailing chain is shown in Figure 6. The conceptual framework comprises six needs that should be

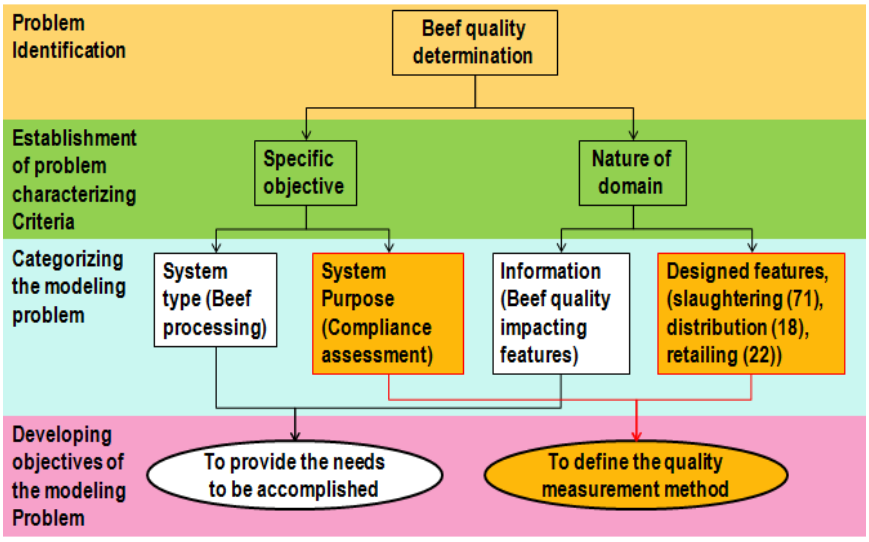

Figure 5. A conceptualized flow chart showing the features and governance in the beef quality determination framework.

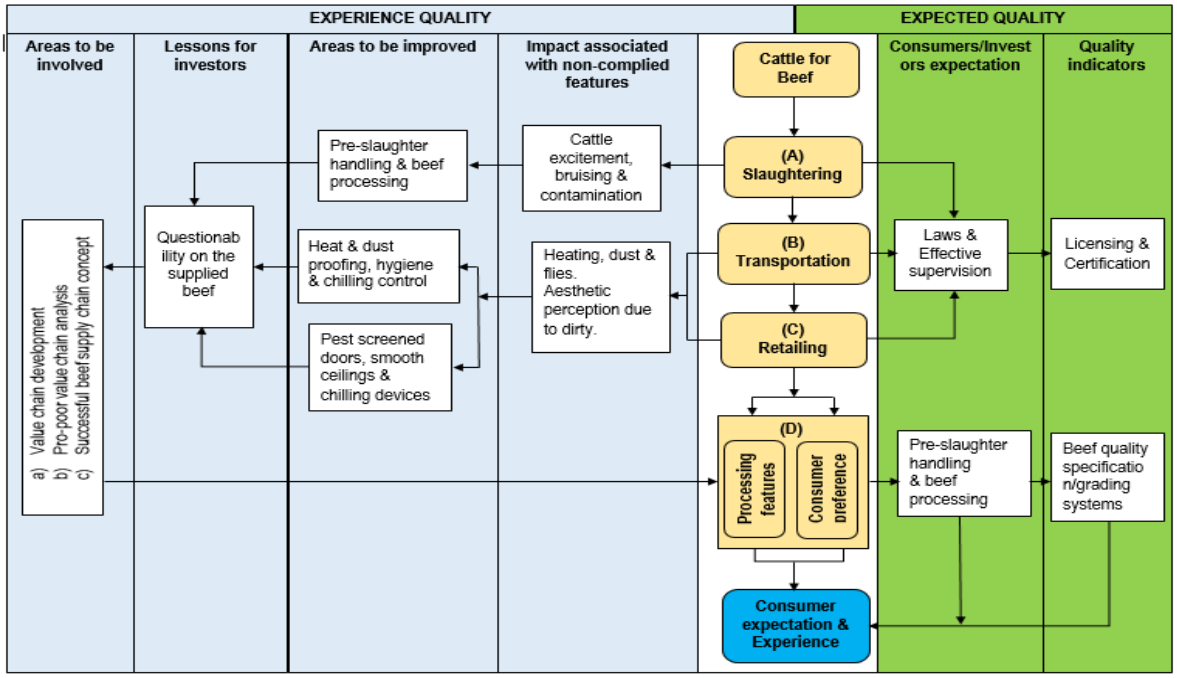

Figure 6. A conceptualized flow chart showing the framework for beef quality determination across the slaughtering, distribution and retailing chain.

accomplished for determination of the level of quality of beef across the supply chain. These are areas to be involved, lesson to be learnt, areas for improvement, impact associated with non-complied features, consumers/investors expectation and quality indicators. The first four needs formulate experience category of

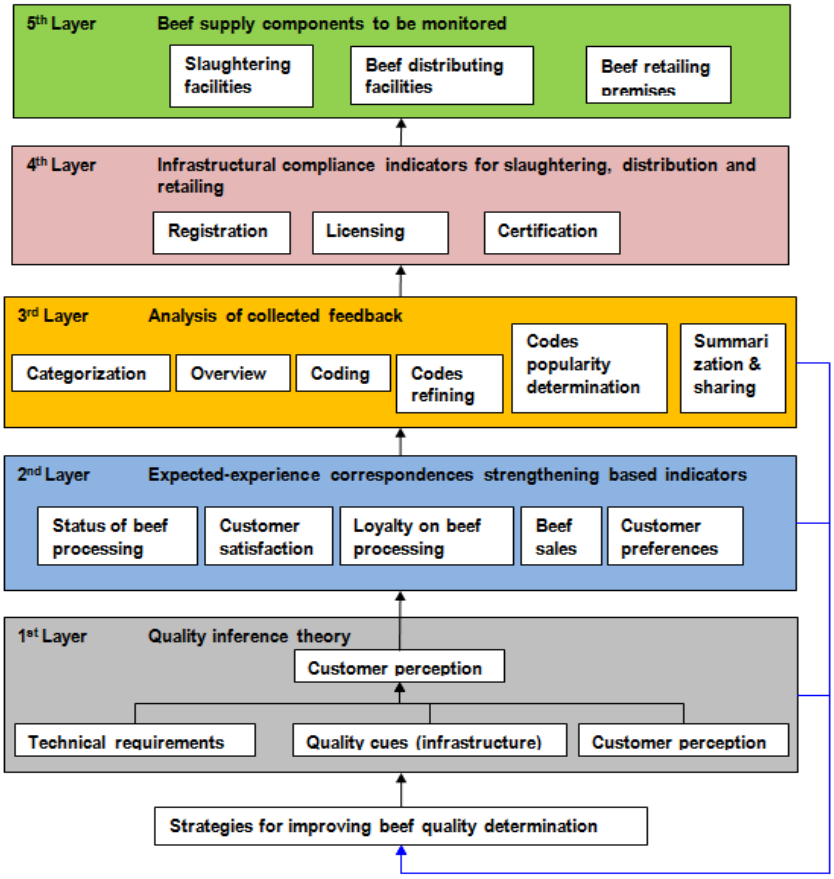

Figure 7. Flowchart indicating theories implemented during development of the quality determination framework.

quality, whereas the last two are the components of expected quality. The level of correspondence between the former and the latter categories of quality is described as the parameter for measuring the needs to be accomplished.

The quality of beef in this concept can be described based on the mechanisms involved with cattle slaughtering unit as an example. In this respect, the cattle excitement, bruising and beef contamination were identified as the measures of the impacts associated with non-complied BPDFs. These requirements were connected with pre-slaughter handling and beef processing as the needs for areas to be improved. The questionability on the handling and beef processing was described as another important requirement for investors to learn the needs for processing suitable beef. After learning the whole system, the areas to be involved, that is, value chain, pro-poor value chain analysis and successful beef supply chain concept were established. Consumers/investors expectation was also taken as another requirement that is indicated by laws and effective supervision. The last need to be accomplished in this design was quality indicators that were indicated by licensing and certification of slaughtering activities. This scenario has also been shown in other studies in which private sectors process and distribute beef for niche market (Kamugisha et al., 2017; Muzzo and Provenza, 2018).

Figure 7 shows the implemented theories for framework development within five main blocks. Beef quality inferring process is shown as one of the layers that show requirements – quality cues based interaction theory. The block inter-relates four main elements (technical requirements, quality cues, customer perception, and customer requirements) that involved in beef quality extrapolation. First layer is another block in this designing that describes correspondence between expected quality and experienced quality was shown by five customer feedback based indicators. This layer is important in the framework development as it provides

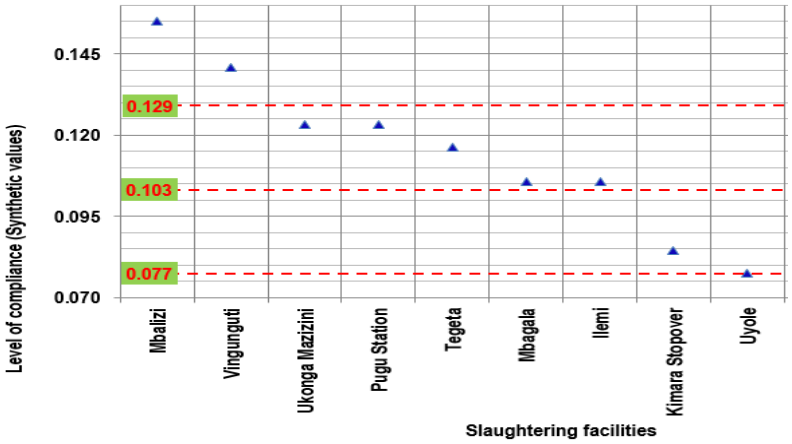

Figure 8. Synthetic outcome measure values in the surveyed slaughtering facilities (N_{sf} = 6 for Dar es Salaam and 3 for Mbeya).

necessary data for customer experience and their overall satisfaction levels between experienced and expected qualities. The means at which the collected feedback is analyzed are shown in the second layer of the implemented theories. This activity includes seven steps: data collection, data categorization, quick overview, feedback coding, code refining, codes popularizing and summarizing. Infrastructural compliance indicators for beef supply are shown in the third layer of the theory implementing diagram. This layer involved conformity checking practices, that is, registration, certification and licensing of beef processing and distribution facilities. Fourth layer comprised SFs, BDFs and BRPs as the important facilities for measuring the level of quality beef.

Application of the designed framework

A list of 39 observed infrastructural based beef processing features among 71 in the surveyed SFs was established for Dar es Salaam and Mbeya. The list includes the level of subsequent quality cues of beef in the processing infrastructural based perspective. The list also includes components of synthetic outcome measure in three types of performance variables, namely, nominants (where the best value is implied); destimuli (where the low values indicate better performance); and stimuli (where the high value means better performance). This is a compliance-based result for the assessed SFs with 0 and 66.7% as the lowest and highest evaluated levels for the surveyed cities. The surveyed SFs with respect to the assessed beef processing facilities were ranked into three groups as shown in Figure 8. The highest ranked group includes Mbalizi (0.155) and Vingunguti (0.141) is bounded by 0.129 and 0.155 limits. This is followed by the group that comprised Ukonga Mazizini (0.123), Pugu Station (0.123), Tegeta (0.116), Mbagala (0.106) and Ilemi (0.106) SFs that were within 0.103 and 0.129 levels of synthetic outcome. The lowest group observed in this slaughtering assessment includes Kimara Stopover (0.085) and Uyole (0.077) as the lowest complied SFs in this study.

It is worth noting that the pattern discrepancy between the surveyed SFs in this analysis as shown in Figure 9 supports the failure of accepting null hypothesis that the assessed SFs have equal medians. In this respect, it was shown that the level of compliance of fourteen features in Mbeya was higher than in Dar es Salaam. In addition to that, the level of compliance of eighteen features in Dar es Salaam was higher than in Mbeya. Despite the mentioned discrepancies, similarity of the assessed cities is shown based on seven BPDFs, that is, flappers in cattle receiving area, slippery treatment area, waste drainage in eviscerating point, obstacle receiving area, dead-end raced receiving area, cattle holding entrance gate and straightness to stunning area.

A list of components of synthetic outcome measure in three types of performance variables, namely, nominants, de-stimuli and stimuli was also included in this study as shown in Figure 9. In this case, the level of compliance was 0% and 81.7% as the lowest and highest evaluated levels, respectively. The compliance of the surveyed SFs with respect to the assessed beef transportation was ranked into three groups as shown in Figure 10. The first group in this respect includes Mbagala and Kimara as the assessed SFs with the highest-level compliance. The second one comprises SFs that have moderate level of outcomes and consist of Ukonga Mazizini, Tegeta and

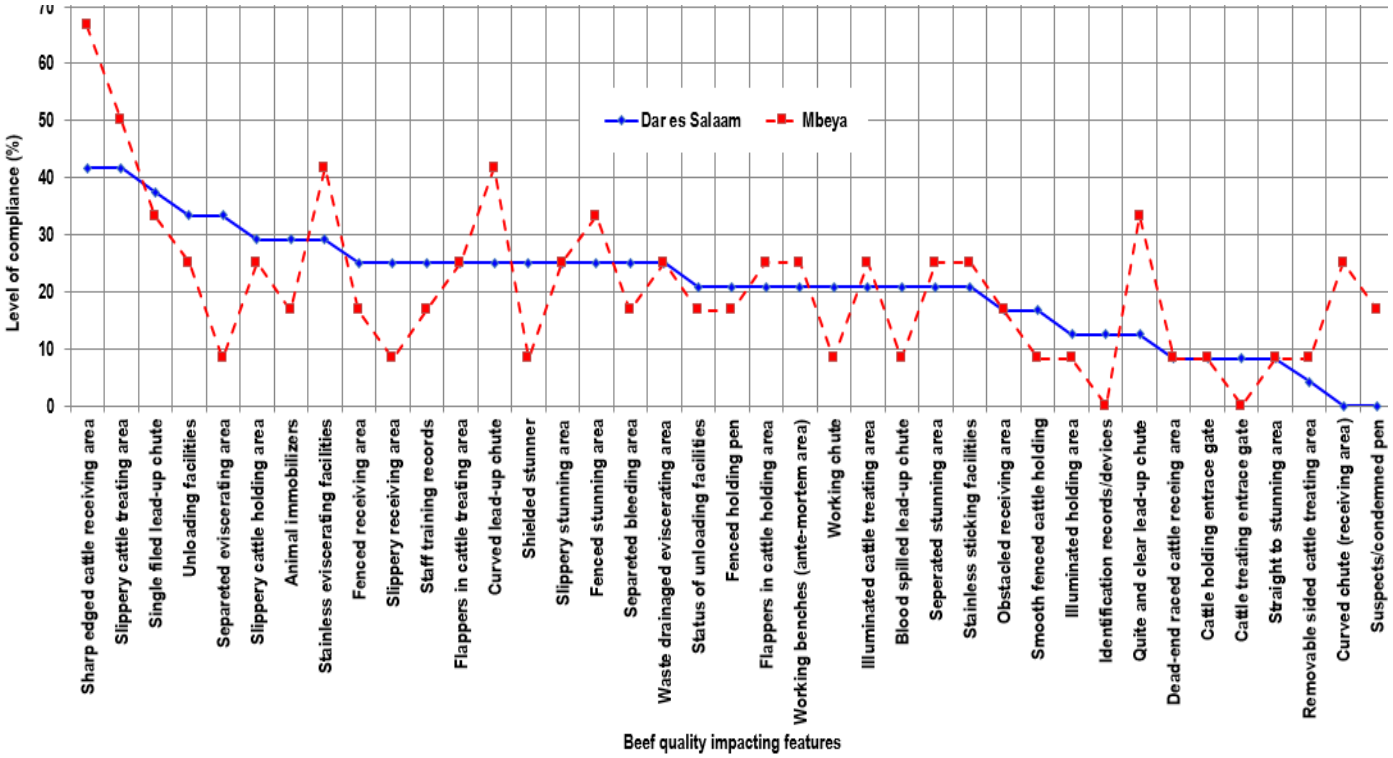

Figure 9. Running charts for the beef quality features established by using the designed beef quality determination framework (N_{sf} = 6 for Dar es Salaam and 3 for Mbeya).

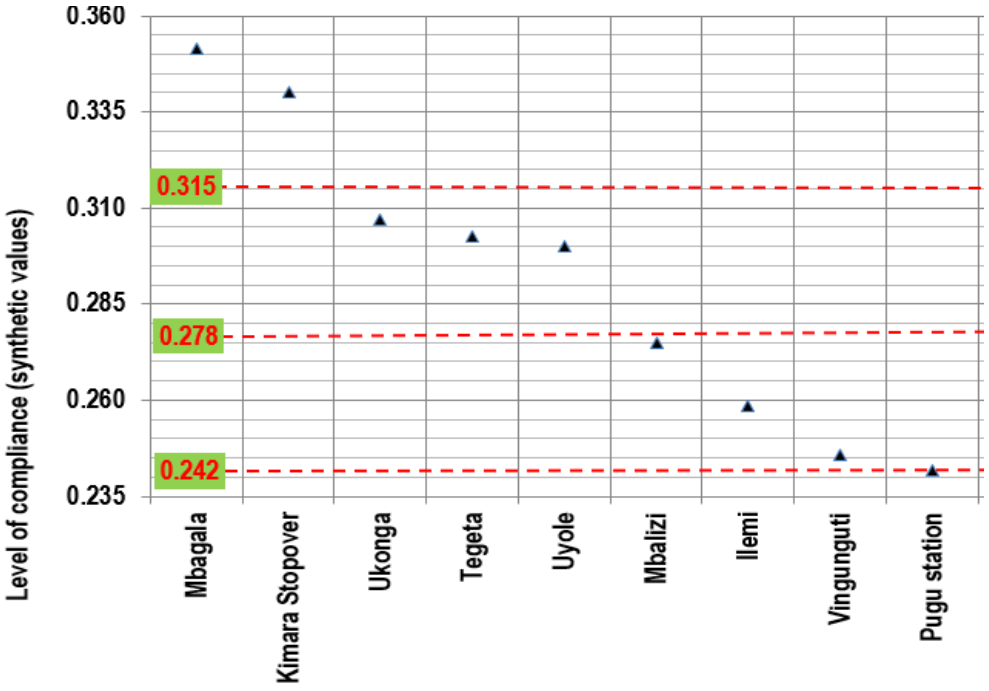

Figure 10. Assessment results for the beef quality impacting factors in beef transporting vehicles in the surveyed slaughtering facilities (N_v =10 perslaughtering facility.

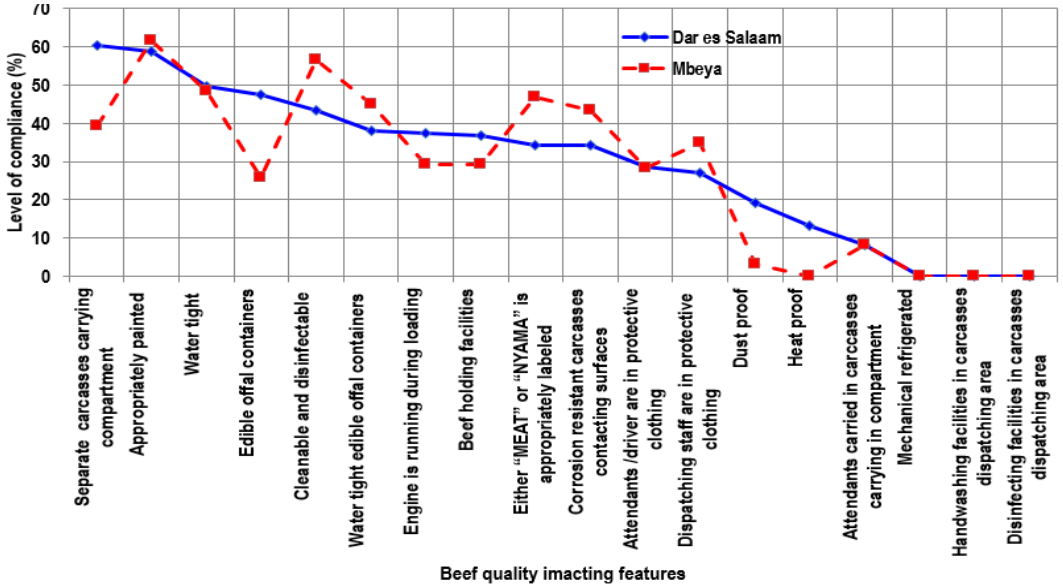

Figure 11. Running charts for beef quality impacting factors from the beef distributing facilities in the surveyed slaughtering facilities assessed by using BQDF (N_v = 10 per Slaughtering Facility).

Uyole. The last ranked group in this case includes Mbalizi, Ilemi, Vingunguti and Pugu Station that were found to have the lowest complied BDFs.

Results were further interpreted by using synthetic outcome measure values of compliance chart that classifies beef transporting vehicles into three groups as shown in Figure 10. The first group is the one that is identified by 0.129 and 0.155 boundary limits with 0.148 as the average scored level whereas Mbalizi and Vingunguti are SFs in this group. Another group is described by 0.103 and 0.129 limits with 0.115 as an optimum scored level and has UkongaMazizini, Pugu Station, Tegeta, Mbagala and Ilemi. The final group that has Kimara Stopover and Uyole is composed of 0.077 and 0.103 as minimum and maximum points with 0.081 as the average scored level.

The pattern discrepancies between beef distributing vehicles in the surveyed SFs in this study support the rejection of null hypothesis. In this case it was noted that at least one of the medians of the assessed vehicles in SFs appears to be different from the others. This situation is shown in Figure 11 where the level of compliance of seven features in Dar es Salaam was higher than Mbeya. In addition to that, level of compliance of six features in Mbeya was higher than in Dar es Salaam. Despite the mentioned discrepancies, similarity of the assessed cities is shown based on five BPDFs: attendant in protective gears, attendants in beef carrier, mechanical refrigeration, hand-washing facilities and disinfectants.

Measurement in beef retailing premises

The analytical level of quality measurement with respect to beef retailing for Dar es Salaam and Mbeya is shown in Figure 12. In this respect, a total of 22 values include components of synthetic outcome measure in nominant and stimuli only. In this respect, the compliance of the surveyed BRPs in 18 surveyed wards with respect to the assessed beef selling was ranked. The first group retailing premises in this regard includes four wards with the highest-level compliance: Kijitonyama, Iyunga, Ilomba and Kariakoo. The second is a group with wards that have retailing premises with an average level of compliance that includes Miburani, Mtoni, Toangoma, Kunduchi, Makuburi, Itezi, Saranga, Kawe, Maendeleo, Charambe and Wazo. The last group in this regard includes Uyole, Iyela and Ruanda as premises with the lowest complied beef transporting facilities. Results were further interpreted by using synthetic outcome measure values of compliance chart that classifies the assessed butchers in the surveyed wards into three groups. The first group is the one that is identified by 0.496 and 0.580 boundary limits with 0.543 as the average scored level whereas Kijitonyama, Iyunga, Ilomba and Kariakoo were the wards with the complied retailing premises in this group. Another group is described by 0.413 and 0.496 limits with 0.462 as the average value and contained the highest number of the surveyed wards in this study. The last group that has Uyole, Iyela and Ruanda is composed

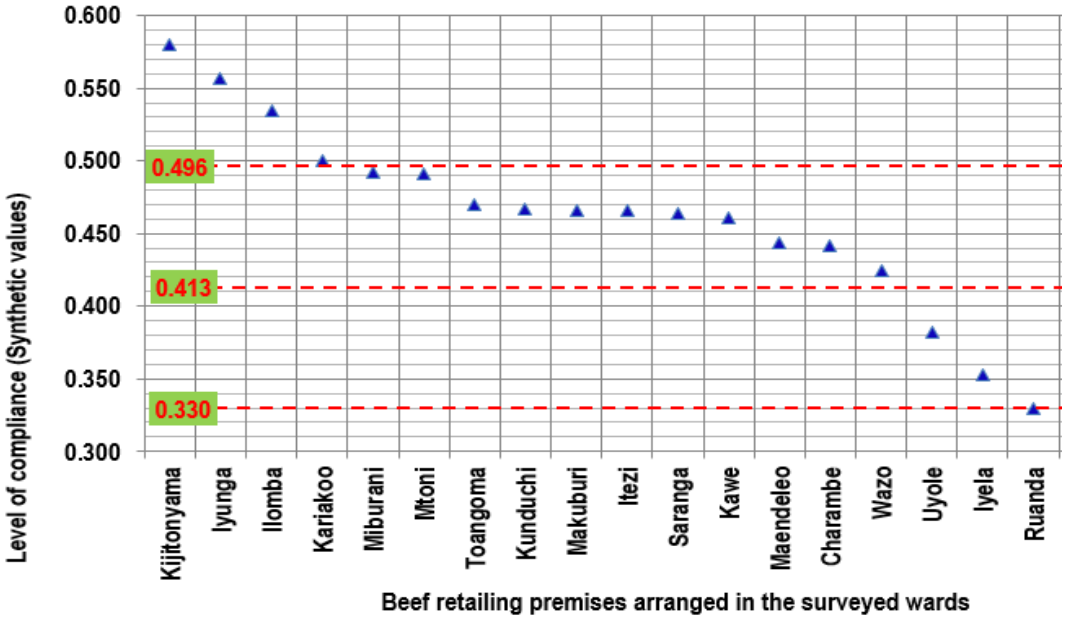

Figure 12. Assessment results for the beef quality impacting factors in beef retailing premises arranged based on the assessed wards (N_b = 95 for Dar es Salaam and 29 for Mbeya).

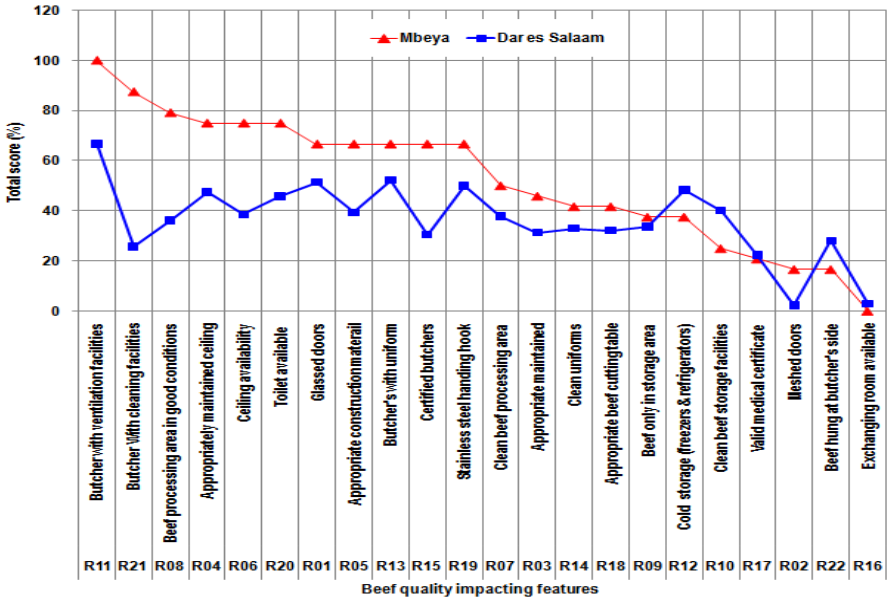

Figure 13. Running charts for beef quality impacting factors from the assessed beef retailing premises in the surveyed wards (N_b = 95 for Dar es Salaam and 29 for Mbeya).

of 0.330 and 0.413 as lowest and highest points with 0.355 as the average scored level.

The pattern discrepancies between the wards in the surveyed cities in this study support the rejection of null hypothesis that at least one of the medians of the assessed retailing premises in the surveyed wards appears to be different from the others. In this respect, Figure 13 shows level of compliance of seventeen

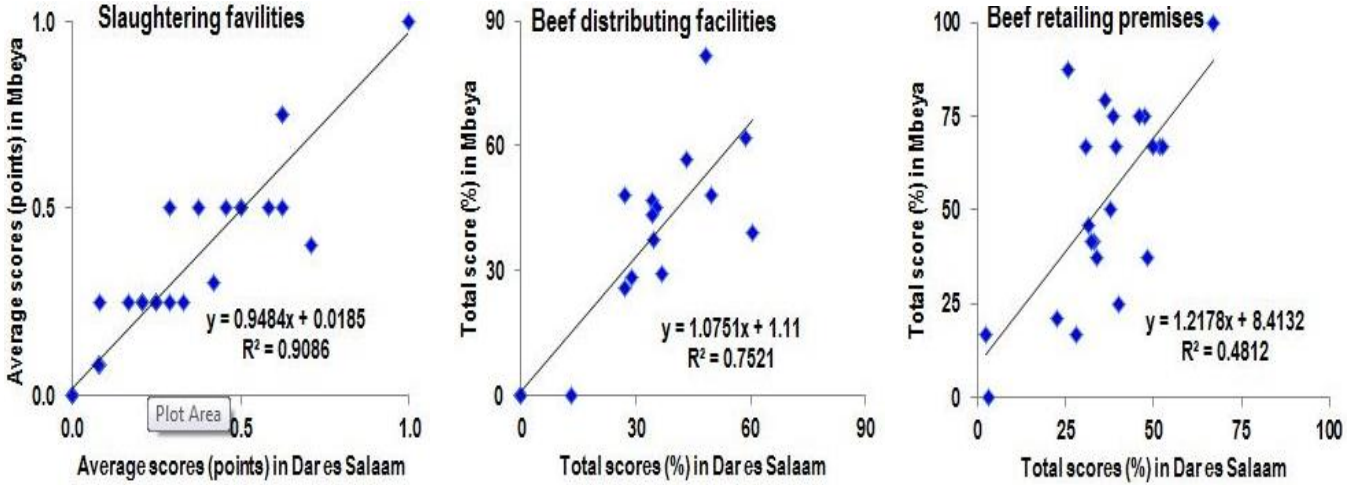

Figure 14. Linear regression of Mbeya city and Dar es Salaam with respect to the assessed beef quality impacting features across the slaughtering, distribution and selling chain.

features in Dar es Salaam was higher than Mbeya. In addition to that, level of compliance of five features in Mbeya was higher than in Dar es Salaam. The dotted lines show the overall average scores for each city, which shows the beef retail shown in Mbeya has a better infrastructural feature compared to Dar es Salaam.

Results were further statistically analyzed and summarized by using linear regression plots between the cities as shown in Figure 14. The scores in each city were further compared using linear regression plots, which compare the values of scores for Dar es Salaam (on horizontal axis), vs those of Mbeya facilities, (vertical axis), for slaughtering facilities, carcass transportation facilities and beef management in retail shops. The closeness of scores between the cities was assessed using the parameters for the linear Equation of the form $y = mx + b$. That is the slope m and the value of regression coefficient, R^2. The higher the slope ($m > 1.0$) the better the beef supply infrastructure in the city of Mbeya than in Dar es Salaam. Based on the line $y = x$ inserted on the plots, a linear best fit above $y = x$ implies better facilities in Mbeya and vice versa. Also, value of $R^2 \approx 1.0$ indicates stronger correlation or resemblance of the facilities design features between the two cities, while the lower value $R^2 << 1.0$ indicates wide difference between the two cities. The results have shown that there is positive correlation of BQIF across the beef supply chain between the assessed cities. This correlation proves the existence of similarities in beef processing in the Tanzanian cities. Similar observation was made by other studies, during which it was reported on the none-adherence to the required sanitation and hygiene requirements in Nairobi and Isiolo counties in Kenya (Chepkemoi et al., 2015).

In general, the study has brought an idea as an additional component in a set that also includes organized rules and beliefs items. Idea in this regard is connected to the thoughts and suggestions of using infrastructural BPDFs to determine the quality of beef. Rules, on the other hand, include the organized principles, that is, test statistics, mathematical expression that were applied in measuring the extent of compliance of various BPDFs.

This work has positive academic contribution to not only engineering management but also in food science and technology in general. This is discussed based on the fact that beef processing infrastructural features were conceptualized and designed to the idea for modeling the quality determination of beef. In this regard, BPDFs across the supply chain were used to measure the quality of the processed beef. This was implemented as the study: identifies basic connections of quality production, quality experience and quality evaluation during beef processing; elaborates aspects of quality processes that influence the means by which the quality is conceptualized and managed; shows the quality states residing within the quality processes; and indicates the theoretical and managerial implication for quality determination

Conclusion

In this study, ideas, rules and beliefs were organized in a set known as a framework that conceptualized, designed and applied for determining the quality of beef. Beliefs in this case are connected to the situation of accepting the idea of using the Likert based data collection technique

for quality determination. In this respect, beef quality determination was described by using BPDFs across the slaughtering, distribution and retailing chain. This determination was implemented by organizing the primary and secondary information in an understandable format for beef quality perception for all stakeholders across the supply chain.

As observed in other studies, the use of Likert based information in quality determination was noted as the gap in this study. This observation is taken as an appropriate justification for proposing further study on quality determination by using Likert based techniques. Another limitation of the study was connected to the assumption that the developed framework is working under assumption that the pre-slaughtering processes like breeding, grazing and cattle transportation should be complied with the technical requirements. The study was also not concerned with the situation across the post retailing activities to the beef preparation at household levels. This was also taken as another area for further study of development of a framework for measuring the quality of beef.

NOMENCLATURE

BSC, Beef supply chain; **BPDFs,** Beef processing designed features; **BDFs,** Beef distributing facilities; **SFs,** Slaughtering facilities; **BRPs,** Beef retailing premises; **BTQRs,** Beef quality technical requirements; **FAO,** Food and Agriculture Organization; ***r*,** Requirements; ***x*,** Requirement variable; ***n*,** Number of designed features of a given requirement; X_1, Sum of a given variables in two dimension; ***R,*** Range; **X∈S,** A set of all stimulant factors; **X∈D,** A set of all de-stimulant variables; ***Max,*** Maximum; ***Min,*** Minimum; ***z,*** Normalized beef processing designed feature; **ZUM,** Zero unitilization method; ***r* × *n,*** a two dimension arrangement of requirements and number of designed features of a given requirement; **Q,** Synthetic variable; q_i, Sum of complied normalized processing designed features; **CI,** Compliance Index; ***k,*** Group classifying parameter; ***TS,*** Total score; **OV,** Overall.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Physical properties of selected groundnut (*Arachis hypogea* L.) varieties and its implication to mechanical handling and processing

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The physical properties of agricultural materials are functional in solving many glitches associated with machine design during handling and mechanical processing. Physical properties of five groundnut varieties ("*Obolo*", "*Yenyawoso*", "CRI *Nkatie*", "*Agbeyeyie"* and "*Pion*") and their relations to the design of food processing equipment were studied. *Obolo* variety recorded the maximum axial dimensions, 1000 mass grain, angle of repose, unit volume, and porosity. However, the values of bulk and true densities for *Obolo* variety were minimal compared with the other four varieties. Data for the angle of repose for the groundnut varieties were 17.74° (*Yenyawoso*), 18.02° (*Pion*), 18.73° (*Agbeyeyie*), 18.71° (*Cri-Nkatie*), and 18.89° (*Obolo*). The porosity of the kernels ranged from 21.97 to 24.54%. The mean greatest porosity was found in *Obolo* (24.54%), followed by *Yenyawoso* (24.38%), while *Agbeyeyie* recorded the least mean porosity of 21.97%. The coefficient of friction was greater for the galvanized steel surface than the other experimental surfaces for all the groundnut varieties studied. Analysis of variance (ANOVA) revealed varietal differences among some means of the physical attributes at $p < 0.05$. Except for the angle of repose, the geometric, gravimetric and frictional properties showed some significant differences at $p < 0.05$. *Obolo* variety was statistically different compared with the other four varieties for all the parameters studied. In selecting or designing equipment for processing, *Obolo* variety will require separate equipment different from that of the other four varieties. Additionally, the study provides pertinent data for use in the selection and designing of machines for processing groundnut kernels.

Key words: Postharvest processing equipment, geometric mean diameter, bulk density, frictional properties, groundnut kernels, angle of repose.

INTRODUCTION

The rate of population growth in the world coupled with demand for quality and safety of food materials require appropriate postharvest machineries for handling, processing, preservation and storage. An empirical data on engineering properties of agricultural biological materials will provide a suitable basis for designing and

selection of the right equipment for the various postharvest operations to ensure that the processed biological material is of quality and safe for consumption. Data on engineering properties such as thermal, physical, mechanical, sensory, frictional, electromagnetic and aerodynamic properties, among others are vital for designing and selection of equipment for mechanical handling and processing of any agricultural biological materials. Among these properties, physical properties of biological materials are of great significance (Ofori et al.., 2019).

The knowledge in physical property provides a basis for better option during designing and selection of appropriate equipment for mechanical handling and processing of agricultural products. A determining factor for selection and designing of equipment for processing is linked-up with the physical attributes of the product. Among these attributes include, 1000 grain mass, axial size, volume, mass per unit volume, porosity, angle of repose, and the frictional properties. The importance of these physical attributes are valuable in choosing sieve separators, aeration, heating, estimating cooling, determining power requirement during size reduction process, displaying of grain drying, and scale draw of preharvest and postharvest equipment (Khan et al., 2019). The use of equipment for sorting, grading, cleaning and processing into different items all require data on the physical attributes of the biological materials (Stroshine and Hamann, 1995). Equipment and operating variable for size reduction, receptacle containers, grain silos, grain hopper dimensions, and holding facilities all depends on the engineering properties of biological materials of which physical attributes form great significance (Khan et al., 2019; Serpil and Servet, 2006).

Experiential knowledge on data abounds with physical properties of biological materials for bambara groundnut (Baryeh, 2001), tiger nut (Abano and Amoah, 2011), *Moringa oleifera* seeds (Aviara et al., 2013) and inter alia. Physical properties of several other local varieties of groundnut pods and kernel have been examined in different countries (Krishnappa et al., 2017; Firouzi et al., 2009; Olajide and Igbeka, 2003; Akcali et al., 2006). Data on anatomical structure of grain is critical for conducting movement of heat during processes such as drying, cooling, freezing and thawing. The rate of drying of biological materials is dependent on the nature of the exposed surface as well as the volume of the material; the more exposed of the surface and lesser volume, the quicker the rate of dispersion of water from the material. Density, size and shape of biological materials are product determinants for estimating terminal velocity in from product with different varieties. Equipment design for processing will be affected if consideration to the physical properties is compromised (Bala, 2017). Safety and quality of processed material is paramount to consumers, hence, appropriate design of equipment for processing are key. Designing of efficient processing equipment significantly depends on the kernel physical properties. Frequent equipment reinvention without consideration to empirical data of the biological material in question contributes to environmental pollution which affects the climate. The operational effectiveness and output to input ratio of processing equipment such as seed cleaners, grading, sorting, conveyors, and seed metering mechanism systems are dependent on the physical attributes of the biological material, and the data on these properties must be carried out and characterized appropriately.

With groundnut production and consumption cutting across all the sixteen regions of Ghana, empirical data on the kernels are momentous in selecting or designing machine for processing and storage. The need to investigate these common elite varieties of groundnut kernels recently released by the Crop Research Institute (CRI), Fumesua, Ghana, to assist in making well informed choices cannot be overemphasized. This research offers appropriate data on geometric, gravimetric and frictional properties of the selected groundnut varieties as a basis for designing and selection of equipment for processing and storage. This study therefore, seeks to determine the geometric, gravimetric and frictional attributes of these groundnut varieties and their correlation to the designing of mechanical equipment for food handling, processing and storage.

MATERIALS AND METHODS

Five groundnut varieties namely: "*Obolo*", "*Yenyawoso*", "CRI *Nkatie*", "*Agbeyeyie"* and "*Pion*" (Figures 1 to 5) used for the study were obtained from the warehouse of Crop Research Institute (CRI), under the Council for Scientific and Industrial Research (CSIR) at Fumesua, Ghana. These samples were manually cleaned free from dirt, leaves, pest and weeds. A sample size of 2 kg each was used for the analysis. The storage moisture contents (MC) of the groundnut kernels when measured were 7.2, 7.9, 7.8, 7.9 and 7.8% wet basis (wb), respectively, for "*Obolo*", "*Yenyawoso*", "CRI *Nkatie*", "*Agbeyeyie"* and "*Pion*". The initial MC of the kernels was obtained by using the method essayed by Ofori et al. (2019) as in Equation 1.

$$\%\ MC_{wb} = \frac{M_r}{M_i} \times 100 \quad (1)$$

where MC_{wb} = percentage moisture content in wet basis, M_r = mass of water removed and M_i = initial mass of sample before drying.

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Figure 1. Sample of the *Obolo* (ICGV 97049) variety.

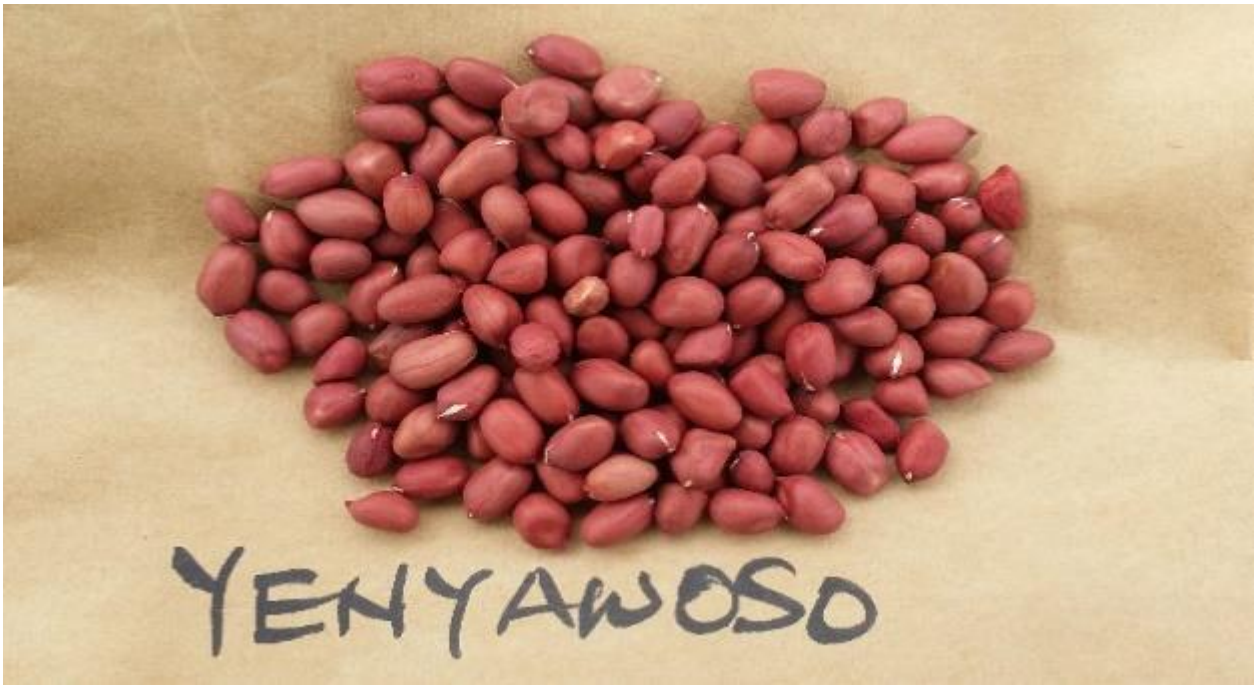

Figure 2. Sample of the Yenyawoso (ICGX SM 87057) variety.

A sample size of one hundred kernels were stochastically chosen from the 2 kg mass sample and the principal axial dimensions of the kernels were determined (Figure 6). From each sample size, three axial dominant dimensions, that is length (L), width (W) and thickness (T) were determined with a digital vernier caliper of precision of ±0.01 mm (Aviara et al., 2013). The geometric parameters [Arithmetic mean diameter (D_a), geometric mean diameter (D_g), sphericity (Ø), surface area (S) and aspect ratio (R)]

Figure 3. Sample of the CRI-Nkatie variety.

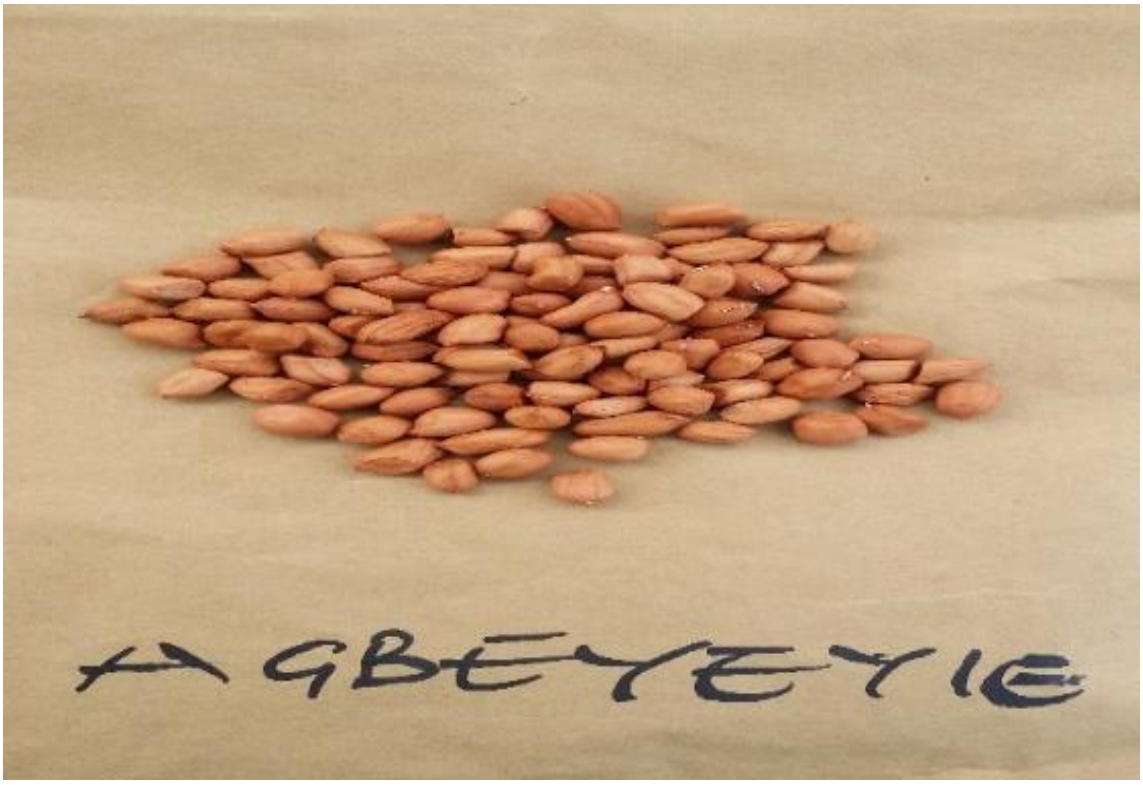

Figure 4. Sample of the CRI-Agbeyeyie variety.

were then estimated from the sample measured axial dimensions.

Equations 2, 3 and 4 were used respectively, to determine arithmetic mean diameter (D_{am}), geometric mean diameter (D_{gm}) and sphericity (Ø) (Aviara et al., 2013; Khan et al., 2019; Mohsenin,

Figure 5. Sample of the CRI-Pion variety.

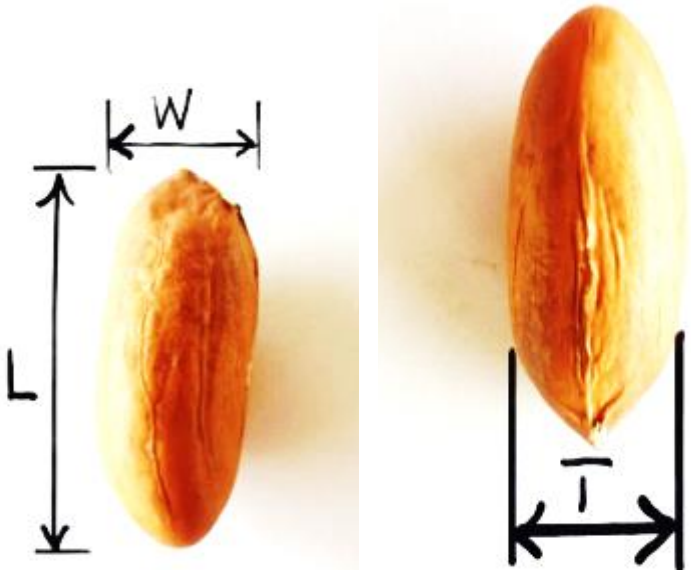

Figure 6. Principal dimensions of groundnut kernel.

1986; Vengaiah et al., 2015).

$$D_{am} = \frac{(L+W+T)}{3}, mm \quad (2)$$

$$D_{gm} = (LxWxT)^{1/3}, mm \quad (3)$$

$$\text{Ø} = \frac{(LxWxT)^{1/3}}{L} x100\% \quad (4)$$

Equations 5 and 6 were used to obtain for the surface area (S) and aspect ratio (R) of the kernels, respectively.

$$S = \pi (D_g)^2, \text{ mm}^2 \quad (5)$$

$$R = \frac{W}{L} \quad (6)$$

Mathematical Equation 7, ascribed to Jibril et al. (2016) was used to compute for the unit volume (V) of 100 individual kernels.

$$V = \frac{\pi LWT}{6} \quad (7)$$

A mass of 100 randomly sampled kernels was measured using a digital balance of precision 0.001 g. To obtain the thousand (1000) grain mass (TGM) of the groundnut kernels, the measured mass of the 100 kernels was multiplied by 10 (Aviara et al., 2013).

The method described by AOAC (1980), was used to estimate the bulk density. A cylinder of known volume 500 ml was filled with groundnut kernels from a height of 15 cm, the additional kernels were taken-off using a flat rule carefully to prevent compaction. Quantity of kernels that filled the cylinder was weighed with a digital balance. Bulk density was computed for using Equation 8.

$$\rho_b = \frac{m_b}{v_b} \quad (8)$$

where ρ_b = Bulk density (g/cm^3), m_b = Mass of the kernel sample (g) and v_b = Volume of sampler (cm^3)

The particle (true) density was determined using Equation 9 as reported by Khan et al. (2019) and Vengaiah et al. (2015). A quantity of known mass of seeds were poured into a measured volume of toluene (C_7H_8) as liquid for displacement in a graduated cylinder, and the true density of the seed found as the ratio of the mass of sample of seeds to the solid volume occupied by the sample.

$$\rho_t = \frac{mass(g)}{v_2 - v_1(cm^3)} \quad (9)$$

where ρ_t = true density, g/cm^3; v_1 = initial volume of toluene, cm^3; and v_2 = final volume of toluene, cm^3.

The method described by Mohsenin (1986) as in Muhammad et al. (2015) in Equation 10 was used to compute for the porosity (ε) of the bulk kernel.

$$\varepsilon = \frac{\rho_t - \rho_b}{\rho_t} \times 100\% \quad (10)$$

A method reported by Serpil and Servet (2006) was employed to obtain the angle of repose. A circular plate of known diameter was placed under an open-ended cylinder of size 150 mm in diameter and 220 mm in height. Grain samples were poured into the cylinder from a pre-determined height until it was full. The cylinder was carefully raised for kernels to form a cone on the circular plate. The angle of repose was calculated by the ratio of the height to the base radius of the heap formed.

The coefficient of static friction (μ) was assayed with three different structural surfaces, viz, glass, polished wood and galvanized steel. To measure the static friction, an open-ended PVC cylinder 110 mm in diameter and 90 mm in height was stuffed with kernel and placed on an inclined plane. The PVC cylinder was elevated about 2.5 mm off-contact from the slanted board. The slanting board was lifted bit by bit with the aid of an adjustable mechanism until the cylinder began to move. The angle of tilt was measured with a protractor. Measurement was repeated three times and for each repeat, the sample in the container was poured out and refilled with a fresh sample. Equation 11 was used to calculate for the static coefficient of friction (Vengaiah et al., 2015).

$$\mu = \tan \theta \quad (11)$$

where μ is the coefficient of static friction and θ is the angle of tilt of table.

Data analysis

Analysis of variance (ANOVA) was performed on the data of the physical attributes measured and computed using SPC for Excel v5 (trial) hosted on Microsoft Excel 2016 at 5% significance level. A trial of least significance difference (LSD) was performed on the means of the physical attributes measured for the five varieties of the groundnut kernels.

RESULTS AND DISCUSSION

Results of the principal dimensions for the five groundnut kernels are depicted in Table 1. Figures 7 to 11 depict the variations of the kernel three axial magnitudes (length, width and thickness). The degree of variations in the minimum and maximum widths and thicknesses among the kernels studied were minimal as shown in Figures 7 to 11. However, differences among kernels minimum and maximum lengths were clearly evident, of which the highest value was found in *Obolo* variety (8 mm), followed by *Agbeyeyie* (7.5 mm) and the lowest was recorded in *Yenyawoso* and *pion* at 4.5 mm each. Grading to obtain uniform sizes is imperative for planting with the use of a metering mechanism. *Obolo* variety had the highest minimal and maximal range of sizes 15.00 to 23.00 mm, 7.47 to 11.67 mm and 8.90 to 11.95 mm, respectively, for the length, width and thickness. The lowest minimal and maximal range of sizes for length, width and thickness were registered for *Yenyawoso* variety which were, respectively at 9.37 to 15.42 mm, 6.16 to 9.27 mm and 6.14 to 10.11 mm. These seed dimensions are useful for selection or designing sieve apertures in the segregation compartment of machine for shelling (Maduako and Hamann, 2005). The obtained data in Table 1 shows that among the five groundnut varieties studied, *Obolo* variety recorded the largest sizes in terms of length, width and thickness. However, the remaining four varieties were ostensibly similar in axial dimensions. Empirical data on axial dimensions of any biological material is imperative with the reason that equipment for processing of these bio-materials is dependent on the physical attributes (Davies, 2010).

Table 1. Geometric parameters of selected physical properties.

Geometric parameter	Number of observations	Variety				
		Obolo	Yenyawoso	CRI Nkatie	Agbeyeyie	Pion
Moisture Contents (%, wb)		7.2	7.9	7.8	7.8	7.8
Length (mm)	100	18.92 (1.63)	12.35 (1.09)	13.64 (1.11)	12.44 (1.16)	12.62 (0.96)
Width (mm)	100	9.63 (0.84)	7.82 (0.51)	7.79 (0.57)	8.11 (0.66)	7.99 (0.57)
Thickness (mm)	100	10.23 (0.65)	8.24 (0.69)	8.40 (0.56)	8.59 (0.59)	8.54 (0.54)
Arithmetic diameter (mm)	100	12.92 (0.69)	9.47 (0.48)	9.94 (0.56)	9.71 (0.53)	9.71 (0.47)
Geometric diameter (mm)	100	12.28 (0.61)	9.25 (0.46)	9.62 (0.53)	9.52 (0.51)	9.50 (0.45)
Sphericity (%)	100	65.21 (4.34)	75.26 (5.38)	70.79 (4.31)	76.93 (5.67)	75.55 (4.57)
Surface area (mm^2)	100	474.98 (46.74)	269.31 (26.78)	291.50 (32.65)	285.43 (30.57)	284.13 (26.98)
Aspect ratio (%)	100	0.51 (0.07)	0.64 (0.07)	0.57 (0.06)	0.66 (0.08)	0.64 (0.07)

Values for standard deviation depicted in parenthesis.

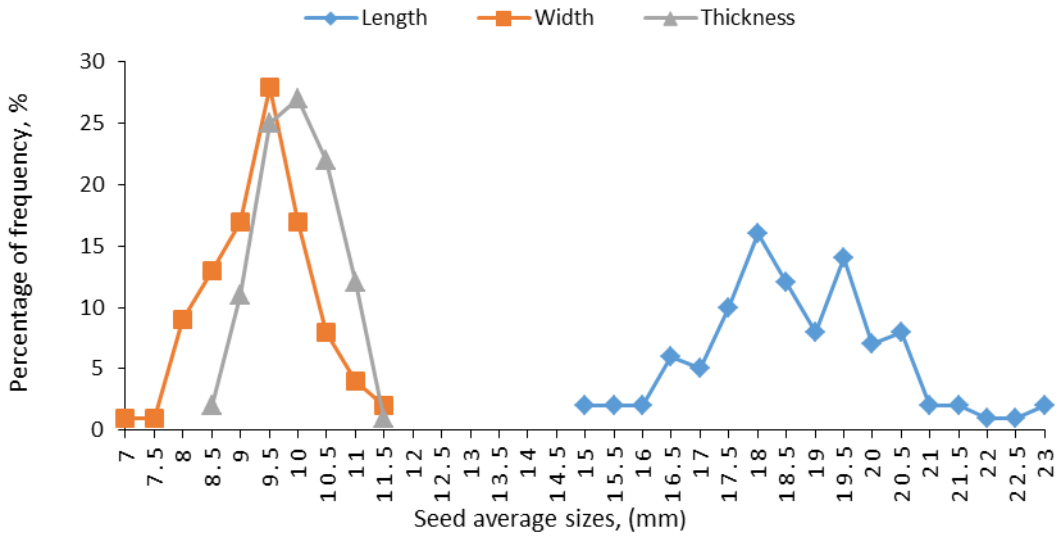

Figure 7. Frequency distribution curves of ICGV 97049 (*Obolo*) seed dimensions.

Hence, in designing sieves for cleaning, *Obolo* variety will have different sieve-size due to its immense axial dimensions distinct from that of the other four varieties.

Geometric and arithmetic mean diameters

The geometric and arithmetic mean diameters for the kernels had averages of 12.28 and 12.92 mm, 9.25 and 9.47 mm, 9.62 and 9.94 mm, 9.52 and 9.71 mm, and 9.50 and 9.71 mm, respectively, for the *Obolo, Yenyawoso, CRI-Nkatie, Agbeyeyie* and *Pion* varieties as presented in Table 1. Geometric mean diameters and the arithmetic mean diameters for the varieties studied are in variance with those of Manipintar, Local I and Local II (Muhammad et al., 2015) as well as RMP-9, ICGV and RMP-12 groundnut varieties (Vengaiah et al., 2015). This variation may be due to varietal differences. Having the requisite data on these properties is significant in the determination of the clearance within the concave openings of groundnut decorticating and separating machines. Also, these attributes are important in screening out solids to remove foreign materials as well as designing equipment for grading.

Sphericity

Sphericity depicts the shape of a biological material in variance to sphere. The mean values of sphericity were 65.21% (*Obolo),* 75.26% (*Yenyawoso),* 70.79% (*CRI-Nkatie),* 76.93% (*Agbeyeyie)* and 75.55% (*Pion)* for the kernels as presented in Table 1. *Agbeyeyie* variety had the highest mean sphericity while the least mean

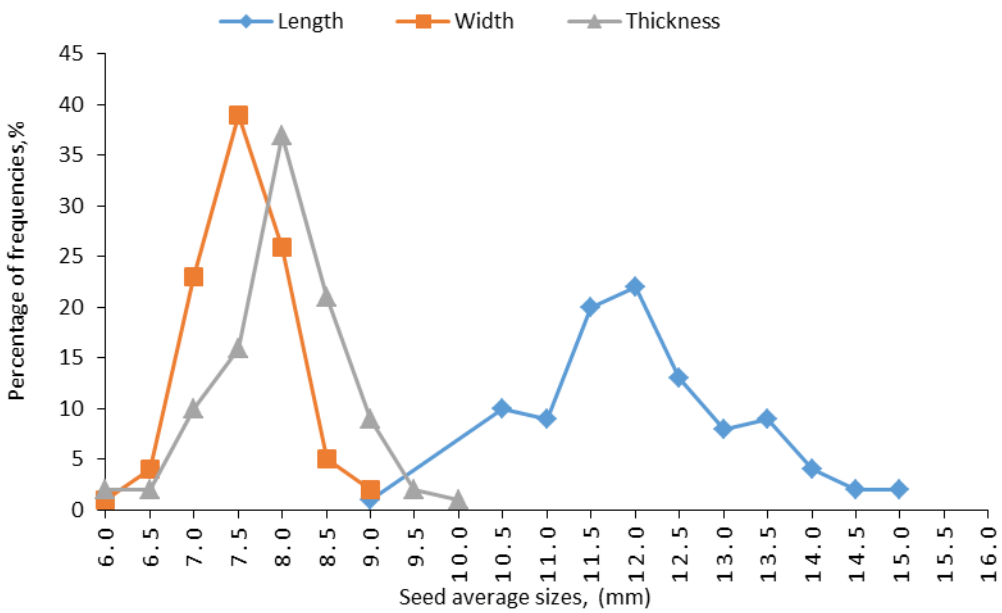

Figure 8. Frequency distribution curves of ICGX SM 87057 (*Yenyawoso*) seed dimensions.

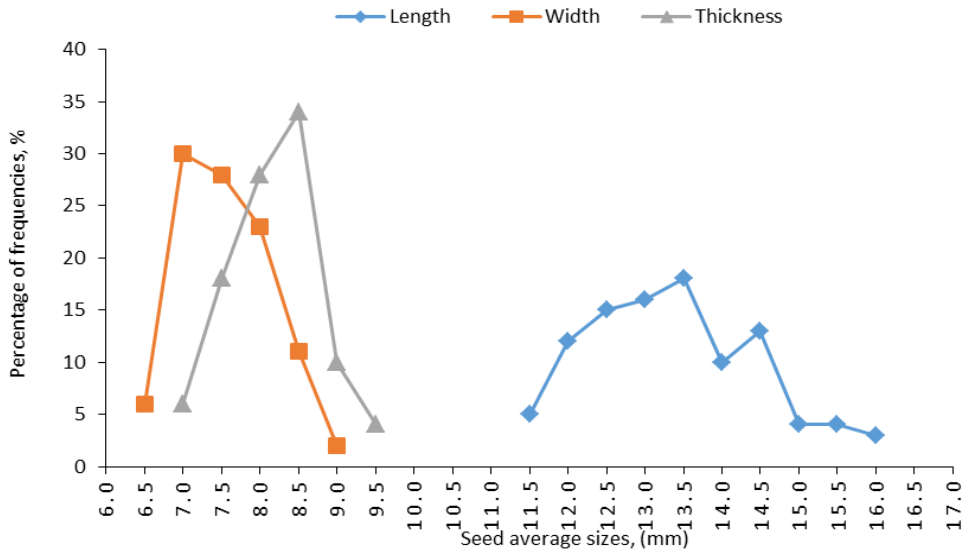

Figure 9. Frequency distribution curves of (CRI-Nkatie) seed dimensions.

sphericity was found in *Obolo*. A sphericity value of a biomaterial between 50 and 100% is an indication of the ability of that material to slide on the surface in contact with it (Muhammad et al., 2015). These values obtained for sphericity indicate that all the groundnut kernels have the propensity to roll on its axis. A spiral separator cleaner can be used for cleaning and removal of immature and shrivelled kernels due to the rolling ability of the kernels. A similar trend was found by Muhammed et al. (2015) for Local II and Manipintar with sphericity of 64.20 and 78.24%, respectively.

Surface area

Surface area is vital for quantifying the rate of heat, water and gas transfer during processing such as drying and roasting of kernels. The larger the surface area of the material, the higher the exposure of kernel to the heat source and the greater the heat absorption and

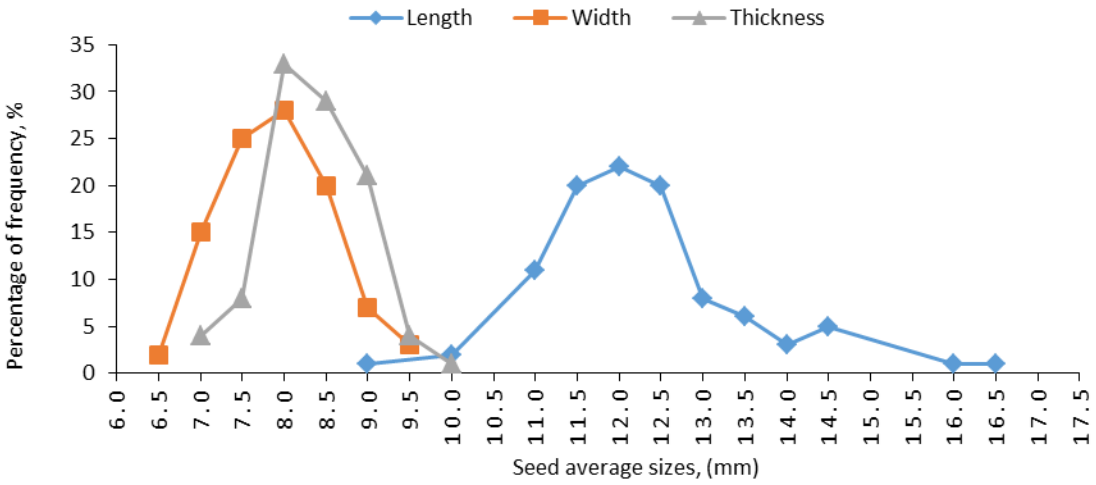

Figure 10. Frequency distribution curves of (CRI Agbeyeyie) seed dimensions.

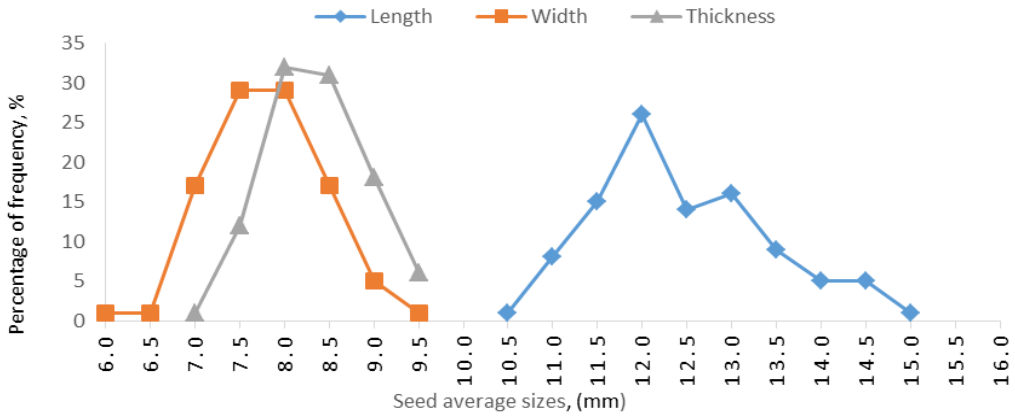

Figure 11. Frequency distribution curves of (CRI Pion) seed dimensions.

desorption during processing that involves heating (drying or roasting) and cooling of kernels (Bala, 2017). From Table 1, the surface areas of the kernels were 269.31, 284.13, 285.43, 291.50 and 474.98 mm^2 for *Yenyawoso, Pion, Agbeyeyie, Cri-Nkatie* and *Obolo,* respectively. Odesanya et al. (2015) found surface areas of 149 and 97 mm^2, espectively, for Samnut 22 and Ex-Dakar. This variation may be due to varietal differences in the products investigated.

Thousand (1000) seed mass

The mean values for the 1000 grain mass of the groundnut kernels were 843.70, 416.66, 420.30, 429.30 and 452.10 g for *Obolo, Yenyawoso, Cri-Nkatie, Agbeyeyie* and *Pion*, respectively (Table 2). Among the five varieties, *Obolo* had the highest mean of thousand grain mass of 843.70 g while *Yenyawoso* had the least value of 416.66 g. The thousand grain mass is substantial in calculating the size of grain holder units (hoppers) and shelling compartments of machines for processing. The 1000 grain mass property is also useful for estimating machine stability during operations such as size reduction and planting (Muhammad et al., 2015).

True and bulk densities

The minimum bulk density of the groundnut kernels was found in *Obolo* variety (758.05 kg/m^3) and the maximum was found in *Agbeyeyie* variety (799.71 kg/m^3). Also, *Obolo* variety recorded the minimum true density of 1004.43 kg/m^3 while the maximum was recorded in Pion variety (1033.67 kg/m^3) as shown in Table 2. The results obtained attest to that recorded by Muhammad et al. (2015) for bulk density range of 0.55 to 0.82 g/cm^3 and true density from 0.87 to 1.08 g/cm^3 for Local II and

Table 2. Gravimetric parameters of selected physical properties.

Gravimetric parameter	Number of observations	Variety				
		Obolo	Yenyawoso	CRI Nkatie	Agbeyeyie	Pion
Moisture contents (%, wb)		7.2	7.9	7.8	7.8	7.8
Unit volume (mm^3)	3	976.82 143.77)	417.07 (62.07)	470.12 (79.93)	455.34 (73.52)	451.83 (64.32)
1000 Grain mass (g)	3	843.70 (0.10)	416.66 (1.15)	420.30 (0.26)	429.30 (0.10)	452.10 (0.10)
Bulk density (kg/m^3)	3	758.05 (1.51)	797.77 (11.04)	792.77 (8.05)	799.71 (4.22)	792.79 (2.03)
True density (kg/m^3)	3	1004.43 (1.37)	1029.98 (2.60)	1020.733 (5.04)	1024.33 (0.67)	1033.67 (2.31)
Porosity (%)	3	24.53 (0.11)	22.48 (1.44)	22.34 (0.13)	21.96 (0.41)	23.43 (0.14)

Values for standard deviation depicted in parenthesis.

Table 3. Frictional parameters of selected physical properties.

Frictional parameter	Number of observations	Variety				
		Obolo	Yenyawoso	CRI Nkatie	Agbeyeyie	Pion
Moisture contents (%, wb)		7.2	7.9	7.8	7.8	7.8
Angle of repose(deg.)	3	18.89 (0.69)	17.74 (0.45)	18.71 (0.23)	18.73 (0.44)	18.02 (0.63)
Coefficient of friction						
Glass	3	0.39 (0.019)	0.32 (0.015)	0.32 (0.015)	0.32 (0.012)	0.32 (0.008)
Galvanized steel	3	0.46 (0.008)	0.34 (0.010)	0.33 (0.012)	0.35 (0.010)	0.34 (0.012)
Polished wood	3	0.42 (0.021)	0.34 (0.006)	0.32 (0.014)	0.33 (0.010)	0.33 (0.010)

Values for standard deviation depicted in parenthesis.

Manipintar, respectively. It also concurs with that found by Maduako and Hamman (2005). These properties are useful tools for evaluating maximum load that seed separators can resist without breaking down during groundnut shelling. The particle and the bulk densities are valuable for estimating the aero and hydrodynamic separation of groundnut kernels from foreign material and imposed pressures during design of silo bottoms.

Porosity

Table 2 depicts the porosity of the groundnut kernels. This attribute provides significant information when developing equipment for material handling such as drying, storage, aeration and ventilation. It is also a useful determinant property for estimating material transport in pneumatic conveyors. The porosity for the five kernel varieties ranged from 21.97 to 24.54%. *Obolo* variety recorded the highest mean porosity of 24.54%, followed by *Yenyawoso* with 24.38%. *Agbeyeyie* recorded the least mean porosity of 21.97%. The experimental data obtained concurs with that obtained by (Firouzi et al., 2009) for Local I, Manipintar and Local II with porosities of 24.70, 28.89 and 37.00%, respectively. Boukouvalas et al. (2006) indicated that porosity is among the single most significant properties by which the shape of food materials can be described. Porosity is a critical parameter among others for equipment design.

Angle of repose

Data obtained for the angle of repose were 17.74° (*Yenyawoso*), 18.02° (*Pion*), 18.73° (*Agbeyeyie*), 18.71° (*Cri-Nkatie*) and 18.89° (*Obolo*) as in Table 3. These values are closely related to that of Maduako and Hamman (2005), who obtained 20.03° for ICGV-SM-93523, 20.05° for RMP-9 and 20.8° for RMP-12 groundnut seeds, but lower than that obtained by Muhammad et al. (2017). As depicted in Table 3, the angle of repose for kernels of smaller axial dimensions was less than that for bigger seeds. The study unraveled that smaller seeds have smaller and smoother surface area as compared to larger seeds which have larger and rougher surface area, hence flowability is reduced with decreasing angle. This property is valuable for determination of optimum sides for planting machine seed hoppers, silos and storage containers to allow easy sliding of materials (El-Fawal et al., 2009). Also, this attribute is significant for conveyor width analysis and bottoms of storage equipment (Galedar et al., 2008).

Coefficient of static friction

The mean values for static coefficients of friction for the

Table 4. Test of significance on varietal differences.

No.	Physical properties (Parameter)	Computed F- ratio
1	Length	365.08
2	Width	397.97
3	Thickness	176.44
4	Dg	331.95
5	Sphericity	362.48
6	Surface Area	364.25
7	Bulk density	20.89
8	True density	17.59
9	Porosity	7.06
10	Angle of repose	2.94
11	Coefficient of Friction:	
	Glass	10.02
	Polished wood	77.02
	Galvanized steel	29.38

Table 5. LSD test for varietal difference on selected geometric properties.

Groundnut variety	Length		Width		Thickness		Dg		Sphericity	
	Mean	LSD	Mean	LSD	Mean	LSD	Mean	LSD	Mean	LSD
Obolo	18.92	a	9.63	a	10.23	a	12.28	a	65.21	a
Yenyawoso	12.35	b	7.82	b	8.24	b	9.25	b	75.26	b
CRI-Nkatie	13.64	c	7.79	b	8.4	b,c	9.62	c	70.79	c
Agbeyeyie	12.44	b	8.11	c	8.59	c	9.51	c	76.93	d
Pion	12.62	b	7.99	c	8.54	c	9.5	c	75.55	b

Means with the same letters are not significantly different.

kernel varieties on the three structural surfaces viz polished wood, galvanized steel and glass were 0.42, 0.46, and 0.39 for *Obolo*, 0.34, 0.34, and 0.32 for *Yenyawoso*, 0.32, 0.35 and 0.32 for CRI-*Nkatie*, 0.33, 0.35, 0.32 for *Agbeyeyie* and 0.33, 0.34, and 0.32 for *Pion,* respectively (Table 3). It was observed that the highest static coefficient of friction was found with galvanized steel and the least was found with glass in all the varieties studied. Coefficient of static friction has effect on the nature of the surface in contact with the kernel. An increase in frictional resistance was associated with rough surfaces as compared with smooth and polished surfaces. This observation depicts that the smoother and more polished the structural surface, the lower the static coefficient of friction of the samples and vice versa. The values obtained for this property concurs with findings from Baryeh (2001), Davies (2009) and Muhammad et al. (2015) for bamabara groundnut, groundnut grains, and groundnut pods and kernels, respectively. The design dimension of hoppers, bunker silos and other bulk solid storage and handling structures are dependent on the static coefficient of friction. This property is a significant bench mark for calculating the angle of inclination in inclined grain transporting equipment like chutes (Gharibzahedi et al., 2010). Coefficient of friction is a dependent variable needed for selecting materials for fabrication and power required for transporting a given biological material.

Analysis of variance on varietal differences

Table 4 depicts a test of variance performed on the means of the physical attributes to examine the deviations among means of the kernel varieties. There were apparent significant differences at $p \geq 0.05$ among the means of the physical attributes studied. The computed *F Critical* at 5% for all the parameters studied was 3.48. Except for the mean angle of repose; the properties for geometric, gravimetric, and frictional attributes were in variance. LSD was used to establish the veracity of differences among the means of the attributes as indicated in Tables 5 to 7. From Tables 5 to 7, the mean length, width, thickness, geometric mean diameter, sphericity and surface area of *Obolo* variety were statistically higher as compared to the other four

Table 6. LSD test for varietal difference on selected gravimetric properties.

Groundnut variety	Bulk density		True density		Porosity	
	Mean	LSD	Mean	LSD	Mean	LSD
Obolo	758.05	a	1004.43	a	24.53	a
Yenyawoso	797.77	b	1029.98	b,d	22.48	b,c
CRI-Nkatie	792.77	b	1020.73	c	22.34	b,c
Agbeyeyie	799.71	b	1024.33	b,c	21.96	b
Pion	792.79	b	1033.67	d	23.43	c

Means with the same letters are not significantly different.

Table 7. LSD test for varietal difference on selected frictional properties.

Groundnut variety	Glass		Galvanized steel		Polished wood	
	Mean	LSD	Mean	LSD	Mean	LSD
Obolo	0.39	a	0.46	a	0.42	a
Yenyawoso	0.32	b	0.34	b	0.34	b,c
CRI-Nkatie	0.32	b	0.33	b	0.32	b
Agbeyeyie	0.32	b	0.35	b	0.33	c
Pion	0.32	b	0.34	b	0.33	b,c

Means with the same letters are not significantly different

varieties. The mean bulk density, true density and porosity of *Yenyawoso*, *Cri-Nkatie, Agbeyeyie* and *Pion* were statistically similar but differ from *Obolo*. Similarly, the mean coefficient of friction on the glass, polished wood and galvanized steel for *Yenyawoso, CRI-Nkatie, Agbeyeyie* and *Pion* were statistically similar but differ from *Obolo*. These differences are very pertinent when deciding on designing of mechanical equipment for handling, processing and storage.

Conclusion

The study carried out on five groundnut varieties, namely, *Obolo*, *Yenyawoso*, *Cri-Nkatie, Agbeyeyie* and *Pion*, respectively, at moisture contents (% wet basis) of 7.2, 7.9, 7.8, 7.8 and 7.8 revealed the following results:

(1) Among the groundnut varieties studied, *Obolo* had the maximum geometric dimensions and this is evident on its physical appearance.
(2) For the 1000 mass grain and porosity, *Obolo* variety recorded extremely high values; however its bulk density was the least among the varieties studied.
(3) The results obtained for frictional properties indicate that the static coefficient of friction varies with the property of the frictional surfaces of the material and this was maximum for galvanized steel, followed by polished wood and glass surface for all the five varieties studied on the structural surfaces.
(4) The means of some of the physical attributes for the groundnut varieties studied were statistically different.
(5) Statistically, *Obolo* variety had geometrical properties different from the remaining four varieties studied. The implication is that, to design or select an equipment for cleaning, winnowing, conveying, storage and grading, these four varieties (*Yenyawoso*, *Cri-Nkatie, Agbeyeyie* and *Pion)* may use a single equipment for the pre-harvest and post-harvest operations as compared with *Obolo* variety which will require separate equipment for processing.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Microbial analysis and factors associated with contamination of ready-to-eat chili pepper sauce in Buea municipality, Cameroon

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Chili peppers sauce is a dietary complement largely consumed in Cameroon. It is consumed in a powder or wet (pepper sauce) form or directly introduced into cooked food. In this study, the microbiological quality of chili pepper sauce used as food complement in the Buea municipality was assessed. The study was an observational and cross-sectional study involving 70 chili pepper sauce samples from food vendors. The samples were cultured on Salmonella-Shigella agar, violet red bile agar, plate count agar and the colonies isolated were enumerated and identified using the Enterosystem 18R. Factors associated with microbial count were identified using a multiple linear regression model. Bacteria isolate from chili pepper sauce were mainly *Entrobacter cloacae* (31.57%), *Citrobacter freundii (*15.78%) and *Klebsiella pneumonia* (15.78%) and other Enterococcal species. Factors associated with bacteria count were: age of the vendor, number of customers served, types of food and food storage conditions (covering, heating, type of storage containers). Chili pepper sauce used as food complement in Buea Municipality were contaminated with Enteric microorganisms and may represents a potential public health hazard to consumers. The presence of these microorganisms from chilli pepper sauce could result from poor handling.

Key words: Chili pepper sauce, contamination, enteric bacteria, health hazard.

INTRODUCTION

Foodborne diseases are important cause of morbidity and mortality, and a significant impediment to socioeconomic development worldwide. Billions of people are at risk and millions fall ill every year; many die as a result of consuming unsafe food (Kirk et al., 2015). Unsafe food poses a global health threats and creates a

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vicious cycle of diarrhoea and malnutrition, threatening the nutritional status of the most vulnerable. Every year 220 million children contract diarrheal diseases and96 000 die as a result of food contamination (Kirk et al., 2015). Where food supplies are insecure, people tend to shift to less healthy diets and consume more “unsafe foods” in which chemical, microbiological and other hazards pose health risks (WHO, 2017). The incidence of infections transmitted through food has remained largely unchanged over years (Marder et al*.,* 2018). Foods may become contaminated by infected food handlers, water and soil or from fecal contamination.

Chili peppers are dietary complements largely consumed worldwide especially in several parts of Africa and particularly in Cameroon. This high consumption is certainly due to its taste and its nutritional benefits. Chili pepper has characteristic taste and pungency (Yazdizadeh et al*.,* 2013). The red peppers are a source of Vitamin A, C and Folate (Davis et al*.,* 2016). Vitamin A helps in the vision while vitamin C is a strong anti-oxidant (Yang and Xu, 2016). Fresh peppers have high quantities of ascorbic acid and carotenoid pigments such as β-carotene with pro-vitamin A activity which have proven to be effective at scavenging free radicals (Deepa et al*.,* 2006).

In Cameroon, several peppers species have been domesticated and the most widely consumed peppers include the Chili peppers (hot and sweet peppers of the genus Capsicum), bell pepper (green pepper), Penja white pepper (*Piper nigrum*) and the Jalpeno pepper. In the Buea municipality, chili pepper is used on a daily basis to complement various foodstuffs, for example, “Eru”, “Ndole”, Roast fish, “Fish roll”, “Scotch egg”, boiled egg, chicken, “accra banana”, pork, “soya”, snail etc. It is a habit in Cameroon to consume foodstuff with chili pepper complement. Chili pepper is consumed fresh, dry (dusty), stewed, stir fried or moist as a dietary complement to increases flavour of most foodstuffs. However, moist pepper sauce (especially those prepared using additional ingredients such as garlic, ginger, tomatoes, onion, green spices) may provide a very conducive environment for the growth of microorganisms. Street foods sold in Buea are mostly prepared and processed manually by vendors and sold to the public. Most at times the safety requirements are ignored or not known by the vendors. These practices raise some doubts on the safety and the microbial quality of such food complement. Previous studies have shown that foodborne illnesses constitute a major health problem associated with food sold to the public (Tabashsum et al*.,* 2013). Street vended chili sauces in Mexico were shown to be faecally contaminated, harboring sufficient enterotoxigenic *Escherichia coli* to cause an infection (Estrada et al*.,* 2002). In this study, the microbiological quality of chili pepper sauce used as food complement in the Buea municipality was assessed and factors predisposing to contamination were investigated.

MATERIALS AND METHODS

Study design

The study was an observational and cross-sectional study in which pepper sauce samples used as food complement were randomly selected from food vendors and analysed in the laboratory for their microbiological content. Data on the sociodemographic characteristics as well as the handling and preservation of the pepper sauce was also collected.

Settings

The study was carried out in Buea, the head quarter of the South West Region of Cameroon from May to July 2018. Buea is located on the eastern slopes of the mount Cameroon and lies between latitudes 4°12’N and longitudes 9^0 12’ E with surface area of 870 km^2. The total population estimated by the health population denominators (2017) revealed 169,745 inhabitants. The population is mainly made up of students and civil servants with a high density in the university surroundings. The samples were collected in Molyko, Mile 17 Motor Park and Muea market. The choice of these areas for sample collection was motivated by the fact that these areas are very prolific in restaurants, mobile and stationed food vendors.

Participants

The study population was food vendors operating in the Buea Municipality. Only those food vendors that sell foodstuff with pepper sauce supplements were approached for sample collection. A total of 70 chili pepper sauce samples were collected from 70 food vendors at the University of Buea campus and layout, Mile 17 Motor Park, Malingo and Muea market.

Sample collection

All food vendors in the study site who sold food stuff with chili pepper sauce as complement and who gave their consent were recruited into the study. Samples were collected from vendors selling boiled egg, roasted fish, snails, pork “Accra banana” “Gateau” (fish roll, fish pie, Scotch egg) and chicken. These samples were served into sterile sample collection tubes by the vendors themselves with the same utensil they use to serve their customers. A questionnaire was administered to the vendor to obtain demographic information, food types sold, mobility of the vendor, preparation, handling and preservation of pepper sauce and the number of times the sauce was heated. Observations were also made on the environmental and hygienic conditions of the food vendor. The pepper sauce samples were transported to the laboratory in a cool box for analysis.

Laboratory processing of samples

One millilitre of the pepper sauce was diluted in peptone water to obtain a uniform distribution of the micro-organisms before inoculation. A loop full of the homogenized samples was inoculated by streak plate method on Salmonella-Shigella (SS) Agar, Violet Red Bile (VRB) Agar, and the plates were incubated at 37°C for 24

Table 1. Demographic characteristics of the vendor population enrolled in the study.

Variable		Frequency	Percentage
Gender	Female	46	65.7
	Males	24	34.3
Age group (years)	11 - 20	26	37.1
	21 - 30	26	37.1
	> 30	18	25.7
Education level	Higher	2	2.9
	Primary	9	12.9
	Secondary	59	84.3

h after which they were observed for bacterial growth. In order to determine the total bacteria count, a 10-fold serial dilution was carried out on the samples and 0.1 ml of the 10^{-3} dilution was inoculated by the spread plate technique onto the Plate Count Agar (PCA) and incubated at 37°C for 24 h. Colonies were counted and recorded as colony forming units (CFU/ml).

A Gram stain was carried out for each of the colonies that grew on the plates and observed under the light microscope at 100X Objective. Colonies that were Gram negative were sub-cultured on Nutrient Agar and incubated at 37°C for 24 h to obtain pure cultures used for bacteria identification. Bacteria were identified using the Enterosystem 18R, which identifies Gram negative, Oxidase negative Enterobacteria of clinical significance. In this kit, identification is based on biochemical tests performed in wells containing specific culture media. The test kit employs 18 standardised and miniature biochemical tests. Sample collection and laboratory processing was done under aseptic conditions to prevent contamination which could introduce biases in the study.

A culture was considered positive for bacteria when growth was observed on any of the culture media used. A culture was considered positive for enteric microorganisms when positive growth was observed on VRB or SS agar and negative when no growth was observed on any of the media used in culturing. The independent variables or predictors were the demographic characteristics of food vendors and pepper handling.

Statistical methods

Demographic data from food vendor, data on the handling of pepper as well as laboratory culture results were entered in a template developed in MS Excel and analysed in SPSS version 21.0. A generalized linear regression model was fitted to investigate the relationships between vendor demographic characteristics, handling of pepper and bacteria count in chilli pepper sauce. The response bacterial count was approximated to normal distribution and normality checked before fitting the model. All tests were performed at a 5% significant level.

Ethical consideration

The protocol of this study was approved by the Faculty of Health Sciences Institutional Review Board (Ref. 2018/0241/UB/SG/IRB/FHS) of the University of Buea, Cameroon. Prior to data collection, the purpose of the study was clearly explained to the participants. A verbal informed consent was obtained from adults whereas for the minors, a written inform consent was obtained from the guardian or parents.

RESULTS

Demographic characteristic of food vendors

Table 1 shows the demographic characteristics of food vendors from whom pepper sauce samples were collected. There were more females (65.7%) than males (34.3%). The mean age of vendors was 25. 84 ± 10.56 of which 37.1% were of ages 11 – 20 years, 37.1% of ages 21 – 30 years and 25.7% were 30 years and above. Most of the vendors had attained secondary education level (84.3%).

Characterisation of the foodstuff from which pepper sauce was collected

Table 2 shows the site of collection and the various foodstuffs from which the pepper sauce was sampled. A great proportion of samples were collected within the University of Buea Layout (37.1%), followed by Malingo street (27.1%), Mile 17 motor park (21.4%) and Muea market (14.3%). The chili pepper sample collected were complements of roasted fish (20%), snails (15.7%), “gateaux” (12.9%), boiled egg (11.4%), pork (10%), chicken (11.5%) and others. A greater proportion of the food handlers were stationary (67.1%).

Pepper sauce handling conditions

Of the 70 respondents, 52 (74.3%) reported that they prepare the pepper on a daily basis. However, 11 (15.7%) of them reported that they prepare the pepper within 2-days interval and 7 (10%) reported doing it weekly. For the heating of pepper sauce during the sale period, 30 (42.9%) did not heat pepper sauce at all. For those who heated pepper sauce, 29 (41.4%) heated it once whereas 3 (4.3%) heated it thrice during the sale period. At the time of sample collection, 9 (12.9%) pepper samples were hot and the rest were cold (87.1%). Most of the pepper sauce were stored in plastic containers

Table 2. Characteristics of the food stuff from which pepper sauce were sampled.

Variable		Frequency	Percentage
Site of food collection	Malingo	19	27.1
	Mile 17	15	21.4
	Muea Market	10	14.3
	University Layout	26	37.1
Food types	"Accra Banana"	6	8.6
	Boiled Egg	8	11.4
	Chicken	8	11.5
	Eru	4	5.7
	Fish	14	20.0
	Gateaux	9	12.9
	Pork	7	10.0
	Snails	11	15.7
	Soya	3	4.3
Mobility of vendor	Mobile	23	32.9
	Station	47	67.1

(54.3%), followed by food storage jar (22.9%), dishes (14.3%) and pots (4.3%). Some of the storage containers were covered (58.6%) while others were not (41.4%) (Table 3).

Isolation and enumeration of bacteria from pepper

The percentage bacterial growth on the various culture media was as follows: On PCA, 95.7%, 68.6% on SS agar and 74.3% on VRB agar. The bacterial colony count on PCA agar ranged from $1x10^3$ to $3x10^5$ CFU/ml.

Bacterial speciation

Table 4 shows the bacteria identified in chili pepper sauce using the Entrosystem 18R kit. A total of 20 isolates randomly selected from different culture media were identified using the Enterosystem 18R. The most prevalent bacterial identified were *Entrobacter cloacae* (31.57%), *Citrobacter freundii* and *Klebsiella pneumonia* (15.78%). Other bacterial identified were *Serratia liquefaciens, Enterobacter alvei, Enterobacter aerogenes, Proteus mirabilis, Salmonella spp. and Escherichia coli.*

Factors associated with bacterial count in pepper sauce

It was observed that the bacterial count was significantly associated with the vendor's age, types of food, pepper storage and handling conditions (Table 5).

For the food types, bacterial count was significantly low in pepper sauce used to sell pork (p=0.041) compared to cow meat. Pepper stored in flask and plastic containers had significantly (P< 0.001) lower bacterial count than that stored in pots. Pepper stored in uncovered containers had a significantly higher bacterial count compared to pepper store in covered containers (p<0.001). Similarly, the bacterial count was significantly higher (p<0.001) in pepper heated once or two times compared to pepper sauce heated 3 times per day. There was a significant positive relationship between the bacterial counts and the number of persons requesting for pepper (p=0.001) and a negative relationship (P<0.001) between the age of the vendor and the bacterial count. The microbial count decreased with the increase in the age of the vendor.

DISCUSSION

Seven species of entrobacteria were isolated from chili pepper sauce including *Enterobacter* spp*. (E. aerogenes, E. alvei, E. cloacae*), *P. mirabilis, C. freundii, S. liquefaciens, E. coli, K. pneumonia* and *Salmonella* ssp. Similar organisms were isolated in food in previous studies (Powers et al*.,* 1975, Filiz, 2001). The bacterial isolates were all members of the *Enterobacteriocea* family since the bacteria identification kit used in this study was for Enterobacteriocea. This group of organisms includes several species of bacteria that cause primary infections of the human gastrointestinal

Table 3. Characteristics of the pepper sauce at the time of sample collection.

Variable		Frequency	Percent
Food sold in a clean environment	No	16	22.9
	Yes	54	77.1
Periodicity of pepper preparation	Daily	52	74.3
	Two days	11	15.7
	Weekly	7	10.0
Types of storage container	Bottle	3	4.3
	Dish	10	14.3
	Food jar	16	22.9
	Plastic container	38	54.3
	Pot	3	4.3
Pepper sauce covered	No	29	41.4
	Yes	41	58.6
Heating of pepper sauce during sale period	No	30	42.9
	Yes	40	57.1
No. of times pepper sauce is heated per day	One time	29	41.4
	Two times	8	11.4
	Three times	3	4.3
Pepper temperature at time of sample collection	Cold	61	87.1
	Hot	9	12.9

Table 4. Bacterial species isolated from pepper cultures.

SN	Species	Media	Frequency	%
1	*Enterobacter aerogenes*	VRBA	1	5.26
2	*Enterobacter alvei*	VRBA	1	5.26
3	*Entrobacter cloacae*	VRBA/SSA	6	31.57
4	*Proteus mirabilis*	SSA	1	5.6
5	*Citrobacter freundii*	VRBA/SSA	3	15.78
6	*Serratia liquefaciens*	VRBA	2	10.52
7	*Escherichia coli*	SSA	1	5.26
8	*Klebsiella pneumonia*	SSA	3	15.78
9	*Salmonella species*	SSA	2	10.52

SSA, Salmonella Shigella Agar; VRBA, Violet Red Bile Agar.

tract infections including septicaemia, pneumonia, meningitis and urinary tract infections. The presence of enteric bacteria in food may indicate faecal contamination probably as a result of poor hygiene practice by the food handlers. These findings clearly indicate that chili pepper sauce used as food complement may represent a potential public health hazard to the consumers. Bacterial counts of up to $3x10^5$ per millilitre of chilli pepper sauce are indications of the load of bacterial people are exposed to when consuming the pepper sauce. The fact that only few vendors heat pepper after preparation could account for the high bacterial count observed in some samples. In fact the bacterial count was significantly associated with number of times pepper was heated. Pepper samples that were heated a least three times a day had less bacterial counts than those that were not heated at all or once per day after preparation. But the fact that these heated samples were still contaminated probably suggests that these pepper sauces were improperly handled after heating. This raises

Table 5. Relationship between food vendors demographic characteristics, food types, pepper handling and bacterial count.

Variable		Coefficient	Standard error	Wald Chi-Square	P-value
Age of food vendor	Age	-2.46	0.631	15.21	0.000
No. of customer requesting pepper per day	Pepper request	1.24	0.253	24.05	0.000
Site of sample collection	Malingo street	46.58	17.80	6.85	0.009
	Mile 17 motor park	31.96	22.54	2.01	0.156
	Muea market	38.13	26.59	2.06	0.152
	University layout	0	.	.	.
Food types	Accra banana	-87.10	48.60	3.21	0.073
	Boiled egg	-46.01	36.43	1.60	0.207
	Chicken	15.08	41.09	0.14	0.714
	Eru	8.59	49.08	0.03	0.861
	Fish	-23.86	47.29	0.26	0.614
	Gateaux	22.78	41.21	0.31	0.580
	Pork	-126.33	38.14	10.97	0.001
	Snails	-97.71	51.85	3.55	0.059
	Soya	0	.	.	.
Types of storage container	Bottle	-171.42	34.14	25.21	0.000
	Dish	-28.24	27.14	1.08	0.298
	Flask	-196.15	25.62	58.64	0.000
	Plastic container	-113.921	25.77	19.54	0.000
	Pot	0	.	.	.
Pepper sauce covered	No	37.89	10.57	12.86	0.000
	Yes	0	.	.	.
No. of time pepper was heated per day	One time	177.04	25.14	49.61	0.000
	Two times	195.85	26.42	54.96	0.000
	Three times	0	.	.	.

a general hygiene problem as far food handling is concerned

This study also investigated the factors that accounted for high bacterial count in pepper sauce. It was observed that the bacterial count was associated with demographic characteristics of the vendors and handling of food. The bacterial count was associated with education level of the vendors. More educated vendors are much more aware of food hygiene compared to less educated vendors. This result is in line with those of findings of Ackah et al. (2011), and Feglo and Sakzi (2012) in Ghana, which reported that low levels of education among food vendors contributed greatly to the increase of food contamination.

This studied revealed that the type of storage containers affects bacterial count. The microbial count was significantly higher in opened containers than closed ones. Covering of pepper sauce had an impact on the bacterial count but not on the contamination since samples from covered and uncovered were equally contaminated with the only difference being the number of CFU counted in both situations. Covering of pepper therefore did not prevent contamination of the sauce. The process of pepper preparation as reported by vendors involves boiling which may kill potential microbial contaminants. It is likely that contamination of pepper observed in this study occurred during post handling process. The presence of faecal contamination indicators like *E. coli*, *Klebsiela* and *Salmonella* species could be as a result of poor hygiene and/or poor handling of the food. This is in line with findings of Gallo et al. (1992), who reported faulty food handling, especially storage of food at improper temperatures for long periods of time. Ghosh (2009) also reported an increase prevalence of microbial contamination as a result of poor hygiene and sanitation practices.

A greater proportion of the vendors heated pepper at least once per day while others did not. Among those who heated pepper, the frequency of heating per day also varied (once, twice, and thrice). The frequency at which the pepper was heated had an influence on the

microbial count of the pepper. Samples that were heated three times a day had significantly low bacterial counts than those heated once or twice a day. This finding is in line with a study by Schwab et al. (1982).

The type of storage container also played an important role in chili pepper sauce contamination. Bacterial counts were higher in pepper stored in pots and dishes compared to pepper stored in bottles. Environmental and personal hygienic as well as food handling practices of the vendor can increase the level of microbial contamination of food. This study revealed viable bacterial counts with values ranging from $1x10^3$ to $3x10^5$ CFU/ml. The Centre for Food Safety and EHD Microbiological Guidelines for ready to eat food (Centre for Food Safety, 2014) classified food into satisfactory, borderline and unsatisfactory based on bacterial load in food. Based on these guidelines, the viable bacteria count revealed by this study makes the pepper sauce unsatisfactory for consumption.

Chili pepper sauce used to complement all sampled food types were contaminated. The only difference between the various food types was the bacteria count which varied significantly from one food type to another with highest counts observed in pepper sauce used to complement chicken and gateau, whereas the least count was observed in pepper sauce used as complement for pork and snails.

Conclusion

More than 95% of pepper samples examined were contaminated with bacteria. Microorganisms isolated from chili pepper sauce include: *Enterobacter* spp*. (E. aerogenes, E. alvei, E. cloacae*)*, P. mirabilis, C. freundii, S. liquefaciens, E. coli, K. pneumonia* and *Salmonella* ssp. The presence of theses microorganisms in chili pepper sauce suggest food contamination which could be potentially cause disease in humans. The age and level of education of the vendor, handling of pepper sauce after preparation (heating, covering and storage) were factors that significantly influence bacterial count. The identification of bacteria in this study was limited to enteric microorganisms. Further studies that will check a wider scope of microorganisms will give a clearer picture of the microbial contamination of this highly consumed food complement.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Probiotic potential of lactic acid bacteria isolated from traditionally fermented legume products of Western Kenya

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Fermentation as a processing technique has been used for a long time by people of Western Kenya to improve the quality of raw food material. During fermentation, lactic acid bacteria (LAB) synthesize vitamins and minerals, produce biologically active peptides with enzymes such as proteinase and peptidase, and remove some ant-nutrients. The medicinal and flavor enhancing properties of fermented foods are due to the presence of probiotics. This study aims at isolating and biochemically characterizing potential probiotic lactic acid bacteria from spontaneously fermented legume-based products from two locations in Vihiga County; Emuhaya and Mbale, Kenya. The results from the findings are presumptive indicator of probiotic bacteria in fermented legumes. $RC0_{PU}2$, $SB0_{PU}3$ and $CP0_{PU}1$ isolated form Emuhaya can survive at low pH of 2 while $SB0_{PU}3$ and $CP0_{PU}1$ in addition can survive a salt concentration of 3%. $SB0_{PU}3$ and $CPO_{PU}2$ and $RC0_{PU}2$, $SB0_{PU}2$ and $CPO_{PU}3$ can grow at high temperature of 45°C. $RC0_{PU}2$, $SB0_{PU}2$, $GG0_{PU}1$, $CP0_{PU}1$ and $CPO_{PU}2$ can tolerate a salt concentration of 3%. Only two isolates $RCO_{PU}2$ and $CPO_{PU}2$ isolated from Mbale samples survived at the pH of 2. The findings from this study indicated that fermented legumes are potential sources of probiotics with unique characteristics.

Key words: Probiotic potential, legumes, lactic acid bacteria.

INTRODUCTION

Legumes are the most abundant and widely cultivated food crops in Western Kenya. They are produced in large quantities and therefore make up the staple food for Luhya people. The use of legumes as alternative sources

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of protein and other micronutrients (Margier et al., 2018; Oluwafemi et al., 2017) could be assisted in improving dietary diversity of low income rural households. The protein of legumes is rich in essential amino acid lysine but poor in sulphur containing amino acids methionine and cystine (FAO, 2016; Anon, 2017).

Fermentation is a desirable process of biological modification of primary food matrix that is brought about by microorganism and their enzymes action The utilization of fermented legume foods containing probiotics would be one avenue by which the health of the children and community at large may be improved (Huan et al., 2019). Probiotics are live microorganisms which are produced during fermentation or intentionally added to foodstuffs and when consumed at certain levels in nutrition, stabilizes the gastrointestinal tract microflora conferring health benefits on the consumer (Markowiak and Katarzyna, 2017). Probiotic organisms also contain biologically active components which can have a positive impact on the wellbeing of the consumer (Alemayehu et al., 2017; Anusha et al., 2015). Several studies have indicated that LAB plays a positive role in modulating the host immune system and displaying of antimicrobial activities against common food-borne pathogens and in preventing and treating diarrhoea (Mokoena et al., 2016). The demand for non-dairy probiotic fermented foods has increased as consumers become more health conscious and at the same time expect particular and most times exact fermented product specifications (Ranadheera et al., 2017). Most indigenous fermentation products are valued for the taste, aroma and other active components produced. The consumption of plant proteins has evolved and is driven by the influence of continued need of consumers for health foods or for partial replacement of animal proteins with plants that possess better and cheaper nutritional components (Oluwafemi et al., 2017). Based on these findings and the increasing demand for probiotic foods from plant materials, this study was carried out to isolate probiotic LAB from fermented legumes, identify the isolates and biochemically characterize them.

MATERIALS AND METHODS

The legume samples were obtained from cereal store traders from Lwanda and Mbale market in Vihiga County, Western Kenya. They were transported in kaki bags to the Food and Microbiology Laboratories, Technical university of Mombasa where the experiment was carried out.

Fermentation of legume products

The legumes (beans, soy bean, cow peas and green grams) were cleaned by winnowing to remove husks and other light foreign materials. They were then sieved to remove stones and sand. 50 g each of the legume was ground using a blender separately to obtain flour, with sterilization of the blender with 70% ethanol after every sample. The sample flour was mixed with two parts water and fermented by incubating at 30°C for 48-72 h in a sterile covered flask. 10 g sample was taken aseptically from each for lactic acid bacteria screening.

Isolation of probiotic potential lactic acid bacteria from legumes

The samples were suspended appropriately and diluted in sterile saline. From each legume fermented, 10 ml of sample was homogenized with 90 ml of 0.85% (w/v) sterile sodium chloride solution to make an initial dilution (10^{-1}). Serial dilutions up to 10^{-7} were made for each sample. 1 ml sample from each of the corresponding dilutions (10^{-5} and 10^{-7}) was plated out onto MRS agar plates by spread plate technique in duplicates. Inoculated plates were then incubated at 37°C for 48-72 h under anaerobic conditions.

Characteristics of the probiotic potential lactic acid bacteria isolates

MRS broth was used in these series of studies but with 0.17 g/L bromothymol blue added as pH indicator (pH 7). Universal bottle with screw caps was each filled with 20ml of the MRS broth and autoclaved. A 24 h culture of each isolate was used as the inoculum whereby the cells were spun down, re-suspended in 0.85% normal saline and a loopful of the suspension was inoculated into each of the test tubes. The temperature tested was 15, 37, 45 and 55°C, the concentration of NaCl tested was 2, 3, 6.5 and 10% (w/v), while the pH tested was 2, 3, 4 and 6. The MRS broth was adjusted with 1M phosphoric acid and 1M NaOH to prepare the initial pH. At the end of 24 h the colour change of each test tube was noted as a simple indication of growth or no growth.

RESULTS

The results are summarized in Tables 1 and 2. This study has shown that probiotic potential lactic acid bacteria could be isolated from fermented legumes. Nine LABs were isolated from Emuhaya legume samples; 6 of these are possible *Lactococcous* species and 3 *Lactobacillus* species. The total isolates from Mbale are ten: 6 are presumptive *Lactococcous* species and 4, *Lactobacillus* species.

All of the isolates from Emuhaya and Mbale were Gram-positive and catalase-negative bacteria, generally unable to grow at 55°C and NaCl concentration of 6.5% but able to grow at pH 4.0 to 6.0. $RC0_{PU}2$, $SB0_{PU}3$ and $CP0_{PU}1$ from Emuhaya can survive at low pH of 2 while $SB0_{PU}3$ and $CP0_{PU}1$ in addition can survive at a salt concentration of 3. $SB0_{PU}3$ and $CPO_{PU}2$ (Table 1) and $RC0_{PU}2$, $SB0_{PU}2$ and $CPO_{PU}3$ (Table 2) can grow at high temperature of 45°. These lactic acid bacteria are possible thermophiles like those of yoghurt culture. $RC0_{PU}2$, $SB0_{PU}2$, $GG0_{PU}1$, $CP0_{PU}1$ and $CPO_{PU}2$ can tolerate a salt concentration of 3%. Only two isolates ($RCO_{PU}2$and $CPO_{PU}2$) from Mbale survived at the pH of 2.

DISCUSSION

There is an overwhelming increase in relation to utilization

Table 1. Phenotypic characteristics of representative strains isolated from traditionally fermented Legume from Emuhaya-Vihiga.

Organisms	Gram's reaction/cell shape	Cultural characteristics	Catalase test	Growth at temperatures (°C)				Growth in NaCl concentration (%)				Growth at pH				Possible isolate
				15	37	45	55	2	3	6.5	10	2	3	4	6	
$RC0_{PU}1$	+Rod	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactobacillus spp
$RC0_{PU}2$	+Cocci	Ppc	-	-	+	-	-	+	+	-	-	+	+	-	+	Lactococcus spp
$SB0_{PU}1$	+Rod	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactobacillus spp
$SB0_{PU}2$	+Cocci	Ppc	-	+	+	-	-	+	-	-	-	-	-	+	+	Lactococcus spp
$SB0_{PU}3$	+Cocci	Ppc	-	-	+	+	-	+	+	+	-	+	-	+	+	Lactococcus spp
$GG0_{PU}1$	+Cocci	Ppc	-	-	+	-	-	+	+	-	-	-	-	-	+	Lactococcus spp
$GG0_{PU}2$	+Cocci	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactococcus spp
$CP0_{PU}1$	+Cocci	Ppc	-	-	+	-	-	-	+		-	+	+	+	+	Lactococcus spp
$CPO_{PU}2$	+Rod	Ppc	-	+	+	+	-	+	-	-	-	-	-	+	+	Lactobacillus spp

+indicate growth; - no growth +; Gram positive +; Ppc: Pin Point Colony; LAB isolates: (RC0PU1- RC0PU2)-Rosecoco isolates; (SB0PU1- SB0PU3) - Soybean isolates; (GG0PU1- GG0PU2) –Green gram isolates; (CP0PU1- CPOPU2) –Cowpeas isolates.

Table 2. Phenotypic characteristics of representative strains isolated from traditionally fermented Legume from Mbale-Vihiga.

Organisms	Gram's reaction/cell shape	Cultural characteristics	Catalase test	Growth at temperatures (°C)				Growth in NaCl concentration (%)				Growth at pH				Possible isolate
			-	15	37	45	55	2	3	6.5	10	2	3	4	6	
$RCO_{PU}1$	+Cocci	Ppc	-	-	+	-	-	+	+	-	-	-	-	+	+	Lactococcus spp
$RCO_{PU}2$	+Cocci	Ppc	-	-	+	+	-	+	-	-	-	+	+	+	+	Lactococcus spp
$RCO_{PU}3$	+Rod	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactobacillus spp
$SBO_{PU}1$	+Cocci	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactococcus spp
$SBO_{PU}2$	+Cocci	Ppc	-	-	+	+	-	+	+		-	_	+	+	+	Lactococcus spp
$GGO_{PU}1$	+Cocci	Ppc	-	-	+	-	-	+	+	-	-	-	-	-	+	Lactococcus spp
$GGO_{PU}2$	+Rod	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactobacillus spp
$CPO_{PU}1$	+Cocci	Ppc	-	-	+	-	-	+	+	-	-	_	+	-	+	Lactococcus spp
$CPO_{PU}2$	+Rod	Ppc	-	+	+	-	-	+	-	-	-	+	-	-	+	Lactobacillus spp
$CPO_{PU}3$	+Rod	Ppc	-	-	+	+	-	+	+	-	-	-	-	+	+	Lactobacillus spp

+indicate growth; - no growth; + Gram positive; Ppc: Pin Point Colony; LAB isolates: (RCOPU1- RCOPU3)-Rosecoco isolates; (SBOPU1- SBOPU2) - Soybean isolates; (GGOPU1- GGOPU2) –Green gram isolates; (CPOPU1-CPOPU3) –Cowpeas isolates.

of commercial probiotic LAB strains isolated from indigenous and naturally fermented foods (Jawan et al., 2020). The food products are said to possess medicinal and health-promoting acid tolerant and catalase negative devoid of cytochromes (Frank, 2017). These bacteria produce lactic acid as main fermentation product of carbohydrate benefits. LABs are Gram-positive, cocci, and cocco-bacilli, fastidious, non-spore-forming rods, catabolism

and other organic substances that add to the flavor, texture, and aroma that contribute to organoleptic characteristics of the products (Vishwanathan and Kadirvelu, 2016). Probiotic isolates must be tolerated and survived a stressful environment such as of low pH (acidic condition) in the stomach and high bile salt concentration in the small intestine (Prete et al., 2020). The LABs which have genes responsible for the degradation and utilization of simple sugars and complex carbohydrates are able to better survive and multiply in the gut (Conlon and Bird, 2015).

Probiotic bacteria are required to survive gastric passage, where the pH can be as low as 1.5 to 2.0 and stay alive for 4 h or more (Gupta and Sharma, 2017) before they move to the intestinal tract. The acid tolerance of bacteria is essential both for withstanding gastric stresses and also to enable the strain to survive for longer periods in high acid carrier foods without reduction in their number (Angmo et al., 2016). Most of the investigated isolates can survive low pH and high salt concentration if incubation time could have been reduced to 3 h (considering the experimental time was more, that is, about 16 h). It is therefore expected they will be good probiotics if they pass safety tests and can be recommended in the food preparations of probiotic foods. To assure viability and functionality of potential probiotic isolates, tolerance to acidic conditions is an important criterion considered. The characteristic of probiotic bacteria to exhibit resistance to acidic conditions is species and strain dependent (Papadimitriou et al., 2016). The growth of pathogenic microorganisms has been shown to be suppressed by probiotic lactic acid bacteria through the release of a variety of antimicrobial factors such as bacteriocins, hydrogen peroxide, ammonia, diacetyl and organic acids such as lactic and acetic acids. These compounds reduce the pH of the lumen, making it difficult for the growth of a variety of food-borne spoilage and pathogenic organism (Gupta and Shama, 2017). The safety analysis is an important issue during the selection and evaluation of new probiotics. Thus, characterization of the safety criteria of the probiotic strains is vital in order to avoid their side effects (Ayala et al., 2019). This study revealed in part that fermented legumes are possible sources of probiotic bacteria; however, the LABs are to be assessed for the ability to produce bacteriocin and lactic acid against a number of serious food borne and spoilage causing microorganisms. The susceptibility to selected eleven antibiotics, inability to produce gelatinase and DNase and non-hemolytic nature will reveal their safety status for further use in food and nutraceutical industry.

Conclusion

The lactic acid bacteria isolated from fermented legumes used in this study possess great potential as probiotics for human and as fermentation starter cultures. This was supported by probiotic characteristics such as survivability in acid condition and high salt concentration. The lactic acid bacteria, $RC0_{PU}2$, $SB0_{PU}2$ and $CPO_{PU}3$ that can be grown at high temperature of 45°C can be considered as thermophiles like those of yoghurt culture.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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