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Full Length Research Paper

Effects of processing on nutrient composition in guava- and jackfruit-based snacks

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Guava and jackfruit are popular fruits in East Africa. With consideration of the high post-harvest losses of these two fruits and only seasonal availability, this study aimed to produce nutrient rich fruit-based snacks to decrease this problem and make use of surplus fruits during on-season. Given the nutritional situation in East Africa, these products were also developed to have a high content of desired nutrients. Next to either guava or jackfruit also mango, different nuts and lemon juice was partly added. Processing methods included cooking and drying, which are suitable for local households and small processing groups. Chemical analyses were implemented to determine nutrient contents before cooking, after cooking and after drying. Major results included that bars with guava and lemon juice contained the highest content of ascorbic acid, 81.19 and 48.18 mg/100 g FM before and after cooking, respectively; jackfruit-based samples without lemon juice after drying contained more phenolic content than guava-based samples; fruit bars with lemon juice had higher acidity; samples of guava contained more β-carotene than jackfruit. In conclusion, the fruit-nut-bars can provide a good option to process surplus fruits and provide essential nutrients to the local population in East Africa.

Key words: Guava, jackfruit, fruit-nut-bars, East Africa.

INTRODUCTION

Guava (*Psidium guajava*) and jackfruit (*Artocarpus heterophyllus*) are naturalized in tropical and subtropical areas around the world (Flores et al., 2015). Both of them have high contents of several nutrients that are essential to human beings, such as ascorbic acid and potassium (Lukmanji et al., 2008). However, in East Africa, these fruits have usually high post-harvest-losses which decrease the benefit of local people (Omayio et al., 2019). At the same time, lacking nutrients in the diet, especially micronutrients, also known as hidden hunger, is a major problem for many people in sub-Saharan Africa (Tulchinsky and Varavikova, 2009). On the other hand, also the overconsumption of certain nutrients is of a problem and, for example, diabetes is also widespread among African people (IDF, 2019). The share of adults aged 20-79 years with diabetes is 3.9% of the total population in the IDF Africa Region with an increasing trend (FAO et al., 2020).

There are different types of guava products, such as jam, jelly and juice (Leite et al., 2006). There are also

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studies that investigated the nutrient content of guava together with one other fruit such as orange (Srivastava et al., 2019), papaya (Bisen and Ruchi, 2020) and mango (Sucheta et al., 2017). Products made from the ripe jackfruit already investigated are jackfruit powder (Ahiduzzaman, 2016), jackfruit chips (Yi et al., 2016), jelly, syrup and jams (Mondal, 2013).

This study also focuses on processing options in order to extend the shelf-life of guava and jackfruit which were selected as target fruits by the overall project. One aim is, however, to increase different kind of nutrients by mixing the target fruits with a combination of other fruits and nuts. The form of products in this study was chosen to be fruit-nut-bars, which can be easily carried and should be accepted, especially by children and adolescents similar to dried-fruit-slices that are already available in East Africa (Omayio et al. 2019). However, the nutrient content of fruit-nut-bars which combine guava or jackfruit with other fruits and nuts still lack studies in East Africa.

Given that diabetes is on the rise in East Africa (IDF, 2019), sugar content in this study is considered in particular and there is no extra sugar added to the products. In addition, with the consideration of chemical residuals, there were also no extra chemical preservatives, which, however, can lead to a problem of food safety and reduced long-time storage. Ascorbic acid and β-carotene as well as mineral content inside the fresh fruits were also considered in addition to the degree of color change after drying.

Cooking was chosen because it is a popular food processing method in East Africa and it helps to reduce microbiological contamination (Njoroge et al., 2015). Grinding was applied to make the fruit-nut-mixture to be as uniform as possible. Drying with oven was used to decrease the water content in samples, because bacteria would spoil foods with high moisture (Ponte et al., 1993). If solar drying is not possible, electric power is needed for the oven, which creates some costs and environmental issues (Bieber et al., 2018).

The objectives of this study were as follows:

1) To develop healthy fruit-nut-bars with high essential nutrient content, low sugar content and with potential for a long shelf life.
2) To develop fruit-nut-bars with crispy texture and high acceptability in East Africa.

This study was carried out in the framework of a large study on “Fruit and vegetables for all seasons” (FruVaSe) with partners in East Africa and Germany.

MATERIALS AND METHODS

Although the FruVaSe project and most of its components take place in East Africa, the fruit-nut-bar development and analysis took place at the University of Goettingen in Germany. The fresh guavas and jackfruits required for product development and testing were bought from a supermarket (KIM) in Hannover, Germany, and an online shop (Tropenkost) in Frankfurt, respectively. The origin country of both fruits was Thailand. Because of limited availability, in this study, only the white-fleshed types of guava could be studied. Mangoes and bananas were bought from a local supermarket (Rewe) in Goettingen, Germany, origin country was Spain. Fruits were stored at 4ºC before being processed.

At the beginning, cashew nuts were decided to be used, because cashew trees also grow in East Africa like Tanzania and Kenya (McLaughlin et al., 2018). It is also a good choice for adding more minerals and using local materials. However, in Uganda, cashew nuts are mainly grown in northern and eastern Uganda; most of them have to be bought from other countries (Wanyama et al., 2017). Consequently, some bars were made by using peanuts to replace cashew nuts. Both nuts were bought in local supermarkets in Goettingen and the origin was Egypt for peanuts and Vietnam for cashew nuts.

In order to increase the flavor, desiccated coconut (Renuka Agri Organics Ltd, origin country Sri Lanka) was also added. According to the results of Okafor and Ugwu (2014), when compared with snacks without coconut, those that contained coconut gained higher acceptability in a study in Nigeria.

Preparation of fruit-nut-bars

The routine of making fruit-nut-bars can be divided into preparing, blending, cooking and drying. During the process of cooking, 50% of total fruit-nut-mass of water was added, in order to prevent the samples from sticking to the pot. Specific processes are shown in Figure 1. The final products of this study can be divided into guava-bar with mango or jackfruit-bar with mango; the choice of nuts can be divided into peanuts and cashew nuts; lemon juice is another alternative. Consequently, there are eight final different recipes (Table 1 and Figure 2).

The eight final recipes were chosen after the sensory test. The concentration of lemon juice increased after the sensory test in these final recipes; banana, which was an ingredient in the first set of recipes, was not chosen to be an ingredient of the final recipes; peanuts were added as an alternative of cashew nuts.

Sensory test

Eleven participants from Uganda, Kenya and Tanzania tasted six different fruit-nut-bars with different ingredients (Table 2) at the beginning of this study in order to refine the recipes. This sensory test also included a questionnaire (Appendix Table 1). The questionnaires were given to each participant before the samples were served. Each sample was put in a bowl and served separately to each person. Recipes were not revealed until all questionnaires were filled.

Instrumental methods

Water content and total soluble solids

To determine the water content, about 10 g of the samples were placed in a petri dish and the total weight was determined, and then dried for 19 hours at 60ºC and 4 h at 105ºC. Afterwards the total weight was determined again, and the water content was calculated (Rutter and Slatyer, 1968).

The method of ºBrix was used to determine the content of total soluble solids (Ranganna, 1976). 1 g of the sample after drying were weighed for three replicates, 9 ml water was added

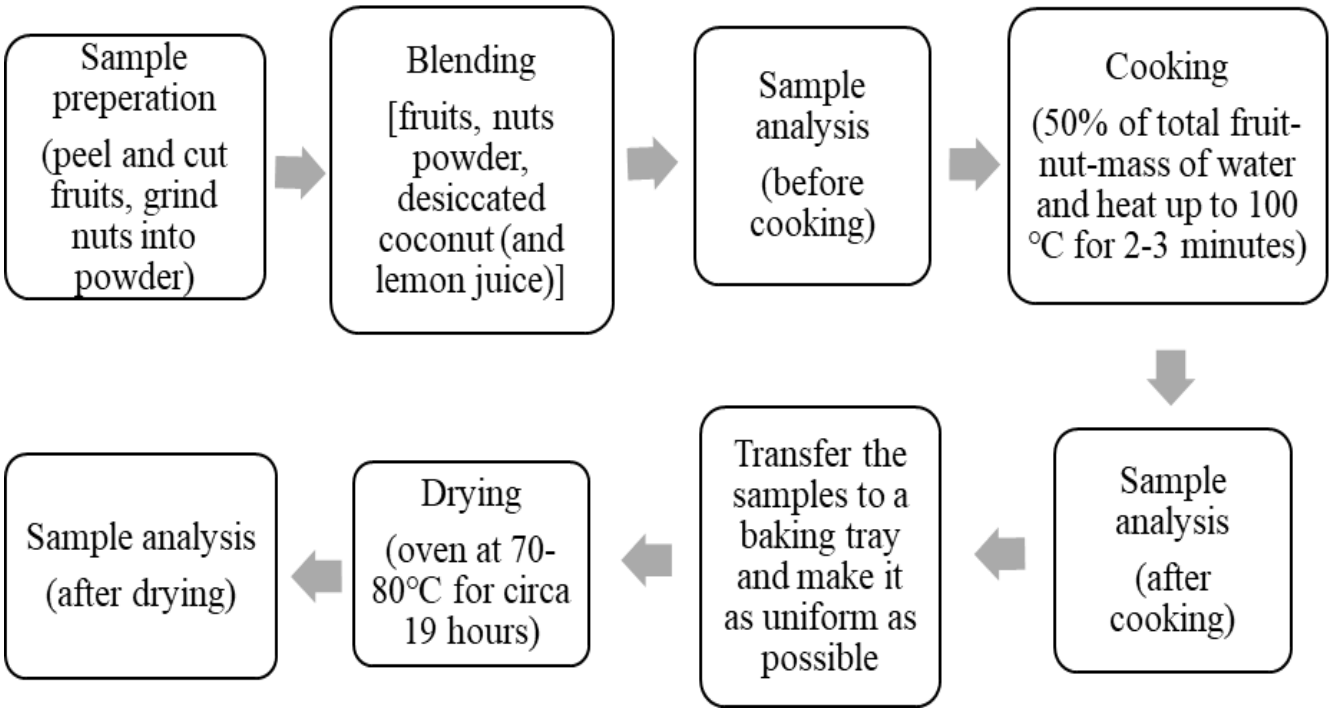

Figure 1. Steps of fruit-nut-bars preparation.

Table 1. Recipes of fruit-nut-bars showing the percentage (%) of all ingredients.

Sample	Jackfruit	Mango	Cashew nuts	Peanuts	Desiccated coconut	Lemon juice*
Jackfruit-cashew-lemon	60	20	10	-	10	10
Jackfruit-cashew	60	20	10	-	10	-
Jackfruit-peanut-lemon	60	20	-	10	10	10
Jackfruit-peanut	60	20	-	10	10	-
Sample	**Guava**	**Mango**	**Cashew nuts**	**Peanuts**	**Desiccated coconut**	**Lemon juice**
Guava-cashew-lemon	60	20	10	-	10	10
Guava-cashew	60	20	10	-	10	-
Guava-peanut-lemon	60	20	-	10	10	10
Guava-peanut	60	20	-	10	10	-

*10% of lemon juice is the weight of lemon juice divided by the total weight of all other ingredients.

afterwards. The samples were mixed (Reamix 2789 Vortex Mixer, MTC, Hamburg) and shaken (shaker Swip, Edmund Bühler) for 1 h, centrifuged for 5000 rpm for 5 min (Centrifuge 5804 R, Eppendorf, Hamburg), then Pasteur pipettes were used to place few drops of the supernatant on a refractometer (handheld refractometer, A. Krüss Optronic GmbH, Hamburg) to obtain the value of °Brix.

Mineral content

The mineral content was determined as described by Koch et al. (2019) with slight modification. In brief, about 100 mg of milled samples were weighed in a Teflon vessel and digested with 4 ml of 65% (v/v) nitric acid and 2 ml of 30% (v/v) hydrogen peroxide in a microwave (Ethos 660; MWT AG, Heerbrugg, Switzerland) for 75 min at 200ºC and 40 bar. Afterwards, the samples were filled up to 25 ml with distilled water. Before samples were measured, 2 ml solution of samples were taken out and 8 ml distilled water was added for dilution. The concentrations of macro- and micronutrients were measured with inductively coupled plasma optical emission spectrometry (Vista-PRO CCD Simultaneous ICP-OES; Varian Inc., Palo Alto, CA).

Titratable acidity and ascorbic acid

Titratable acidity content was determined by titration method (0.1N NaOH solution) according Kanski et al. (2020) with modification.

Figure 2. Final products of eight fruit-nut-bars From left to right: Jackfruit-Cashew-Lemon; Jackfruit-Cashew; Jackfruit-Peanut-Lemon; Jackfruit-Peanut Guava-Cashew; Guava-Cashew-Lemon; Guava-Peanut; Guava-Peanut-Lemon.

Table 2. Composition of samples for sensory test.

Sample	Jackfruit (g)	Guava (g)	Mango (g)	Banana (g)	Desiccated coconut (g)	Peanuts (g)	Lemon juice (g)
Guava-banana-lemon	-	240	-	80	40	40	20
Guava-banana	-	240	-	80	40	40	-
Guava-peanut-lemon	-	240	80	-	40	40	20
Guava-mango	-	240	80	-	40	40	-
Jackfruit-mango-lemon	240	-	80	-	40	40	30
Jackfruit-peanut-lemon	240	-	80	-	-	80	30

About 0.2 g of the milled samples after drying was weighed into a beaker and 20 ml water was added together with a magnet. The beaker was put on a magnetic stirrer for 15 min. The solution was titrated with a solution of 0.1 N NaOH to pH 8.1 (pH-titrator Titro line 96, SCHOTT AG, Mainz).

The ascorbic acid content was determined according to Sonntag et al. (2020) with modifications. 5 g of samples and 20 ml of meta-phosphoric acid were mixed with Ultra-Turrax (T18 digital Ultra Turrax, IKA, Staufen) for 2 min. The pulp was transferred into a measuring cylinder, filled up to 50 ml with pure water and filtrated; thereafter, 10 ml of the filtrate was transferred into the Erlenmeyer flask (2-3 times for each sample). Finally, samples were titrated against 2,6-Dichlorophenolindophenol (DIP) solution until a light pink end point was reached and could persist for 15 s, then the used amount of ml of DIP solution could be recorded.

Total phenolic content

The total phenolic content was determined by using Folin and

Table 3. Instruments settings for the measurement of β-carotene content (Schex et al., 2018, modified).

Used software was LabSolutions, Version 5.32 (Shimadzu, Duisburg, Deutschland).	
The injection volume was 20 µL. Chromatographic separation was achieved at 30°C using a YMC C30 reversed phase column (250 × 4.6 mm i.d., 5 µm particle size, YMC Europe Dinslaken, Germany) protected by a YMC C30 guard cartridge column (10 × 4.6 mm i.d., 5 µm particle size, YMC Europe).	
HPLC eluents:	Mixtures of methanol/tBME/water (80:18:2, v/v/v, eluent A; 8:90:2, v/v/v, eluent B), containing 0.4 g/L ammonium acetate
The elution gradient at a constant flow rate of 0.6 mL/min:	90 to 40% A (30 min), 40 to 0% A (5 min), isocratic at 0% A (2 min), 0 to 90% A (3 min), isocratic hold at 90% A (5 min).
Total run time was 45 min, post run time 2 min.	
Detection wavelengths were 663 nm (Chlorophyll a), 647 nm (Chlorophyll b), 669 nm (Pheophorbide a) and 407 nm (Protoporphyrin IX). UV/vis spectra were recorded between 300 and 700 nm.	
Compounds were assigned by comparing their retention times (tR) and UV/vis absorption spectra to those of commercially available reference standards.	

Ciocalteu's phenol reagent (Folin-C reagent) and photometrical determination (Singleton and Rossi, 1965). About 0.25 g of each sample powder was weighed and 5 ml 80% Ethanol was added in a centrifugation tube. The tube was vortexed and then centrifuged at 5000 rpm for 10 min (Centrifuge 5804 R, Eppendorf, Hamburg). The supernatant was transferred into a 10 ml graduated flask, and then the extraction was repeated. The supernatants were combined together and filled up to 10 ml with 80% Ethanol and were frozen in a szintilation vessel prior analysis. Before measurement, the supernatants were taken out at room temperature for 1 h.

For measurements, the samples were prepared as follows: water 2.4 ml, NaOH 1 ml, sample solution 500 µl and Folin reagent 100 µl. Afterwards they were measured immediately at 735.8 nm with a photometer (HP 8453, Hewlett-Packard, Waldbronn) and the results were expressed in mg gallic acid equivalents (GAE).

β-carotene

The β-carotene content was determined according to Schex et al. (2018). As the samples after drying were difficult to be grinded into powder, only samples before and after cooking were tested. 100 mg of lyophilized powdered samples were placed in a 2 ml reaction tube together with 600 µl Methanol (MeOH)/ Tetrahydrofuran (THF) (1:1, v/v). The solution was mixed in a thermomixer (ThermoMixer C, Eppendorf AG, Hamburg) for 10 min at 1400 rpm and centrifuged at 8000 rpm for 5 min (Centrifuge 5804 R, Eppendorf, Hamburg). Afterwards 500 µl supernatant was transferred into another 2 ml reaction tube. 500 µl MeOH/THF (1:1, v/v) was added in the old tube and the extraction was repeated twice. The supernatants (1500 µl) were combined together and evaporated to dryness in a rotational vacuum concentrator (RVC 2-25 CDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz) for almost 5 h at 20°C. Then the samples were frozen at -80°C until measurement. 250 µl Methyl tert-butyl ether (MTBE) were added to the samples to re-dissolve the residue and vortexed (Mini Vortexer, Heathrow Scientific, and Vernon Hills). Then, 250 µl MeOH were added and vortexed. Afterwards, the samples were dissolved by using a thermomixer (ThermonMixer C, Eppendorf AG, Hamburg) for 5 min at 800 rpm. Then the solution was filtered through syringe-filter with pore size of 0.2 µm and almost 400 µl filtered solution was transferred into a HPLC vial with insert and measured by HPLC (System "Prominence", Schimadzu). The instrument settings for measurement are shown in Table 3 (Schex et al., 2018).

Color

Color was determined using the machine MINOLTA Chroma Meter - CR310 (Konica Minolta, Inc., Marunouchi, Japan) for samples before and after cooking and by MINOLTA - CR400 (Konica Minolta, Inc., Marunouchi, Japan) for samples after drying to obtain L (brightness), a*(variation from green to red) and b* (variation from blue to yellow) values (Itten, 1997).

Textural profile analysis

The texture properties determined were the hardness and crispness of the fruit-nut-bars. They were measured in terms of the maximum peak force and number of peaks during the first compression cycle with the texture analyzer ('Stable Micro Systems', Winopal, Germany) according to Yadav and Bhatnagar (2017) with the following settings:

Pre-Test Speed: 2 mm/s; Test Speed: 1 mm/sec; Post-Test Speed: 10 mm/s; Force: 10 g; Trigger Force: 10 g; Probe: P/5; 5 mm Dia Cylinder (Figure 3).

Depending on the thickness and breakage of fruit-nut-bars, the parameter of distance was set differently. It was the peak distance reached by the probe, that was deep enough to completely penetrate the bars, but did not touch the platform on which the samples were placed.

Data analysis

Data analysis was mainly carried out with Excel, where the mean values were calculated, the standard deviation as well as the formation of bar graphs was undertaken. The significant difference (POSTHOC Tukey HSD) was calculated using PSPP version GNU pspp 1.4.1-g79ad47.

RESULTS

The following results are for eight final fruit-nut-bars, the recipes are shown in Table 1. The names of these fruit-nut-bars show the varying ingredients (such as Guava-

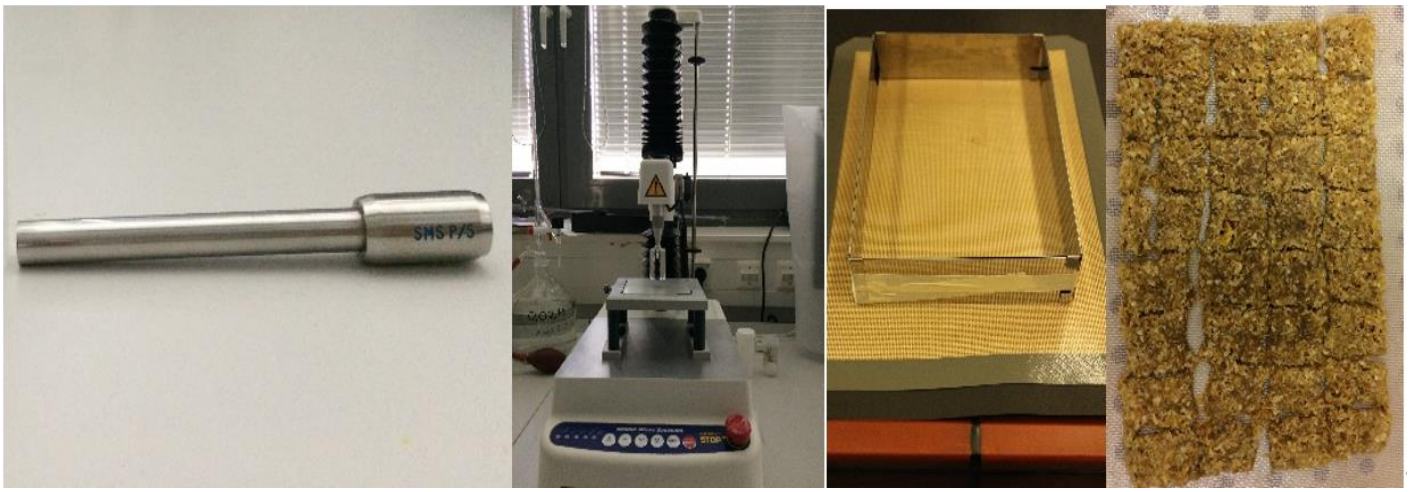

Figure 3. Photos of textural profile analysis. From left to right: 5 mm dia cylinder; textural test machine; baking tray; dried samples.

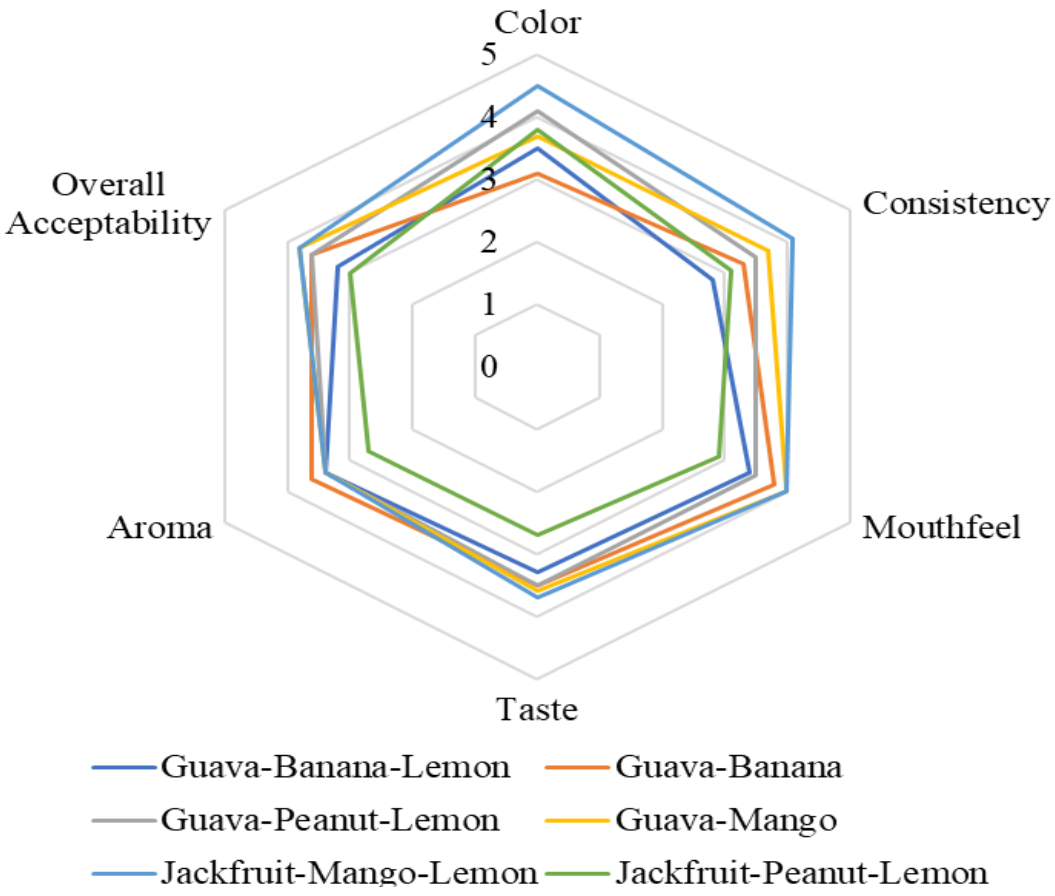

Figure 4. Results of sensory test of six preliminary fruit-nut-bars (11 participants from Kenya, Tanzania and Uganda).

Cashew-Lemon). Ingredients, which were present in all eight fruit-nut-bars, namely mango and desiccated coconut, will not be mentioned explicitly.

Sensory test

Eleven participants that were project partners from

Table 4. Water content (n=1), total titratable acidity and color (n=3, mean ± SD) of guava- and jackfruit-based snacks after drying.

Sample	Water content (%)	Titratable acidity (%)	Color			
			Top side		Bottom side	
			L	b	L	b
Guava-cashew-lemon	10.08	0.75±0.030^{a}	34.73±0.43^{b}	22.32±2.28^{d}	56.71±1.11^{bc}	31.46±0.51^{cd}
Guava-cashew	7.89	0.38±0.015^{b}	33.44±3.11^{b}	23.32±2.23^{d}	55.38±2.73^{c}	27.03±0.49^{e}
Guava-peanut-lemon	5.85	0.73±0.040^{a}	36.75±1.17^{b}	26.70±1.17^{cd}	57.54±3.45^{abc}	34.01±1.55^{bc}
Guava-peanut	6.86	0.44±0.015^{b}	39.12±2.97^{b}	31.35±2.77^{bc}	57.46±0.75^{abc}	30.05±1.23^{de}
Jackfruit-cashew-lemon	8.09	0.63±0.030^{a}	48.10±1.45^{a}	37.53±1.25^{ab}	62.42±1.66^{ab}	36.18±2.25^{ab}
Jackfruit-cashew	7.21	0.71±0.015^{a}	45.97±2.12^{a}	35.75±2.69^{ab}	55.70±1.14^{c}	36.37±1.16^{ab}
Jackfruit-peanut-lemon	4.66	0.42±0.000^{b}	49.76±3.98^{a}	40.85±4.12^{a}	62.82±3.07^{a}	38.59±0.79^{a}
Jackfruit-peanut	8.49	0.41±0.015^{b}	48.25±1.78^{a}	35.65±2.51^{ab}	62.31±0.73^{ab}	36.51±1.60^{ab}

Different letters indicate statistically significant differences ($P < 0.05$).

Table 5. Total soluble solids content (n=3, mean ± SD) of guava- and jackfruit-based snacks in three stages (before cooking, after cooking and after drying).

Sample	Total soluble solids (°Brix)		
	Before cooking	After cooking	After drying
Guava-cashew-lemon	13.13±0.094^{d}	8.80±0.00^{f}	4.13±0.094^{bc}
Guava-cashew	13.67±0.094^{c}	9.20±0.00^{e}	4.20±0.00^{bc}
Guava-peanut-lemon	13.07±0.094^{d}	8.33±0.094g	4.00±0.00^{c}
Guava-peanut	13.53±0.094^{c}	8.47±0.094^{f}	4.07±0.094^{bc}
Jackfruit-cashew-lemon	16.80±0.00^{b}	11.73±0.094^{c}	4.07±0.094^{bc}
Jackfruit-cashew	18.67±0.094^{a}	12.27±0.094^{b}	4.40±0.00^{a}
Jackfruit-peanut-lemon	16.73±0.094^{b}	11.13±0.094^{d}	4.33±0.094^{ab}
Jackfruit-peanut	18.73±0.094^{a}	12.73±0.094^{a}	4.47±0.19^{a}

Different letters in the same column indicate statistically significant differences ($P < 0.05$).

Kenya, Tanzania and Uganda took part in a sensory test by tasting six different preliminary fruit-nut-bars and answering a questionnaire. Average values of each attribute were calculated (Figure 4). As the radar graph shows, Guava-Mango and Jackfruit-Mango-Lemon gained the highest overall points; desiccated coconut was an ingredient in both recipes. Jackfruit-Mango-Lemon showed almost in all attributes the highest points except aroma. As for color and consistency, Guava-Banana and Guava-Banana-Lemon achieved the least points, respectively. For the other three attributes (mouthfeel, taste and aroma), Jackfruit-Peanut-Lemon (without desiccated coconut) showed the least points.

Water content, total soluble solids, titratable acidity and color

Table 4 shows the results of water content, total titratable acidity and color of guava- and jackfruit-based snacks after drying. Table 5 shows the average contents of total soluble solids of guava- and jackfruit-based snacks before and after cooking, and after drying. Only one test of water content after drying was done, so there were no average results or standard deviations. Almost all samples showed water content less than 10% because of drying. Jackfruit-based samples showed higher results of total soluble solids than guava-based samples. Guava-Peanut-Lemon bars had the lowest content among eight samples. Except for Jackfruit-Cashew-Lemon samples, other snacks with lemon juice all showed higher values of titratable acidity. For color test, the data of ‘a’ (variation from green to red) were very low for after drying and so for final products only the data of ‘L’ (brightness) and ‘b’ (variation from blue to yellow) were recorded. In terms of brightness, the bottom side of products showed higher values than the top side (brighter); jackfruit-based samples were significantly brighter than guava-based samples ($P<0.05$). Within samples with the same basic fruit (guava or jackfruit), there was no significant brightness difference (except the bottom side of Jackfruit-Cashew samples). For the top side of jackfruit-based samples, samples with lemon juice were brighter and had more yellowness, which was also the same tendency for

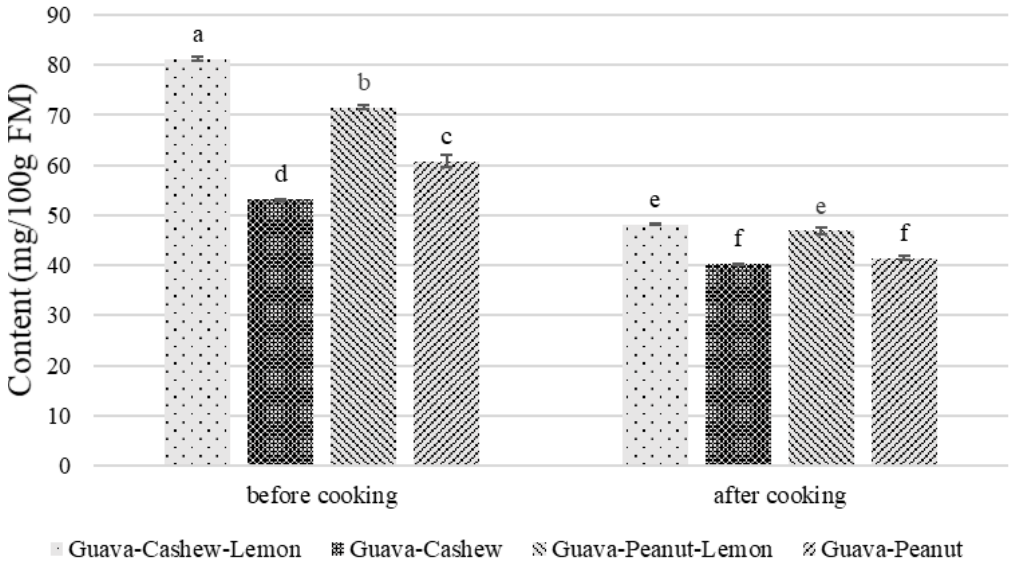

Figure 5. Ascorbic acid content of guava-based samples before and after cooking (n=3). Different letters indicate statistically significant differences (P<0.05).

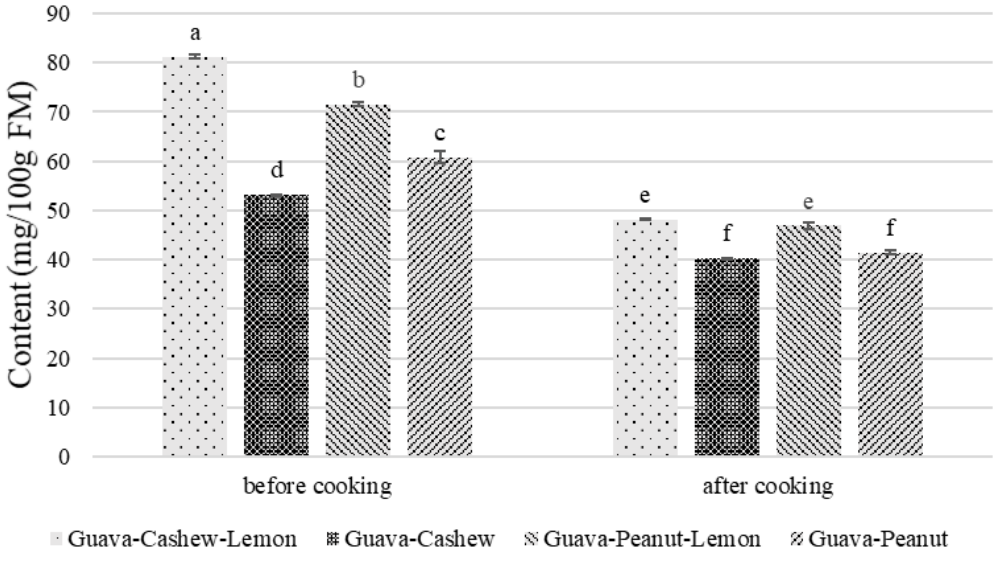

Figure 6. Ascorbic acid content of jackfruit-based samples before and after cooking (n=3). Different letters indicate statistically significant differences (P<0.05).

the bottom side of all samples.

Ascorbic acid, total phenolic content and β-carotene

Figure 5 and 6 show the results of ascorbic acid, namely the average contents in two stages, before and after cooking. As a result of drying, the water content in the samples was very low, so it was not possible to determine ascorbic acid content in them. The cooking process resulted in a significant decrease in ascorbic acid content, with guava-based samples showing higher values than jackfruit samples.

Figures 7 and 8 illustrate the average results of total phenolic content of guava- and jackfruit-based samples in three stages (before cooking, after cooking and after drying). Lemon juice led to a result of higher values of total phenolic content for all samples before cooking. In jackfruit-based samples with lemon juice total phenolic content decreased from before cooking to after drying,

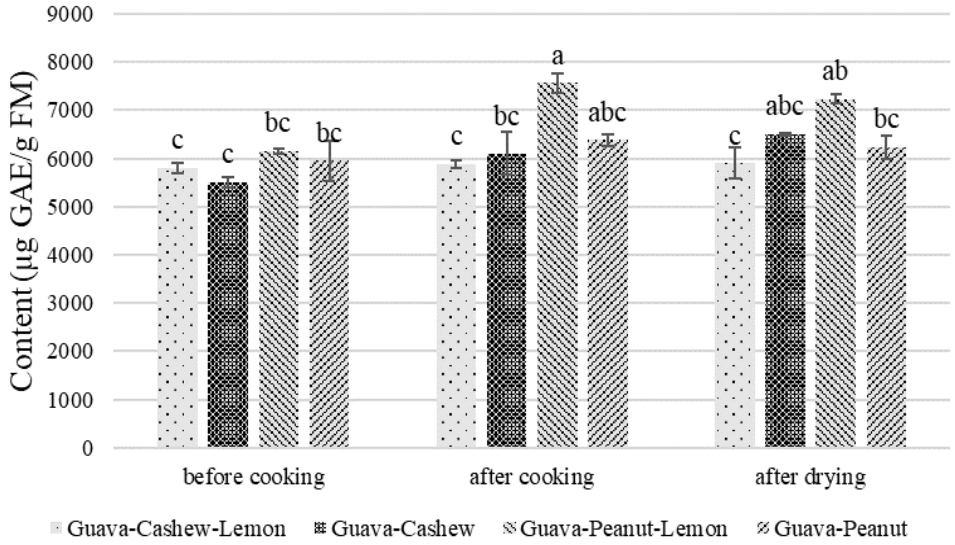

Figure 7. Total phenolic content of guava-based samples in three stages (before cooking, after cooking and after drying) (n=2). Different letters indicate statistically significant differences (P < 0.05).

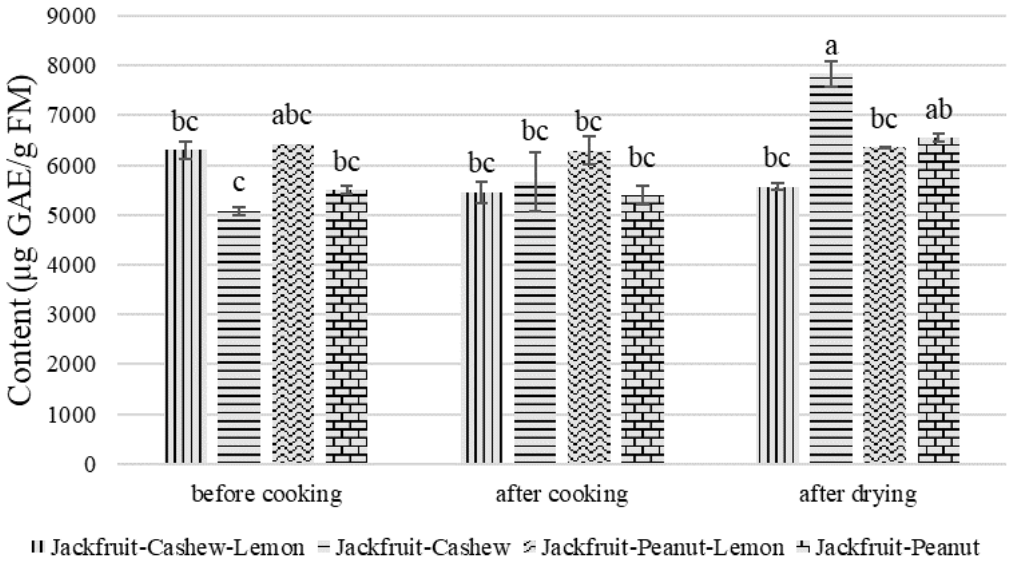

Figure 8. Total phenolic content of jackfruit-based samples in three stages (before cooking, after cooking and after drying) (n=2). Different letters indicate statistically significant differences (P < 0.05).

while samples without lemon juice showed the opposite results.

As in pre-test, β-carotene contents of dried samples were hardly measurable with HPLC, for final tests, only samples before and after cooking were measured (Table 6). Guava-based snacks showed higher values than jackfruit, Guava-Cashew samples before cooking and Guava-Peanut samples after cooking showed the highest value. Jackfruit-based snacks with lemon juice after cooking had higher β-carotene content.

Mineral content

The mineral contents of the fruit-nut-bars are shown in Tables 7 and 8. There are nine minerals in fruit-nut-bars, namely K, P, S, Mg, Ca, Na, Cu, Fe, Zn and Mn. In general, jackfruit-based samples had a little higher mineral content than guava-based samples. Except for K, Mg and Mn in specific guava-based samples, the mineral content among other samples were not significantly different (P<0.05).

Table 6. β-carotene content in guava- and jackfruit-based samples before and after cooking (n=3, mean ± SD).

Sample	β-carotene (mg/100 g DM)	
	Before cooking	After cooking
Guava-cashew-lemon	0.19±0.02^{ab}	0.16±0.01^{bc}
Guava-cashew	0.24±0.00^{a}	0.23±0.04^{a}
Guava-peanut-lemon	0.22±0.00^{a}	0.21±0.00^{a}
Guava-peanut	0.19±0.00^{ab}	0.24±0.01^{a}
Jackfruit-cashew-lemon	0.13±0.01^{cd}	0.22±0.01^{a}
Jackfruit-cashew	0.07±0.00^{ef}	0.09±0.01^{de}
Jackfruit-peanut-lemon	0.14±0.01^{cd}	0.16±0.03^{bc}
Jackfruit-peanut	0.04±0.00^{f}	0.05±0.01^{ef}

Different letters in the same column indicate statistically significant differences (P < 0.05).

Table 7. Mineral content of guava-based products after drying (n=5, mean ± SD).

Mineral (mg/100 g DM)	Guava-cashew-lemon	Guava-cashew	Guava-peanut-lemon	Guava-peanut
K	1150.25±22.26^{a}	1014.37±57.75^{b}	1099.12±47.23^{a}	1093.60±44.09^{a}
P	428.69±83.63^{a}	416.58±70.23^{a}	396.81±73.19^{a}	413.89±87.96^{a}
S	303.41±200.06^{a}	188.45±30.32^{a}	207.91±26.37^{a}	221.74±68.23^{a}
Mg	181.39±1.90^{a}	176.04±4.63^{a}	152.10±4.43^{c}	164.48±8.90^{b}
Ca	79.96±9.20^{a}	66.40±2.90^{a}	81.67±26.39^{a}	65.56±4.32^{a}
Na	45.90±28.79^{a}	45.85±33.23^{a}	48.17±28.19^{a}	51.10±26.92^{a}
Cu	7.48±3.75^{a}	5.62±2.00^{a}	8.67±4.43^{a}	5.78±3.40^{a}
Fe	6.50±3.83^{a}	4.07±2.52^{a}	4.60±1.91^{a}	4.42±2.25^{a}
Zn	6.71±3.78^{a}	7.39±4.66^{a}	5.58±1.52^{a}	4.75±1.59^{a}
Mn	2.33±0.07^{a}	2.14±0.04^{b}	1.34±0.01^{d}	1.62±0.08^{c}

Different letters in the same row indicate statistically significant differences (P < 0.05).

Table 8. Mineral content of jackfruit-based products after drying (n=5, mean ± SD).

Mineral (mg/100 g DM)	Jackfruit-cashew-lemon	Jackfruit-cashew	Jackfruit-peanut-lemon	Jackfruit-peanut
K	1227.56±47.01^{a}	1318.95±113.51^{a}	1214.03±150.30^{a}	1408.01±93.35^{a}
P	378.44±81.78^{a}	390.72.19±90.20^{a}	358.52±89.74^{a}	390.32±71.18^{a}
S	167.23±26.53^{a}	359.61±299.63^{a}	211.00±72.11^{a}	200.39±58.99^{a}
Mg	178.84±14.69^{a}	191.26±7.55^{a}	174.73±13.18^{a}	186.15±9.51^{a}
Ca	96.78±20.97^{a}	82.41±10.18^{a}	80.05±10.18^{a}	85.74±8.00^{a}
Na	43.69±28.68^{a}	44.75±28.90^{a}	40.72±26.69^{a}	49.03±26.89^{a}
Cu	7.99±2.94^{a}	7.36±3.31^{a}	5.91±2.89^{a}	8.04±2.89^{a}
Fe	5.17±2.42^{a}	7.33±2.64^{a}	5.20±2.70^{a}	5.60±2.44^{a}
Zn	6.79±3.31^{a}	5.06±2.32^{a}	6.86±5.59^{a}	6.64±3.86^{a}
Mn	2.36±0.21^{a}	2.58±0.23^{a}	2.32±0.19^{a}	2.29±0.08^{a}

Different letters in the same row indicate statistically significant differences (P < 0.05).

Textural profile analysis

Textural properties of guava- and jackfruit-based products after drying are shown in Table 9. For jackfruit-based samples, there were significant differences (P < 0.05) in the peak distance reached by the probe due to the thickness of the samples. The force to break (max Peak Force) each of the four products was not significantly

Table 9. Texture analysis results of guava- and jackfruit-based samples after drying.

Sample	n	Max peak force (N) Mean±SD	Distance (mm) Mean±SD
Guava-cashew-lemon	6	249.65±145.85[a]	10.00±0.00[a]
Guava-cashew	5	258.98±64.57[a]	10.00±0.00[a]
Guava-peanut-lemon	5	235.17±145.08[a]	10.00±0.00[a]
Guava-peanut	5	168.72±83.54[a]	10.00±0.00[a]
Jackfruit-cashew-lemon	6	203.38±196.86[a]	11.95±0.12[a]
Jackfruit-cashew	7	263.51±220.26[a]	11.38±0.49[a]
Jackfruit-peanut-lemon	5	21.31±7.55[a]	7.00±2.24[b]
Jackfruit-peanut	6	101.40±63.98[a]	10.17±2.04[a]

Different letters indicate statistically significant differences ($P < 0.05$).

different ($P < 0.05$). Appendix Figures 1 and 2 show the curves of hardness and crispness parameters of the investigated samples.

DISCUSSION

The availability of fruits during off-season in order to increase fruit consumption is crucial for balanced and healthy diets. One solution to bridge seasonal gaps can be the processing of fruits into dried fruit-nut-bars which was tested in this study. Through physical and chemical analyses of different fruit-nut-bars combinations based on either jackfruit or guava, we have found partly very different and also promising results in terms of nutrient content.

Sensory test

The aim of this preliminary test at the beginning of the product development was to find out which fruit combinations might most closely match the sensory expectations of the East African consumers and which might not. The sensory test used in this study can be defined as affective testing, attempting to quantify the degree of liking or disliking of a product (Stone et al., 2012). On average, for both guava- and jackfruit-based bars, recipes with mango were relatively more popular than recipes with banana. As a result of this sensory test, banana was no longer used in the final recipes, whereas fruit bars with desiccated coconut were also characterized by a higher acceptability, which is the reason that all final recipes contained desiccated coconut. Higher concentration of lemon juice in jackfruit-based bars gained more acceptability than guava-based bars. However, for guava-based bars, recipes without lemon juice were more popular than those with lemon juice. So in final recipes, lemon juice was added as an alternative choice.

Jafari et al. (2016) scored sensory properties of dried kiwifruit samples, which were dried by two different drying methods, oven drying and refractance window (RW) drying, respectively. Results showed that oven dried samples gained less acceptibility than refractance window dried ones, and the main reason was the changed organoleptic properties. RW drying was also found to preserve more nutrients in fruits and vegetables and to be favourable in terms of color and texture as compared to other drying techniques (Shende and Datta, 2019). When taken into practice, it should be considered to dry products from this study by RW drying when produced in East Africa as it is already tested by other projects in Uganda and Kenya (icipe, n.d.).

Water content, total soluble solids, titratable acidity and color

Samples after cooking were put into the oven until the products became totally dry, so the water content of the dried products was around 10%, which is highly important for the microbiological safety (Samotyja, 2015). Drying time of 19 h was quite long and would need a considerable amount of energy. In order to reduce the drying time, less water than the 50% of fruit-nut-mass which was added during the cooking process could be admixed.

Total soluble solids are determined by a refractometric index of the proportion (%) of dissolved solids in a solution, which not only indicates the content of sugars (sucrose and hexoses; 65%), but also acids (citrate and malate; 13%) and other compounds (polyphenolics, amino acids, soluble pectins, ascorbic acid and minerals) in the product (Balibrea et al., 2006; Kader, 2008). Results showed that jackfruit-based samples had more total soluble solids than guava, which according to USDA (2019, 2020), was a reliable result. In general, dried samples had low concentrations of total soluble solids (ranged from 4.0 ºBrix to 4.6 ºBrix for different recipes), when compared with the results of dried guava-orange bars (73-81ºBrix) (Srivastava et al., 2019) and guava-papaya fruit bars (30-38 ºBrix) in the research of Bisen and Ruchi (2020). These low-sugar snacks would not aggravate the diabetes situation in East Africa; however,

it could possibly influence the acceptability of consumers (Kader, 2008).

Acids influence the taste of the product (Stevens, 1972) in the way that an acid addition can minimize sweetness (Stampanoni, 1993). Acid addition and the resulting pH reduction can extend the shelf-life of the product (Ramachandran et al., 2017). Furthermore, increased ascorbic acid content may have a protective effect against discoloration (Martí et al., 2002), which is important for product development. In the present study samples with lemon juice had higher acidity content than without, as expected. Titratable acidity content in dried Guava-based samples with lemon juice was similar to the result of pure fresh guava pulp (0.704±0.09%) in the study of Srivastava et al. (2019). In dried Guava-based samples without lemon juice they also reported a similar result of a 50%:50% guava-orange bar (0.38±0.006%) (Srivastava et al., 2019). Interestingly, dried Jackfruit-Cashew samples had a higher titratable acidity content (0.705±0.02%) than dried Jackfruit-Cashew-Lemon samples (0.63±0.03%), and both results were higher than in the other two dried Jackfruit-based bars. The reason may be due to the difference of ripeness of mangos that were used in different recipes. Results measured in this study were quite similar to the results of the top portion of the fresh jackfruit in day five after harvesting (0.61±0.14%) (Ong et al., 2006). All in all, contents of titratable acidity in our fruit-nut-bars showed quite similar results when comparing with other studies, not only for mixed pulp and fresh fruit, but also for dried guava-orange bars. Titratable acidity of all dried Guava-based samples and two Jackfruit-based samples without lemon juice increased, comparing to samples before cooking, which showed the same tendency recorded by Toor and Savage (2006) and Khazaei et al. (2008). Increased titratable acidity content could have positive effects on prolonging shelf life (Zomo et al., 2015)

As for color testing, according to Rahman et al. (2020), the darker browning on the top side of the fruit-nut-bars can result from non-enzymatic ascorbic acid oxidation and enzymatic oxidation of polyphenols. In this study, products before drying were much brighter than dried final products, which lost the yellow-orange color. The reason for this is the degradation of β-carotene, when the temperature is higher than 22°C (Kläui and Bauernfeind, 1981; Krokida and Maroulis, 1998). In the final products, the bottom side was brighter than the top side, because the bottom side was protected by the baking mat from direct heating. This uneven discoloration could possibly affect consumer acceptance, the dark brown color at the top side of products may not be appealing to them. In further studies, more attention should be paid to the protection of the product surface.

Ascorbic acid, total phenolic content and β-carotene

In this study, the ascorbic acid content of the Guava-Cashew-Lemon bars before cooking was 81.19±0.37 mg/100 g FM, which was similar to the value of 60:40% guava-orange bar reported by Srivastava et al. (2019). However, for Guava-Cashew-Lemon and Guava-Cashew samples after cooking, the results were less than 50 mg/100 g FM, similar to the result of 40:60% guava-orange fruit bar of Srivastava et al. (2019). This implies that the effect of cooking on the ascorbic acid content is similar to the effect of reducing the share of guava. Vice versa, an increased ratio of guava may lead to an increased content of ascorbic acid. The jackfruit-based bars showed much lower contents than guava-based bars. According to Shwetha and Ranganna (2016), different genotypes of jackfruit could also result in different levels of ascorbic acid, which could range from 3.57 to 5.00 mg/100 g FM. Comparing results of products in this study, lemon juice led to relatively higher ascorbic acid content.

Cooking led to nearly 30% loss of ascorbic acid in guava-based bars, compared with the value before cooking. Afterwards samples were dried under 70ºC to 90ºC for 23 to 19 h, respectively. According to Siow and Hui (2013), ascorbic acid content in guava slices dried by convection for 9 hours at 40ºC decreased 27% compared to fresh fruits. Assuming that in this study ascorbic acid content in guava-based bars would decrease by 54% during the drying process; nearly 20 mg/100 g FM ascorbic acid would still be contained in the final samples. This could be beneficial to children as well as other population groups in East Africa when comparing with the recommended daily allowance. In order to avoid some diseases such as cardiovascular risks and cataract, 110 mg ascorbic acid per day for an adult is recommended (Fain, 2004). For teenagers at the age of 9-13 years old, the recommended dietary allowances (RDAs) of ascorbic acid are 45 mg per day (Institute of Medicine, 1998). Thus, a guava-nut-bar of 100 g could provide about half of the daily requirement of vitamin C for 9-13 years old children.

Phenolic contents in guava samples with and without lemon juice before cooking showed no significant differences. The lowest result of guava-based-bars in this study (550.4±9.9 mg GAE/100 g FM) was higher than the results in the experiment of Patel et al. (2016), although in the current study only white fleshed guava was used. As Chiveu et al. (2019) has tested, phenolic content for red fleshed guava samples were higher than white fleshed ones. Consequently, products produced by red-fleshed guava in East Africa may contain more phenolics than products in this study, which is desirable as phenolic secondary metabolites are, attributed protective effects against different non-communicable diseases (Crozier et al., 2008). Jackfruit-based samples in this study showed higher values than the freeze-dried jackfruit chips measured by Yi et al. (2016) and jackfruit pulp measured by Shafiq et al. (2017). Overall, dried products of this study showed higher phenolic content than dried single

fresh fruit (guava and jackfruit) in other studies. One reason may be the use of different cultivars and the addition of mango with high phenolic content. In the experiment of Ongphimai et al. (2013), mango showed a result of 6646 mg/100 g DM of insoluble phenolic acids and 37 mg/100 g DM of soluble phenolic acids. In general, high phenolic content of products can have protective effects against diabetes, hypertension and cardiovascular disease (Liu et al., 2008; Sun et al., 2002; Visioli and Davalos, 2011; Yi et al., 2005).

According to the results of β-carotene, it can be seen that in samples of guava, almost all samples before cooking had higher content than samples after cooking, which can be attributed to the heat sensitivity of β-carotene. The contents of β-carotene in guava-based samples before drying were less than in the studies of Nwaichi et al. (2015) (0.38 mg/100 g), Leiton-Ramírez et al. (2020) (0.85 mg/100 g dry basis in fresh pink fruits), and Nora et al. (2014) (0.51 mg/100 g dry fruit in red guava). Since β-carotene is lipid-soluble (Palan et al., 1994), peanuts and cashew nuts increased the fat content, which may lead to loss of β-carotene during measurement. After the 5-h-evaporation, there was still lipid inside the reaction tube that was not completely dissolved in MeOH/THF (1:1, v/v). As with the jackfruit samples, the samples with lemon juice in the same processing step showed a higher content of β-carotene than without, which can be explained by the protection effect caused by ascorbic acid, since β-carotene is sensitive to oxygen (Goldman et al., 1983). Result from Ahiduzzaman (2016) was lower than the most values from this study (except Jackfruit-Peanut samples), which might be due to the high content of β-carotene in mango (Godoy and Rodriguez-Amaya, 1989; Mercadante et al., 1997). An average content of 1105 µg of β-carotene per 100 g of ripe mango is given in the food composition table of Kenya (FAO/GOK, 2018). As mango is also highly perishable and seasonal, processing in combination with our key fruits would make a further contribution to prevent food losses and at the same time enhance the nutritional content of the new products.

According to the Institute of Medicine in the USA (2001), β-carotene is one kind of provitamin A, which must be transferred into retinol after absorption in the small intestine. The Recommended Dietary Allowance (RDA) of vitamin A and preformed vitamin A depicted as the form of retinol activity equivalents (RAE) per day, is 445 and 420 µg RAE per day for boys and girls, respectively, 9-13 years old (Institute of Medicine, 2011). Besides, the retinol activity equivalency (µg RAE) ratio for β-carotene from plant sourced food is estimated to be 12:1 (Debelo et al., 2017), which means that every 100 g of the fruit-nut-bars could provide between 3.3 and 20 µg RAE. Overall, products in this study can provide more β-carotene than single fresh guava or jackfruit but less than fresh mango, which can still be regarded as a reasonably good source of β-carotene for 9-13 years old children.

Mineral content

The fruit-nut-bars contained significantly higher amounts of minerals than single fruits. Taking K as an example, guava-based bars ranged from 934-1034 mg/100 g FW, which was higher than values measured by Chiveu et al. (2019) in freeze dried guava samples and values from USDA (2018) in fresh guava. This is due to the high K content of peanuts and cashew nuts (Settaluri et al., 2012; Rico et al., 2016). Comparing results of jackfruit pulp gained from Ojwang et al. (2018), K was double as high as results of jackfruit-based bars in this study; Zn showed almost the same result. On the contrary, Mg was two times less than results of jackfruit-based bars in this study. The difference between results of other literature and fruit-nut-bars in this study may be due to different fruit species in different countries that were used for the experiment (Abedin et al., 2012).

According to the Institute of Medicine in the USA (2011), for teenagers at the age of 9-13 years, the recommended dietary allowances (RDAs) of Fe and Zn are 8 mg each per day. Products in this study contained Fe and Zn from 4 to 7 mg / 100 g DM. Overall, both guava- and jackfruit-based bars of this study combining fruits and nuts can provide considerable amounts of minerals, which could contribute to a balanced diet of local people in East Africa.

Textural profile analysis

From these results, it is quite obvious that each sample had different texture behaviour, which is connected with the drying condition. Texture tests were performed to examine the maximum chewing force for customers to break these final products (expressed as max Peak Force). When the moisture content was less, the bars were quite easy (less force was needed) to be broken. Conversely, samples with higher moisture content were not as easy to break, which may be due to the less crispness (Yi et al., 2016). However, Vijayanand et al. (2000) showed an opposite result that guava leathers with higher moisture content had lower hardness.

On average, the force for biting through the jackfruit-based bars (max Peak Force) was smaller than that of guava-based bars. According to Yi et al. (2016) using a ball probe (p/0.25S), pear chips showed a result of 32.5 N maximum peak force which was similar to values in the pre-test of guava-based bars in this study (33.86 N). After hot air-drying method, pear chips showed the largest value of 59.9 N maximum peak force (Yi et al., 2016), which was considered as a hard structure. In our investigation, most values of hardness (max Peak Force) were similar or even higher than the study of Vijayanand et al. (2000), namely more than 160 N with 7% equilibrium moisture content (EMC). They reported for guava-bars higher EMC (15.4%) and lower hardness

(94.2 N) (Vijayanand et al., 2000). Overall, almost all final samples in this study could be defined as having a very hard structure (except Jackfruit-Peanut-Lemon samples). Moreover, although mouth feel properties are not necessarily related to the force of breakdown, attributes like stickiness and viscosity are still related to it (Stone et al., 2012). So, the value of max Peak Force also showed a relative sticky texture of the final products, which was also seen in the texture test; some products even stuck to the cylinder probe after testing.

Crispness of the fruit-nut-bars was characterized by the number of peaks (Appendix Figures 1 and 2). In the study of Yi et al. (2016), pear chips with more compression peaks had crisper textures. Our results showed the same tendency, namely Jackfruit-Peanut-Lemon samples exhibited more than hundred peaks and had the highest crispness. At the same time, the maximum Peak Force of Jackfruit-Peanut-Lemon samples were the lowest among the investigated samples. Each sample showed a different texture, with almost all guava-based fruit-nut-bars showing smooth curves with few compression peaks, indicating less crispness and a harder texture.

As the acceptance between hard and softer products can vary to a great extent, it is important to understand the preferences of consumers. Bower and Whitten (2000) found a negative correlation between the force of chewing and the acceptance of cereal bars, meaning that increased chewing effort can lead to a decrease of liking. However, different customers have different preferences, and cultural background and age will play an additional role in the acceptance of a product (Köster, 2009). According to the sensory test in this study as tested with eleven East African participants, results showed a relatively moderate point (neither like nor dislike) of the overall acceptability. However, this small number of adult participants cannot be representative of local residents in East Africa, yet, show an acceptability trend. Further analysis of texture acceptability should be done in order to know more about the preference of the local population. The drying time can also be reduced to achieve a relatively higher moisture content (15%), so that the fruit-nut-bars are less hard to the bite, which can lead to better acceptability (Vijayanand et al., 2000).

Conclusion

In this study, eight different fruit-nut-bars based on two main fruits, guava and jackfruit were produced and tested.

The bottom side of products had brighter color than the upper side and bars based on jackfruit showed brighter color than guava, which might have an influence on consumer acceptance. Texture showed quite different results for each piece of bars, which was also influenced by moisture content of the oven during drying procedure and, thus, will vary depending on the production facilities. Ascorbic acid content increased with the addition of lemon juice and can possibly contribute to a longer shelf-life.

Total soluble solids showed an inverse result, which also indicates that fruit-nut-bars are “low-sugar-snacks”. Total phenolic content showed higher values than single fruits in other literatures, which can provide protective effects against several non-communicable diseases. Mineral contents in 100 g DM of fruit-nut-bars such as Fe and Zn could provide sufficient amounts for teenagers aged 9 to 13 years and to combine fruits and nuts seems to be advantageous. The content of β-carotene in samples before drying was higher than in fruit pulp of single fresh guava or jackfruit, however, data of dried product were not obtained and will be much lower because of the heat sensitivity of β-carotene.

Overall, this study provides a simple method of processing local fruits. It is shown that guava- and jackfruit-based fruit-nut-bars can prolong the shelf life of fruits, so that they can be also consumed during off-season when fresh fruits are either not available or very expensive. The bars provide essential nutrients such as ascorbic acid and mineral elements. When producing guava-based bars with different flesh color, the investigated ingredients may also show different values, for example, higher β-carotene values when pink fleshed guavas are used. In conclusion, these products can provide a nutrient rich and low-sugar snack especially during off-season and contribute to a diverse and balanced diet of local communities in East Africa.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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APPENDICES

Table 1. Sensory test of fruit bars.

You are provided with six coded samples of fruit bars. Taste the samples and indicate how much you like or dislike the samples against the tasted attribute using a 5-point hedonic scale as follow: 1 = Dislike very much; 2 = Dislike moderately; 3= Neither like nor dislike; 4 = Like moderately; 5 = Like very much						
Attribute	**Sample code**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Colour						
Consistency						
Mouthfeel						
Taste						
Aroma						
Overall acceptability						
Comments:……						
Age: …… Sex: ……						

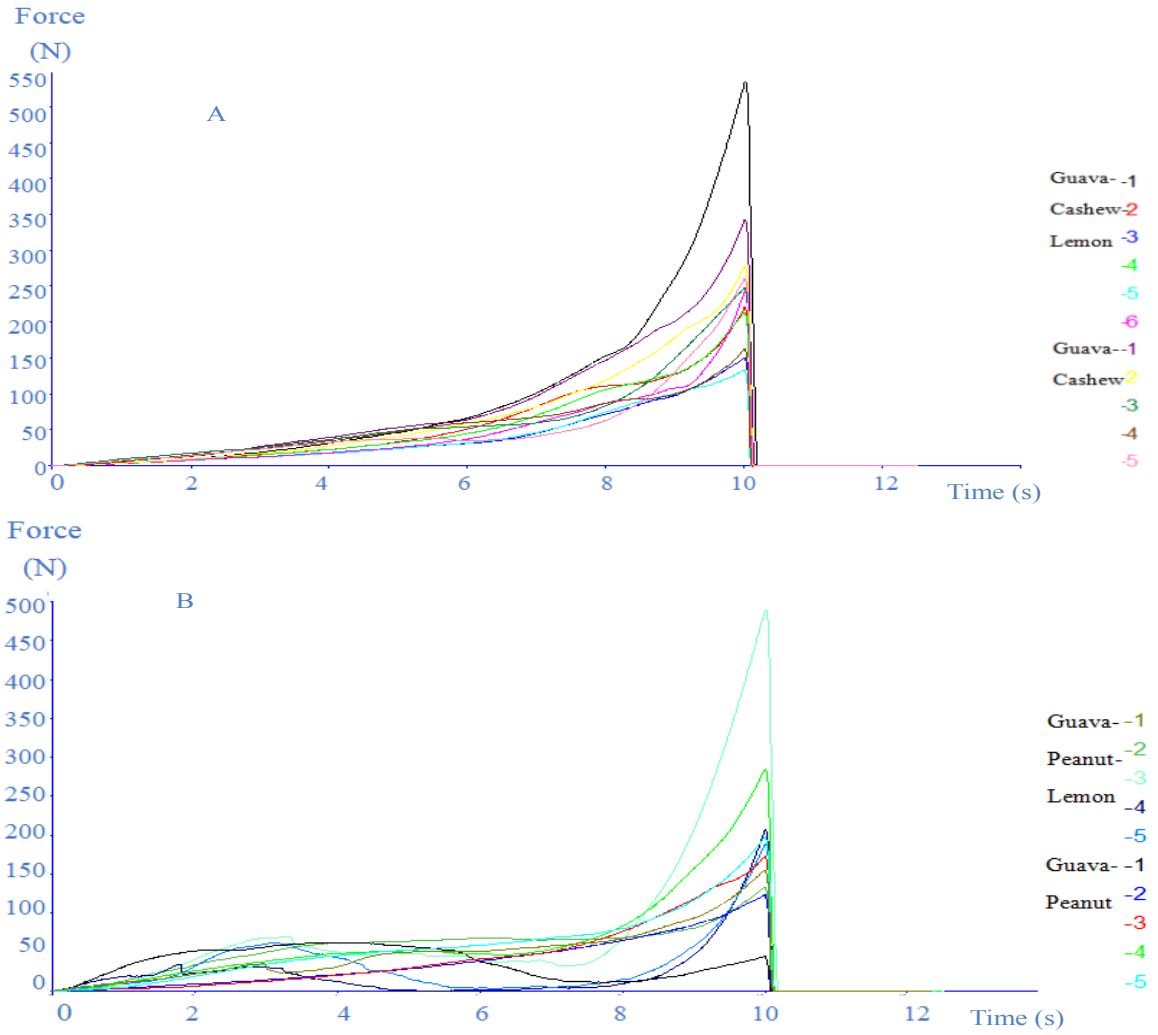

Figure 1. Hardness and crispness parameters of guava-based fruit-nut-bars A: Guava-Cashew-Lemon and Guava-Cashew; B: Guava-Peanut-Lemon and Guava Peanut.

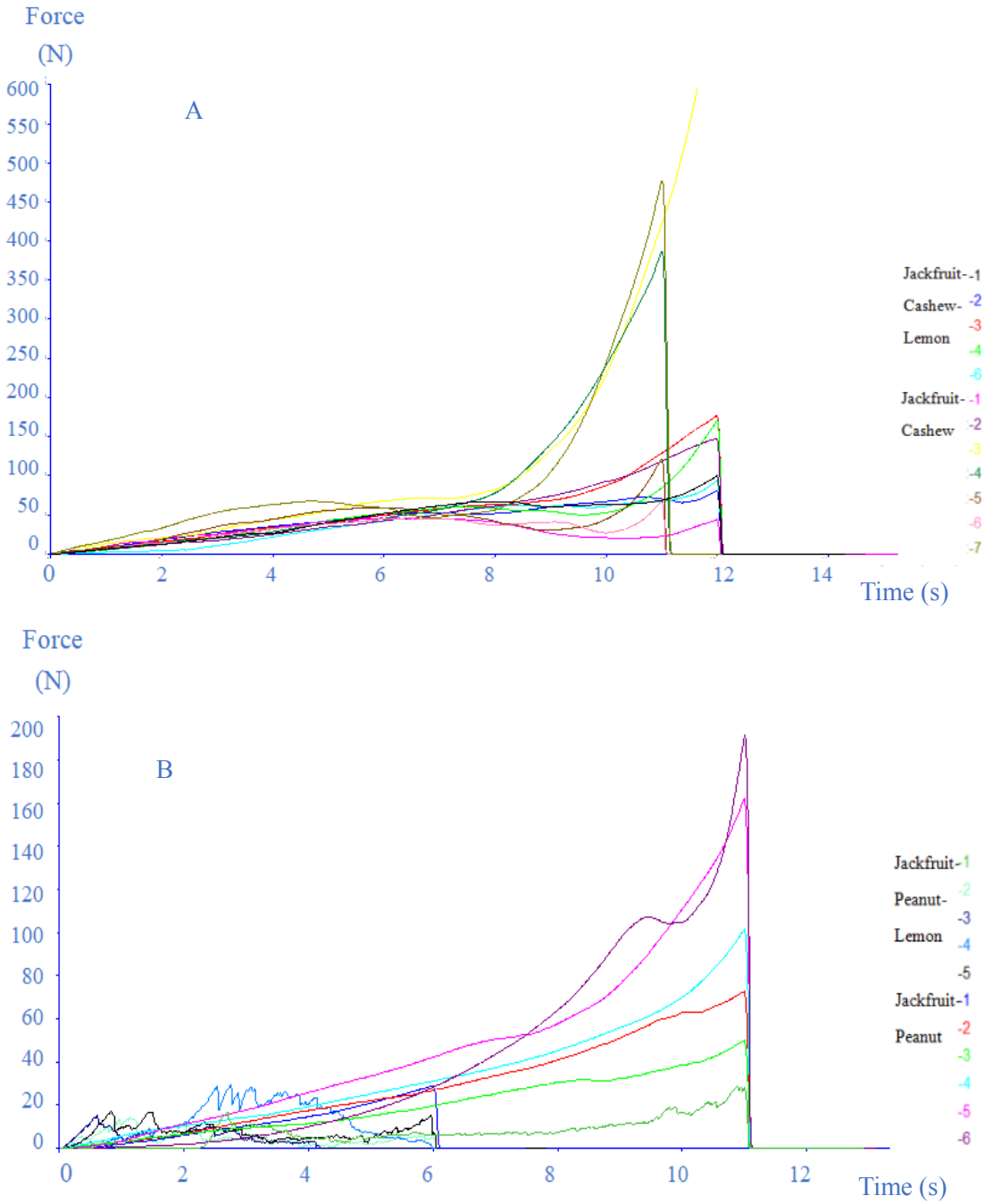

Figure 2. Hardness and crispness parameters of jackfruit-based fruit-nut-bars A: Jackfruit-Cashew-Lemon and Jackfruit-Cashew; B: Jackfruit-Peanut-Lemon and Jackfruit Peanut.

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Phenotypic and genotypic characterization of lactic acid bacteria isolated from spontaneously fermented vegetable amaranth

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Lactic acid bacteria (LAB) are Gram-positive, non-spore-forming, catalase-negative cocci or rod-shaped bacteria that produce lactic acid as a major fermentation product. They are also involved in the production of fermented foods. They have applications in industry and human health, such as food preservation and probiotics. The aim of this research was to isolate, characterize, and classify indigenous lactic acid bacteria from fermented vegetable amaranth, a leafy vegetable native to Africa. The isolates' 16S rRNA gene was amplified using bacterial universal primers 27F and 1492R. From fermented vegetable amaranth, a total of 15 LAB were isolated were grouped into the genera *Lactobacillus*, *Lactococcus*, and *Weissella* based on 16S rRNA gene analyses. *Lactobacillus plantarum* dominated vegetable amaranth fermentation, accounting for 60% of all isolates.

Key words: Lactic acid bacteria, vegetable amaranth, fermentation, 16S rRNA.

INTRODUCTION

Fermentation is one of the oldest food processing techniques. It is a technology that millions of people in the developing world depend on to preserve their food at prices that are affordable to the average consumer (Kalui et al., 2010). Fermented food items are a subset of foods that are distinguished by various carbohydrate breakdowns in the presence of probiotic microorganisms (Mulaw et al., 2019). Africa has a wide variety of fermented foods which include plant-based products from maize, sorghum, millet and cassava (Franz et al., 2014). It has been suggested that vegetables are a good source of lactic acid bacteria growth (Wu et al., 2020). Vegetables also have high contents of vitamins, minerals, dietary fiber, and antioxidant compounds (Wu et al., 2020).

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Fermented foods are now known not only as a source of nutrients, but also as functional foods that offer health benefits against food-borne illnesses in addition to their nutritional value (Mulaw et al., 2019). Fermented vegetables contain a variety of microorganisms, including LAB, which produce lactic acid and, in rare cases, bacteriocins, which are essential for food preservation (Viridiana et al., 2018). In recent years, the probiotic potential of LAB isolated from fermented vegetable has been investigated (Viridiana et al., 2018). The microorganisms present during vegetable fermentation are very diverse and may considerably affect the quality and safety of the final product (Bautista-Gallego et al., 2020). The microbiota that is initially present in lactic fermentation processes comes primarily from the material, though other factors such as brines, ingredients used, and the industry's environment have an effect (Bautista-Gallego et al., 2020).

Lactic acid bacteria are a group of Gram-positive, aerotolerant, acid-tolerant, generally non-sporulating rod or cocci organisms that play an important role in food fermentation by inhibiting pathogenic microorganisms (Anjum et al., 2014). Lactic acid bacteria are present in a wide range of environments, including plant materials, various animal products, human and animal gastrointestinal and urogenital tracts, soil, and water (Ruiz Rodríguez et al., 2019). Lactic acid bacteria (LAB) use the Embden Meyerhof Parnas (EMP) pathway to ferment sugars and generate lactic acid as a final product (Anjum et al., 2014). Homofermentative lactic acid bacteria produce only lactic acid as a major product of fermentation, whereas heterofermentative lactic acid bacteria produce CO_2, hydrogen peroxide, acetic acid, and alcohol in addition to lactic acid (Anjum et al., 2014). Organic acids and other low molecular weight substances produced by these microorganisms have been shown to improve food nutritional, sensory, technical, as well as protection and shelf-life properties (Oguntoyinbo et al., 2016a). Due to their versatile metabolism, LAB has been widely used as starter cultures and probiotics for these purposes (Naeem et al., 2012; Ruiz Rodríguez et al., 2019). Heterofermentative microorganisms usually perform the first steps of vegetable fermentation, producing lactic and acetic acids, which contribute significantly to the final product's flavor and aroma (Breidt et al., 2013). Then, because of their ability to produce lactic acid, which causes a greater decrease in pH but prevents the growth of other microbial classes, they are replaced by more acid-tolerant homofermentative microorganisms (Montet et al., 2014).

Amaranths are a diverse group of food crops with some grown as edible grains and others as edible leaves (Achigan-Dako et al., 2014). Amaranth belongs to the family Amaranthaceae (Zehring et al., 2015). Vegetable amaranth is one of the most widely consumed vegetables in Asia and Africa, and it plays a significant role in the supply of essential proteins and minerals (Achigan-Dako et al., 2014). The consumption of amaranth as a vegetable is mainly exclusive in Africa and Asia, whereas the grain is popular all over the world (Zehring et al., 2015). Amaranth leaves contain secondary plant metabolites such as saponins, flavonoids, betalains, tannins, etc. (Zehring et al., 2015).

Smallholder farmers in rural and peri-urban areas in Africa depend heavily on indigenous leafy vegetables for food protection. Post-harvest losses, on the other hand, may be as high as 50% due to poor production conditions (Abukutsa-Onyango, 2007). Although fermentation of vegetables, particularly cabbage, is common in Europe, fermentation of leafy indigenous vegetables is not yet common in Africa (Oguntoyinbo et al., 2016a). As a result, there appears to be a case for stepping up efforts in Africa to research and introduce this form of biological preservation system for leafy vegetables (Oguntoyinbo et al., 2016a). As a result, spontaneous fermentation was produced and monitored in this study to identify the LAB that enable fermentation and can be used as a viable approach for the preservation and enhancement of the protection of African leafy vegetables.

MATERIALS AND METHODS

Sampling and preparation of sampling materials

Amaranthus dubius was obtained from Agrifood Organics, Ruiru, Kenya. The plant was cultivated for six to eight weeks (22-30°C). Hand-picking was used to harvest the vegetables, which were then shipped to the laboratory for processing. The leaves were destemmed, washed with tap water, and air-dried with paper towels on a clean and sterilized work bench.

Fermentation of vegetable amaranth

Fermentation was performed in 5-L stainless steel buckets. 1 kg of leaves and 3 L of salt and sugar solution were used. The solution consisted of a combination of salt and sugar, 3.0% each. Common table salt and retail sugar were purchased at local stores in Kenya. These components were sterilized by autoclaving for 15 min at 121°C. Weights were used to hold all plant material below the surface of the liquid. Inoculation and sampling were done in a sterile setting. The fermentations were done in duplicates at a constant temperature of 25°C (Stoll et al., 2021).

Sampling and analysis of fermentation brine

Samples were taken and analyzed for pH and microbial counts at 0, 24, 48, 72, and 144 h on MRS and M17 agar plates. The microbial diversity was investigated using amplicon sequencing of the 16S rRNA gene. 1 ml samples from different stages of vegetable amaranth fermentation brine were added to 9 ml quarter-strength Ringer's solution and vortexed to isolate LAB. To obtain the typical LAB associated with fermenting vegetable amaranth, the samples were further diluted in a 10-fold dilution sequence and 10 µl aliquots were spread-plated onto MRS agar (De Man, Rogosa, and Sharpe, M641) and M17 (M929) agar. Under aerobic conditions, plates were incubated at 30°C for 24-48 h (Stoll et al., 2021). For further characterization, colonies were selected at random from the highest

dilution agar plates. The strains were then grown aerobically in MRS broth at 30°C and streaked to ensure purity. All media were purchased at Himedia (Mumbai, India). The isolates stock cultures were stored in MRS broth with 20% glycerol at -80°C (Abdou et al., 2018). The isolates were divided into groups based on phenotypic and biochemical characteristics, and their identity was confirmed through 16S rRNA gene sequencing.

Phenotypic characterization

Presumptive lactic acid bacteria were phenotypically identified using phase-contrast microscopy at 100x magnification (Shimadzu CX41, Japan), as well as standardized tests including catalase activity, gas output from glucose in MRS broth, growth at different NaCl concentrations (4 and 6.5%), and growth at different temperatures (15 and 45°C).

Determination of cell morphology and Gram status

Overnight cultures were introduced on microscopic slides and visualised under a light microscope at 100x magnification. Gram status was determined using 3% KOH as described by Mulaw et al. (2019).

Catalase test

Overnight cultures were introduced on a microscopic glass slide and two drops of 3% hydrogen peroxide added and thoroughly blend as described by Mulaw et al. (2019). The development of gas bubbles during a positive catalase test indicates that the test bacterium is producing catalase enzyme. The absence of gas bubbles indicates a negative catalase test.

Gas production from glucose fermentation

The aim of this test was to determine the homofermentative and heterofermentative properties of LAB isolates. Inverted Durham tubes with 1% glucose were used to measure CO_2 output from glucose in modified MRS broth. Separately, 50 µl LAB culture was inoculated with 9 ml MRS broth in separate tubes containing 1% glucose and inverted Durham tubes. The test tubes were then incubated for 5 days at 30°C. The presence of gas bubbles in Durham tubes over the course of 5 days indicated that the isolates produced CO_2 from glucose fermentation (Mulaw et al., 2019).

Growth at different temperatures

Growth at 15 and 45°C are the most frequently used for the classification of bacilli. To determine the growth at given temperatures, the MRS broth was used. 50 µl of overnight cultures were inoculated into 9-ml test media, incubated at 15 and 45°C and observed for five days for color and growth. Negative control was set using a 9-ml test tube containing the broth without inoculating with the LAB cultures (Mulaw et al., 2019).

Growth at different NaCl concentrations

The resistance of LAB isolates to various NaCl concentrations was tested. For this purpose, 4 and 6.5 % NaCl concentrations were used for testing. Test tubes containing 5 ml of modified MRS broth containing bromocresol purple indicator were prepared according to the concentrations needed and were inoculated separately with 50 µl of each overnight culture. The Test tubes were then incubated at 30°C for 5 days (Mulaw et al., 2019).

Genotypic characterization

Genomic DNA was extracted from overnight cell cultures grown in MRS broth using Quick-DNA^{TM} Fungal/Bacterial Miniprep Kit (Zymo Research, USA) according to the manufacturer's protocols. The final DNA concentration and purification were determined using a NanoDrop spectrophotometer (PCR^{max} Lambda, Staffordshire, United Kingdom) and DNA quality was checked by 1% agarose gel electrophoresis. PCR amplification of 16S rRNA gene for presumptive LAB strains was done using bacterial universal primers 27 F:5`-AGA GTT TGA TCC TGG CTC AG-3` and 1492 R:5` GGT TAC CTT GTT ACG ACT T-3`. PCR was performed in a 50-µl reaction containing 25 µl One Taq® 2X Master Mix with standard buffer (New England Biolabs), 1 µl forward primer, 1 µl reverse primer, and 22 µl RNase free water. Then 49 µl of the mixture was added into a sterile PCR tube, and 1 µl of gDNA was added and used as a template. The condition of amplified gene fragment: pre-denaturation of the target DNA at 96°C for 4 min followed by 30 cycles at 94°C for 1 min, primer annealing at 51.5°C for 1 min, and 30 s and primer extension at 68°C for 8 min. PCR was completed with 10 min elongation at 68°C followed by cooling to 4°C. The reactions were carried out in a thermal cycler (ProFlex PCR systems). The size of the 16S rRNA gene PCR products was confirmed by electrophoresis on a 1% (w/v) agarose gel stained with GelRed and visualized using a Uvitec Cambridge gel documentation system (Uvitec, UK). PCR products were purified using the QIAquick PCR purification Kit (Qiagen, Germany) according to the manufacturer's instructions. The purified amplicons were Sanger sequenced at Human Genomics Macrogen Europe (Macrogen Europe B.V, Amsterdam, Netherlands).

Phylogenetic analysis

The 16S rRNA gene sequences of the bacterial isolates were viewed for quality checks and edited using ChromasPro 2.1.8 software package. They were then compared with available standard sequences of bacteria lineages in the public nucleotide sequence databases in the National Center for Biotechnology Information (NCBI) using nucleotide blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find closely related bacterial 16S rRNA gene sequences. The 16S rRNA gene sequences of the isolates and those of the unknown closely related bacteria strains were aligned using MEGA (Molecular Evolutionary Genetics analysis) 7.0 software package (https://www.megasoftware.net) and phylogenetic trees were constructed using Maximum Likelihood method based on the Kimura 2-parameter model (Kimura M 1990) with MEGA (Molecular Evolutionary Genetics analysis) 7.0 software package (Kumar et al., 2016) (https://www.megasoftware.net). The tree topologies were evaluated using the bootstrap resampling method (Felsenstein, 1985) based on 1000 replicates. *Escherichia coli* was used as an outgroup.

Statistical microbial analysis

The microbial enumeration results were expressed as the mean and standard deviation of duplicate experiments of the counts and sampling sites using two-way ANOVA (GraphPad Prism version 8.4.2 software, GraphPad LLC, San Diego, California, USA), at a significance level of $p<0.05$.

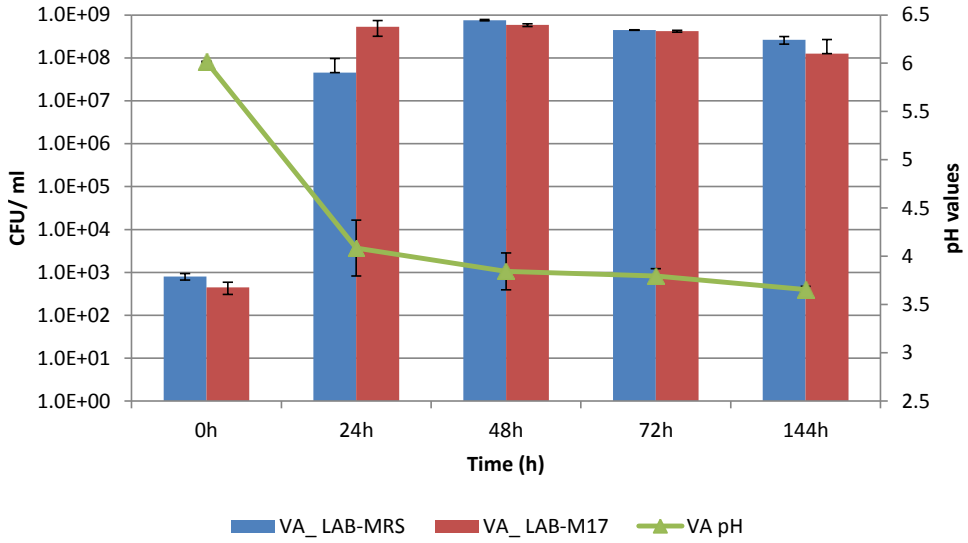

Figure 1. Mean counts and standard deviation (CFU/ml) of lactic acid bacteria of the vegetable amaranth fermentation brine on MRS (orange) and M17 (blue) agar plates. Also, the mean pH values and standard deviations are given (grey). On the basis of duplicate independent fermentations, mean counts were determined. VA stands for vegetable amaranth.

RESULTS

Selection and enumeration of presumptive lactic acid bacteria

The microbial counts varied between the sampling points (Figure 1). The highest microbial counts were recorded between 24-48h. The results also showed a decrease in microbial counts between 72-144h. The ability of LAB to acidify during fermentation is reflected in the pH production. Within 24h of fermentation, the pH had reduced significantly from a pH 6.0 to pH 4.0. Within 48 h, the pH had reduced to pH 3.8, and by the end of the fermentation, it had dropped to pH 3.6. The counts of LAB on MRS and M17 agar were log 2 CFU/ml at the beginning of the fermentation and increased to log 7 and 8 CFU/ml after 24 h of fermentation, respectively. From 24 h to the end of the fermentation, the LAB counts ranged from log 7 to log 8.9 CFU/ml, suggesting that LAB constituted the majority of the isolates on these media (Figure 1). Classic macroscopic techniques of color, type, shape, and elevation of pure colonies were used to morphologically characterize presumptive LAB. Most colonies were able to grow within 2-4 days of incubation at 30°C. Gram's reaction, form, and catalase activity of bacterial species were also investigated. All of the isolates were Gram-positive and catalase-negative. The presumptive LAB strains are shown in Table 1.

Phenotypic characterization

In total, 15 Gram-positive, catalase-negative presumptive lactic acid bacteria were isolated from fermentation brine samples using MRS and M17 agar. Eight coccus-shaped isolates and seven rod-shaped isolates were among the presumptive lactic acid bacteria.

Morphological and physiological characterization

All isolates were identified according to their morphological and physiological properties (Table 1) and were able to grow at 4% NaCl salt concentration. Only 10 were found growing at 6.5% NaCl concentration. Examining the potential of the isolates to grow at 15 and 45°C showed that all isolates were able to grow at 15°C and only 12 isolates grew at 45°C. From the 15 isolates, 8 isolates produced gas from glucose. Thus, 8 isolates were found to be heterofermentative and 7 isolates were homofermentative (Kostinek et al., 2008).

Identification and phylogenetic analysis of isolates

Isolates were blasted on the Blastn search and showed similarities with percentage identities of 98 to 100% (Figure 2). Based on the Blastn search performed against the GenBank and phylogenetic analysis, nine isolates NS489A (MF992229.1), NS442B (MF992227.1), N8481(MN420754.1), N8243 (MT613638.1), M444B (KX057551.1), M723B (MF992227.1), JK248A (MN640561.1), JK 248B (MF992227.1) and JK444A (KX649074.1) were affiliated with *Lactobacillus plantarum*, Four isolates, JK482 (MT613468.1), JK481 (MT613505.1), JK721 (MT613505.1) and JK487

Table 1. Colony morphologies and molecular identities of lactic acid bacteria strains.

Sample ID	Cell shape	Gram stain	Catalase	Closest relative	% Identity	Accession number	GC content
JK 482	Rods	+	-	*Weissella cibaria*	100	MT613468.1	46
JK 481	Rods	+	-	*Weissella cibaria*	99.63	MT613505.1	47
JK 444A	Rods	+	-	*Lactobacillus plantarum*	100	KX649074.1	52
JK 248B	Rods	+	-	*Lactobacillus plantarum*	100	MF992227.1	52
JK 248A	Rods	+	-	*Lactobacillus plantarum*	98.94	MN640561.1	51
JK 244	Cocci	+	-	*Lactococcus garvieae*	98.22	MT611574.1	51
M 723B	Rods	+	-	*Lactobacillus platarum*	99.06	MF992227.1	49
M 444B	Rods	+	-	*Lactobacillus plantarum*	99.27	KX057551.1	50
JK 721	Cocci	+	-	*Weissella cibaria*	99.82	MT613505.1	37
JK 487	Rods	+	-	*Weissella cibaria*	100	MT613505.1	50
N8 243	Rods	+	-	*Lactobacillus plantarum*	99.72	MT613638.1	48
N8 729	Cocci	+	-	*Lactococcus garvieae*	100	MT604790.1	51
N8 481	Rods	+	-	*Lactobacillus plantarum*	96.75	MN420754.1	48
NS 442B	Rods	+	-	*Lactobacillus plantarum*	98.67	MF992227.1	50
NS 489A	Rods	+	-	*Lactobacillus plantarum*	99.76	MF992229.1	48

(MT613505.1) were affiliated with *Weisella cibaria*, and two isolates, JK244 (MT611574.1) and N8729 (MT604790.1) were affiliated with *Lactococcus garvieae*.

DISCUSSION

Fermented foods have a long history in Africa and are found all over the continent. Fermentation is a popular processing method for extending shelf life, increasing micronutrient availability, improving palatability, and improving digestibility (Wafula et al., 2016).

Amaranths, or *Amaranthus* spp., are a non-grass plant belonging to the *Amaranthaceae* family. Plants in this genus are important not only as vegetable and grain crops, but also as a source of vegetable protein for dry-land agriculture (Niveyro et al., 2012). Amaranth vegetable is one of the most popular leafy vegetable in Africa (Achigan-Dako et al., 2014). In many temperate and tropical regions, amaranth is widely grown as a green, leafy vegetable and grain crop. Amaranths have C_4 photosynthesis and develop quickly in hot, dry environments. They also tolerate a wide range of unfavorable abiotic conditions, such as high salinity, acidity, or alkalinity, making them ideal for subsistence farming. Amaranth has the potential to have a positive effect on malnutrition if it is implemented (Maughan et al., 2011). Amaranths contain high contents of essential micronutrients such as calcium, iron, vitamin c, folic acid and b-carotene hence they have excellent nutritional value (Achigan-Dako et al., 2014).

Lactic acid bacteria are a group of microorganisms that produce lactic acid as a result of carbohydrate fermentation. They are normally considered micro-organisms with no pathogenic activities. They are widely used in the production of fermented dairy and non-dairy food products such as yoghurt (*Streptococcus* spp and *Lactobacillus* spp), cheese (*Lactobacillus* spp and *Lactococcus* spp), and sauerkraut (*Leuconostoc* spp) (Biosci et al., 2014). The vegetable amaranth was chosen for this study because it is an indigenous vegetable that is high in micronutrients, as well as being readily available and inexpensive. The minerals and vitamins content in AILVs are higher than those present in most exotic vegetables (Gido et al., 2017). In this study, we investigated the natural fermentation of the AILV vegetable amaranth in Kenya.

The study design was chosen to determine the dominance of lactic acid bacteria involved in the natural fermentation of this vegetable. Vegetable amaranth leaves fermented successfully in a fermentation brine containing 3% sugar and 3% salt. The aim of this study was to discover the diversity of the LAB population found in fermented vegetable amaranth. In present study, LAB counts were log 2 at the beginning of the fermentation and increased to log 7 and 8 respectively by 24h of fermentation. This was because, within the first 24h of the fermentation, the bacteria were at the exponential or log phase of growth whereby the cells are dividing and doubling in number. The LAB counts ranged from log 7 to log 8.9 from 24h of fermentation till the end of the fermentation. In line with this growth, there was a reduction in pH from pH 6.0 to pH 4.0 within the first 24h, and the pH further decreased to pH 3.6 by 144h of the fermentation. Lactic acid bacteria are acid-tolerant and produce acid and bacteriocin which reduces the pH and also inhibits the growth of pathogenic organisms (Masud and Anwaar, 2002; Oguntoyinbo et al., 2016a; Oluwajoba et al., 2013).A pH value of 4.2 or less is regarded as an important factor for food safety (Holzapfel, 2002).

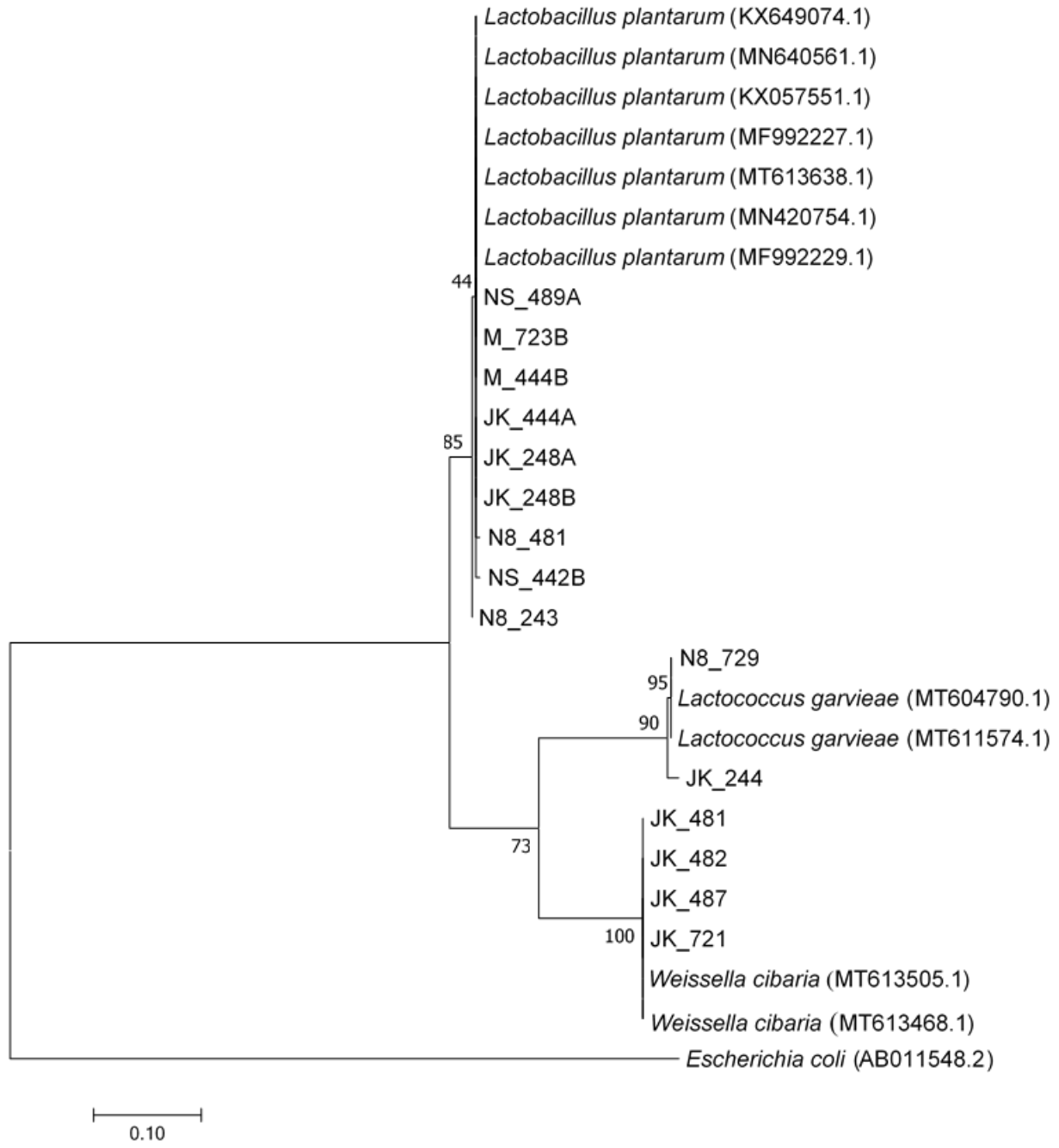

Figure 2. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship among the lactic acid bacterial isolates and between representatives of other related taxa. The scale bar indicates 0.10 substitutions per nucleotide position. The number beside the node is the statistical bootstrap value. Besides the bacterial name is the GenBank accession numbers.

Reduction of pH due to lactic acid bacteria fermentation between underneath 4.0 is critical to hinder the development of various pathogenic organisms (Oguntoyinbo et al., 2016a).

The 16S rRNA gene sequencing showed that the fermentation batches were characterized by a diverse microbiota consisting of strains belonging to the genus *Lactobacillus*, *Weissella* and *Lactococcus* (Oguntoyinbo et al., 2016a) and also found that spontaneous fermentation of African kale leaves was rather variable. The sequence data based on a constructed phylogenetic tree identified the isolates as *L. plantarum, L. garvieae,* and *W. cibaria*. Nine isolates were affiliated with *L. plantaraum.* Four isolates were affiliated with *W. cibaria*

which have been described by Kang et al. (2016) as a Gram-positive, non-pore-forming, non-motile, hetero lactic acid-fermenting, and catalase-negative bacillus that cannot produce dextran from sucrose. Two isolates were affiliated with the genus *Lactococcus* with all two isolates associated with *L. garvieae*. Some genera of LAB isolated from this work like *Lactobacillus* and *Weissella* were also isolated in the previous study of Park et al. (2010) involving the use of 16S rRNA gene sequencing analysis to identify LAB diversity from fermented kimchi (a vegetable dish in Korea). According to the work carried out by other authors, Corsetti et al. (2001), Emerenini (2013), Sánchez et al. (2000), *L. plantarum* in plant materials was dominating the LAB flora. Similar to most other studies on non-dairy fermentation, *L. plantarum* strains were the LAB most dominant in our study (60% isolates). In addition, the study of LAB throughout the fermentation period clearly showed that *L. plantarum*-group strains occurred throughout the fermentation period (Huch et al., 2008). *L. plantarum* is the most common species found in fermented vegetables, owing to its ability to withstand the high saline and acidity content of fermented vegetables such as cucumber, sauerkraut, and olives (Behera et al., 2018). The results of the present study showed strains of *Lactobacillus* species from traditional fermented vegetable amaranth is important and more recurrent in vegetable amaranth fermentation and these findings can help us to have a better fermentation of this vegetable.

Conclusion

This work provides a microbiological and molecular study of fermented vegetable amaranth. The results of this study showed which strain of lactic acid bacteria is present in the fermentation of vegetable amaranth. Lactic acid bacteria were isolated from the fermentation of vegetable amaranth. The molecular results identified the lactic acid bacteria as *L. plantarum, W. cibaria,* and *L. garvieae* with *L. plantarum* being the most abundant species present. The purpose of this study was to use 16S rRNA sequencing to profile and taxonomically identify bacteria isolated from fermented vegetable amaranth. *L. plantarum* was the most dominant preceding lactic acid bacteria followed by *W. cibaria* and finally *L. garvieae*. According to the findings of this study, fermented vegetable amaranth contains a variety of lactic acid bacteria organisms. These bacterial strains could be used in a variety of industrial and commercial settings. As a result, further research is required to evaluate the isolates' functional properties as potential probiotics and starter cultures. Specifically, the molecular and metagenomic procedures would allow for a thorough examination of the function of LAB during fermentation, as well as microbial variations during processing, geographical and varietal effects.

Ethical approval

This study did not use human or ethical subjects, and as such, ethical approval was not required.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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Full Length Research Paper

Improving shelf life of nectarine fruit (*Prunus persica*) by beeswax coating and cold storage application

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This study was carried out to determine the effect of beeswax coating and cold storage condition on the shelf life of nectarine fruit. A total of 240 nectarine fruits were collected and divided into six treatments, each with 40 fruits. The treatments consisted of coated fruits stored at 6°C (T1), coated fruits stored at 1°C (T2), coated fruits stored at ambient temperature (T3), uncoated fruits stored at 6°C (T4), uncoated fruits stored at 1°C (T5) and uncoated fruits stored at ambient temperature (T6). Physico-chemical data and sensory attributes were taken at five days interval. The results showed that there was a significant difference between treatments (p≤ 0.05) for both physico-chemical properties and sensory attributes during the storage time (50 days). The result showed that the highest percentage of titratable acidity (1.45± 0.06) and total soluble solids (15.32±0.91°Brix) as well as highest mean scores of sensory parameters such as flavor (4.23±0.06), sourness (4.12±0.07), appearance (4.49±0.05), taste (4.27±0.06), texture (4.19±0.07) and overall acceptability (4.25±0.05) were recorded for coated nectarine fruits stored at 6°C followed by coating and storage at 1°C. On the other hand, the highest weight loss percentage (25.27±3.67) and pH value (4.29±0.16) and the lowest mean scores values for sensory evaluation were recorded for uncoated fruits stored at ambient temperature (22°C). The shelf life of beeswax coated fruit stored at cold storage was extended by 50 days following the deterioration of uncoated fruit stored at ambient temperature after three days. Therefore, nectarine fruits coated with beeswax and stored at temperatures of 1 and 6°C had prolonged shelf life without affecting its nutritional quality.

Key words: Attributes, bee, coating, nectarine, physico-chemical, properties, sensory, wax.

INTRODUCTION

Despite the remarkable progress made in increased food production at the global level approximately half of the population in the third world does not have access to adequate food supplies. There are many reasons for this, one of which is food losses occurring in the postharvest and marketing system. Postharvest losses of fruits and vegetables are estimated to be 5-20% in developed countries and 20-50% in developing countries (Mashav, 2010). On farm losses occur during storage, when the crop is being stored for auto-consumption or while the farmer awaits a selling opportunity or arise in prices (Shepherd, 2012).

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Agriculture is the mainstay of Ethiopian economy and it provides all the necessary dietary foods, raw materials for food industries and quality products for export market. From a total of 39.7 million tonnes of total crops produced in Ethiopia, 23.1 million tonnes are not highly perishable whereas 6.6 million tonnes are highly perishable (CSA, 2012).

Horticultural crops, such as fruits are perishable products and, therefore, sensitive to greater losses than do non-perishable crops (Parfitt et al., 2010). Their post-harvest life depends on the rate at which they use up their stored food reserves and their rate of water loss. When food and water reserves are exhausted, the produce dies and decays. Perishable commodities need careful handling during harvesting and post-harvest handling; this would make deterioration of produces minimized as much as possible during the period between harvest and consumption. For instance scientists reported total post-harvest loss of banana as 26.5% where 56% of the loss occurred at the retail level due to rot before reaching consumers in Ethiopia. Furthermore, climate and weather conditions, harvesting and handling techniques, packaging, storage and transportation facility, market situation, disease and pest were major causes of post-harvest loss in Dirre Dewa where postharvest loss ranging from 20 to 50% was recorded between marketing and consumption (Mohammed and Afework, 2016).

Even though the horticultural sector in Ethiopia is growing there is low and insufficient support for the improvement and reduction of post-harvest loss; quality deterioration of horticultural crops was reported. An estimate of 15 to 70% of post-harvest losses of horticultural crops in Ethiopia was reported. Thus, such losses during harvest are a major source of food loss and could be seen from food security and poverty reduction aspects in the country because losses have direct effect on people's livelihood and the economy of the country as whole (Misrak et al., 2014).

To increase food availability, it is therefore not enough to increase productivity of agricultural commodities but there is also a need to lower the losses. According to Boxall (1998), farmers growing horticultural crops, especially fruits are facing high economic losses, due to lack of methods to increase the shelf life of those crops. Peaches are extremely perishable fruits and do not lend themselves to prolonged storage; if held too long at or near 0°C they are subjected to chilling injury. The onset of these symptoms determines the postharvest storage potential, because chilling injury development reduces consumers' acceptance (Crisosto et al., 1997).

Application of surface coating on fruits is considered as one the several treatments developed to reduce postharvest losses and to prolong the shelf life of fruits and vegetables (Baldwin et al., 1995). Preserving fresh fruits after harvest and maintaining their quality for longer periods until processing, marketing or consumption is one of the major problems in the value chains of most fruits including peach or nectarine. This situation necessitates the use of proper preservation and optimum storage conditions. Hence, the aim of the study was, therefore to evaluate the potential of beeswax coating and storage temperature to extend the shelf life of nectarine fruits as determined by their physico-chemical properties and sensory attributes.

MATERIALS AND METHODS

Experimental materials:

A total of 240 nectarine fruits were collected from Holetta Agricultural Research Centre located at Holetta, Ethiopia. The fruits were carefully selected, based on uniformity at the commercial maturity stage, colour, shape and those free of physical damage and infection by biotic factors used for the experiment. Harvested fruits were transported to the laboratory and washed in tap cold water to lower the temperature and, after a while, washed again with warm water (45-50°C) to minimize surface load of microorganisms. Then, fruit samples were surface dried by muslin cloth and randomly assigned to six groups. Purified beeswax and DANA edible oil were collected from the Holetta Beekeeping Research Centre, Holetta, Ethiopia and the local super market, respectively. The wax emulsion was prepared, according to Hassan et al. (2014) with little modification, using 120 g of wax dissolved in 200 ml of pure water and heated to 80-90°C. The solution was mixed gently until all the wax was dissolved in hot water. Then, 100 ml of the edible oil was added to the molten wax and, finally, hot water (50-55°C) was added to the solution until it reached 1000 ml.

Treatments

The fruits were randomly divided into six groups of 40 fruits each and treated in the following way: coated fruits stored at 6°C, coated fruits stored at 1°C, coated fruits stored at ambient temperature, uncoated fruits stored at 6°C, uncoated fruits stored at 1°C and uncoated fruits stored at ambient temperature. Fruit coating was performed by the dipping method to cover whole surface of fruits and then by air drying. Physico-chemical properties were measured at 5 days interval for 50 days. However, fruit sensory attributes of the samples were determined based on the shelf-life of each treatment during the storage periods.

Physico-chemical properties

Percentage of weight loss

All sample fruits were weighed on the first day to determine their initial weights. Then, fruits were weighed in triplicates at five 5 days interval and percentweight loss was calculated by using the following formula (Wang et al., 2005).

Total soluble solid (°Brix)

The concentration of total soluble solid was determined by direct reading from sample juice dropped on a refractometer sample platform. A small quantity of fruit juice (3-5 drops) was placed onto a fixed prism surface of the refractometer at 20°C and the result was expressed as o Brix (AOAC, 2006).

Table 1. Effect of beeswax coating and storage temperature on physico-chemical properties of nectarine fruits.

Treatments	pH	TA (%)	TSS (%)	Weight Loss (%)
Coated fruits stored at 6°C	3.42±0.06^{c}	1.45± 0.06^{a}	15.32±0.91^{a}	8.20±1.48^{c}
Coated fruit stored at 1°C	3.62± 0.07^{bc}	1.37± 0.05^{ab}	15.23±0.83^{a}	7.97±1.15^{c}
Coated fruit stored at 22ºC	3.91±0.17^{abc}	1.19±0.11^{abc}	13.82±0.91^{ab}	14.06±3.04^{bc}
Uncoated fruit stored at 6°C	3.99±0.13^{ab}	1.03± 0.11^{bc}	10.73±0.95^{bc}	20.39±3.19^{ab}
Uncoated fruit stored at 1°C	4.01±0.15^{ab}	1.01±0.09^{bc}	10.46±1.15^{bc}	19.78±3.00^{ab}
Uncoated fruit stored (control) at 22ºC	4.29±0.16^{a}	0.93±0.11^{c}	9.36±1.20^{c}	25.27±3.67^{a}

Values are Means ± Standard Error. Valuesfollowed by different letters within a column are significantly different (p≤ 0.05) using Least Significance Difference (LSD).

Titratable acidity and pH

Titratable acidity was measured according to standard procedures (AOAC, 2000). Ten grams of ground nectarine fruit samples were taken from each treatment. Then, the samples were diluted with 250 ml warm water. Ten milliters of the supernatant was titrated with 0.1N NaOH until a pH value of 8.2 was reached. The titratable acidity was expressed asa percentage of citric acid/100 ml of juice. The pH value of the samples were measured using a glass electrode pH meter, which was calibrated by buffer solution at pH 7 and 4, according to the method described by AOAC (2005).

Sensory evaluation

Sensory properties (flavour, texture, appearance, taste, and overall acceptability) were evaluated by ten semi-trained panellists composed of 6 females and 4 males. Panellists were selected based on their previous experience in sensory evaluation.Samples were given to each panellist in a completely randomized order, served on white saucers and labelled with three digit random numbers. Panellists were served water and unsalted crackers to cleanse their palettes in between samples. Using the five point hedonic scale panellists were asked to rank or to score sensory attributes based on preference where; 1= for dislike very much, 2= for dislike moderately, 3= for neither like nor dislike, 4= for like moderately and 5= for like extremely. An average score above 3.5 was considered a limit of acceptability for all sensory attributes.

Statistical analysis

Statistical analysis of the physico-chemical and sensory data was performed by SPSS software version 20 (SPSS, Inc., Chicago, IL, USA). Analysis of variance was performed using two-way ANOVA at 95% confidence interval and 5% level of significance. For comparison of treatments, sensory data were subjected to analysis by Kruskal Wallis test and value of P ≤0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Fruit weight loss

Table 1 shows the change in weight of coated and uncoated nectarine fruits, which were stored at different temperatures. The results indicated that weight loss increased steadily with prolonged storage. There was a significant (p ≤0.05) difference between the treatments. The highest percentage of weight loss recorded for uncoated fruits stored at 22°C (control), while, the lowest percent weight loss was recorded for coated nectarine fruits at 1°C (Table 1). These results were similar with the findings of Joyce et al. (1995), who reported that waxing extended storage life of avocado through the reduction of moisture loss and modification of internal storage, as well as to observations reported by Shein et al. (2008) who concluded that the use of 18% teva wax coating in combination with cold storage can reduce the percentage of weight loss of 'Sai Nam Peung' mandarin orange (*Citrus reticulata* Blanco) up to 30%, and to Patel and Goswami (1984), who reported that storage life of mango fruits was extended by wax coating and co1d storage.The nectarine fruits which were coated and stored at 1 and 6°C had lower weight loss percentage than those fruits coated and stored at ambient temperature. This indicates that, in addition to waxing, especially storage temperatures of the coated fruits determine the shelf life of nectarine fruits. Hence, waxing and storage at ambient temperature might not be act as moisture loss protective as compared to waxing and cold storage. This might be, the ambient temperature affects moisture content of the wax and the wax may not help moisture loss barrier from surface of the nectarines fruit.

Titratable acidity

There was statistically significant difference (p≤ 0.05) amongst the treatments for titratable acidity. The highest mean value of titratable acidity (1.452%) was recorded for coated fruits maintained at 6ºC during the storage periods, while uncoated fruits stored at ambient temperature (22°C). Relatively higher values of titratable acidity were observed for coated fruits as compared to those uncoated and stored at higher temperatures. This could probably be due to the effect of waxing and lower storage temperatures (6 and 1°C) to slow down the change in fruit acidity and fruit starch hydrolyzed to simple sugars during metabolic activities. The decrease in acid content of fruits stored at high temperature could also be caused by the use of acids in the fruit as a source of energy and conversion of organic acids to form sugars (Burton, 1985; Wills et al., 1998).

Table 2. Mean score values of sensory attributes as affected by beeswax coating and storage temperature of nectarine fruits.

Treatments	Flavour	Sourness	Appearance	Taste	Texture	Overall acceptability
Coated fruits stored at 6°C	4.23±0.06^{a}	4.12±0.07^{a}	4.49±0.05^{a}	4.27±0.06^{a}	4.19±0.07^{a}	4.25±0.05^{a}
Coated fruit stored at 1°C	4.16±0.04^{a}	4.01±0.06^{a}	4.31±0.06^{a}	4.15±0.05^{a}	4.22±0.07^{a}	4.19±0.04^{a}
Coated fruit stored at 22ºC	2.67±0.18^{b}	2.62±0.17^{b}	2.61±0.18^{b}	2.61±0.18^{b}	2.48±0.17^{b}	2.59±0.17^{b}
Uncoated fruit stored at 6°C	1.43±0.20^{c}	1.46±0.20^{c}	1.47±0.20^{c}	1.45±0.20^{c}	1.47±0.20^{c}	1.45±0.20^{c}
Uncoated fruit stored at 1°C	1.42±0.20^{c}	1.45±0.20^{cd}	1.46±0.20^{cd}	1.45±0.20^{c}	1.47±0.20^{c}	1.45±0.20^{c}
Uncoated fruit stored (control) at 22°C	0.77±0.16^{d}	0.82±0.16^{d}	0.82±0.16^{d}	0.81±0.16^{d}	0.81±0.17^{d}	0.81±0.16^{d}

Values are Means ± Standard error. Values

Fruit juice pH

Table 1 shows that there was a significant difference ($p \leq 0.05$) amongst the treatments for pH of fruit juice. The highest mean pH value (4.29) was observed for uncoated fruits stored at ambient temperature (control), while the lowest value (3.42) was recorded for coated fruits stored at 6°C (Table 1). The result indicated that coated fruits stored at low temperatures had lower mean pH values than the uncoated fruits stored at higher temperatures. This might be due to combined effect of waxing and cold storage slowing down oxidation of acids found in nectarine fruits. The result of the present study was in agreement with the work of Diaz et al. (1996) who reported increases in pH of the control samples compared to pH of the mangoes coated with malto dextrin and methyl cellulose. The result of the present study is also in line with previous findings by Medlicott et al. (1987), who observed that the rate of increase in pH of control samples was higher than in coated mango fruits stored at 25°C

Total soluble solids (TSS)

The result presented in Table 1 shows the difference in TSS of the coated and uncoated nectarine fruits which was significant ($p \leq 0.05$) during storage period. The result indicated that the highest mean (15.32°Brix) TSS was observed for coated fruits stored at 6°C while the lowest value (0.93°Brix) was for the control treatments. The reduction in TSS in uncoated fruits stored at higher temperature could be faster gas exchange metabolic rates (Mahajan et al., 2006). The mean TSS content for coated fruit stored at 6 and 1°C, at ambient temperature, and uncoated fruit stored at 6 and 1°C and at ambient temperature (control) at the end of the storage period were 15.32, 15.23, 13.82, 10.73, 10.46 and 0.93°Brix, respectively (Table 1). The increase in mean TSS with prolonged storage time could be probably due to the effect of waxing and cold storage, as waxing and cold storage condition slows down the rate of respiration and, thus percentage of TSS increased slowly with storage period. This result is in agreement with the findings of Patel et al. (2008), who reported that changes in TSS content are natural phenomena that correlate with hydrolytic changes in carbohydrates during storage.

Flavour and taste

It was observed that there were significant differences ($p \leq 0.05$) between the treatments for mean score value of flavour and taste (Table 2). The highest mean score (4.23) of flavour was observed for coated fruits stored at 6°C, while the lowest value (0.77) was for the control treatment fruits (uncoated) stored at ambient temperature throughout the storage time (Figure 1). The overall mean score for coated fruits stored at different temperatures was higher than the corresponding value for uncoated fruits. The same trend was observed for mean scores of taste with the highest value (4.27) for coated fruits stored at 6°C, and the lowest (0.81) for the control treatments (Figure 3). In line with this Karakurt et al. (2000) reported that none melting flesh peach cultivars which were evaluated as low flavoured had reduced soluble sugars and total soluble solids. Hence, results of the present study indicate that waxing and storing fruits under cold condition maintain sensory attributes without significant changes for longer period, as there were no significant differences ($p > 0.05$) in flavour and taste between coated fruits stored in cold storage during the experimental period. The mean score for flavour had similarity with the mean score of taste. This could be due to the fact that the same biochemical constituents contribute to both flavour and taste of the fruits.

In agreement with results of the present study, Rapaille et al. (2003) reported that sorbitol as one of alcohol sugars is more beneficial than other sugars with regard to diet control and dental health (reducing caloric intake) and it improves the fruit's taste and texture, as texture and physical properties of a fruit have, in turn, influence on fruit taste. Similarly, it has been reported that fruit quality can be properly preserved under cold conditions for long periods of time, resulting in only a small reduction in flavour quality (Abad et al., 2003).

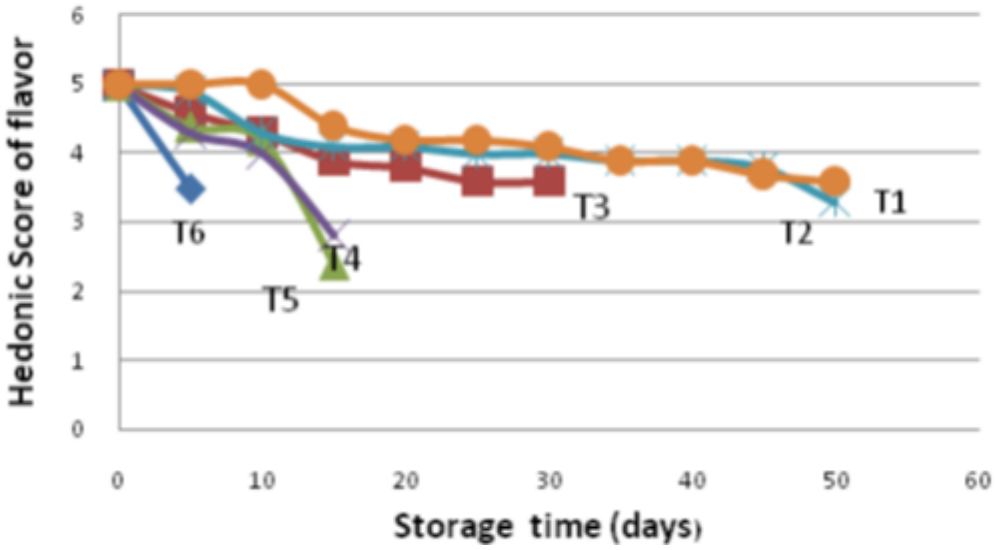

Figure 1. The effects of beeswax coating and storage temperature on the flavor nectarine fruits during storage period.

Sourness and texture

Highest mean score values of sourness (4.12) and texture (4.22) were observed for coated fruits stored at 6 and 1°C, respectively (Table 2), while the lowest mean score parameters were recorded for the control fruits (uncoated and stored at ambient temperature). The result indicated that coated fruits stored at 1 and 6°C exhibited the highest mean scores of sourness and texture as compared to other treatments. But, the mean score values of coated fruits stored at ambient temperature were higher than the overall mean values of uncoated fruits (Figures 4 and 5) stored at 22, 1 and 6°C. Hence, the result of the present study indicates that coating by beeswax and storing cold temperature (1 and 6°C) may help to maintain the sensory quality of nectarine fruits. The study also indicated that coating alone may not help much, as shelf life of coated nectarine fruits depends on storage temperature. In agreement with this, Patricia et al. (2005) reported that refrigerated strawberry coated with gluten based films had better firmness retention than the control. Similarly, apple coated with paraffin oil and jojoba was found to have higher mean scores for visual appearance, texture and overall acceptability; while the control apple sample had the lowest mean scores values for those parameters (EL-Anany et al., 2009).

Appearance and overall acceptability

Table 2 shows that there was statistically significant difference ($p \leq 0.05$) among the treatments for appearance and overall acceptability. The highest means score values of appearance and overall acceptability were observed for coated nectarine fruits stored at 6°C, while the lowest mean value was recorded for control treatments (uncoated) stored at ambient temperature (22ºC). The mean scores of appearance and overall acceptability for coated fruits stored at 6°C were 4.49 and 4.25, respectively (Table 2). Furthermore, overall acceptability values were consistently higher and maintained for longer period for coated fruits stored at 1 and 6°C than for the other treatments (Figure 6).

This result was in agreement with the findings of Hassan et al. (2014) who found that the highest score of sensory attributes (colour, texture, odor, freshness, appearance, fruit firmness, taste, and overall acceptability) was observed for 12% wax coated tangerine citrus var. Siam Banjar fruit stored at 5°C.

Shelf life determination (acceptability assessment)

Sensory attributes (flavour, taste, appearance, sourness and overall acceptability) were evaluated by ten semi-trained panellists at 5 days intervals during the storage period. Treatments which failed the acceptability assessment during the storage periods were discontinued from further sensory evaluation.

Results of the present study indicated that, there were significant variations between the treatments for all sensory attributes during the storage period. There was a sharp decrease in the mean value of flavour in T6 (uncoated fruits stored at ambient temperature) on the 5th day of storage when other treatments had acceptable

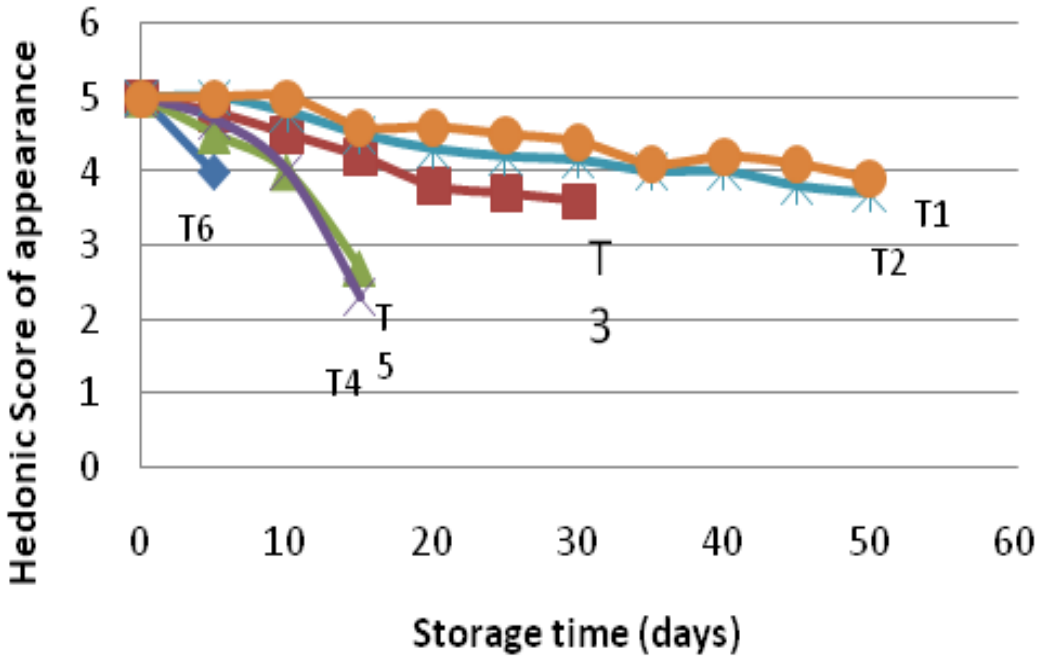

Figure 2. The effect of beeswax coating and storage temperatures on the appearance of the nectarine fruits during the storage period.

values (Figure 1). However, the mean flavour of the coated fruits stored both at 6 and 1°C was not much affected and gradually declined during the storage period. This indicates low temperature and coating can maintain flavour and slow down the rate of biochemical reaction of fruit.

Hence, this shows that storing nectarine fruits at ambient temperature (22°C) had a detrimental effect on flavour through its effects on biochemical constituents of the fruits. In line with this, David et al. (2013) reported that though ethyl-based esters were most typical in sweet and aroma, their presence might lead to off-flavour if over-abundant in the fruit. Similarly, there was a sharp decline in the “appearance” of uncoated fruit stored at ambient temperature (22°C); the decrease observed in the coated fruits stored at 6 and 1°C was not significant ($p<0.05$) and gradual during the storage period (Figure 2). In general, flavour and appearance of fruits in all treatments decreased with prolonged storage though coating nectarine fruit with beeswax and storing in cold temperature (1 and 6°C) significantly retained the appearance and flavour as well as other sensory attributes of the fruits for longer periods. In agreements with this result David et al. (2013) reported that holding mandarins at warm temperatures such as 20°C could be very harmful to flavour quality. The combined effect of waxing and storage temperature on fruit internal atmosphere and citrus quality has also been shown in some previous works (Eaks and Ludi, 1960; Baldwin et al., 1995; Chun et al., 1998).

The score values of taste and texture also decreased with the time of storage (Figures 3 and 4) and showed the same trend of response to the treatments as did flavour and appearance values. Retention of taste and texture was by far better for coated nectarine fruits than for uncoated fruits and for cold (1 and 6°C) than for ambient temperature (22°C) during 50 days of storage. Similar results were also reported by Alam and Paul (2001) who studied the effects of cellulose-based coating (carboxyl methyl cellulose) on the shelf life of Kinnow fruits and found that carboxyl methyl cellulose coating (0.5%) extended shelf life up to 40 days without adversely affecting the quality but taste scores were lowered when storage life increased.

At the initial stages texture of the fruits was firm but eventually the fruit structure disintegrated. It was, probably because of physiological and biochemical changes. In line with this, it has been reported that, during ripening and maturation, protopectin (insoluble form of pectin substances) is gradually broken down to lower molecular weight fractions, which are more soluble in water and cause softening of fruits (Wills et al., 1981). Similar results have also been reported for sweet orange by Muhammad (2007) indicating that the scores values of taste decreased during fruit storage time from 0 to 56 days.

The hedonic score values of overall acceptability (Figure 6) and sourness (Figure 5) decreased with prolonged storage. However, mean score of overall acceptability and sourness decreased rapidly for

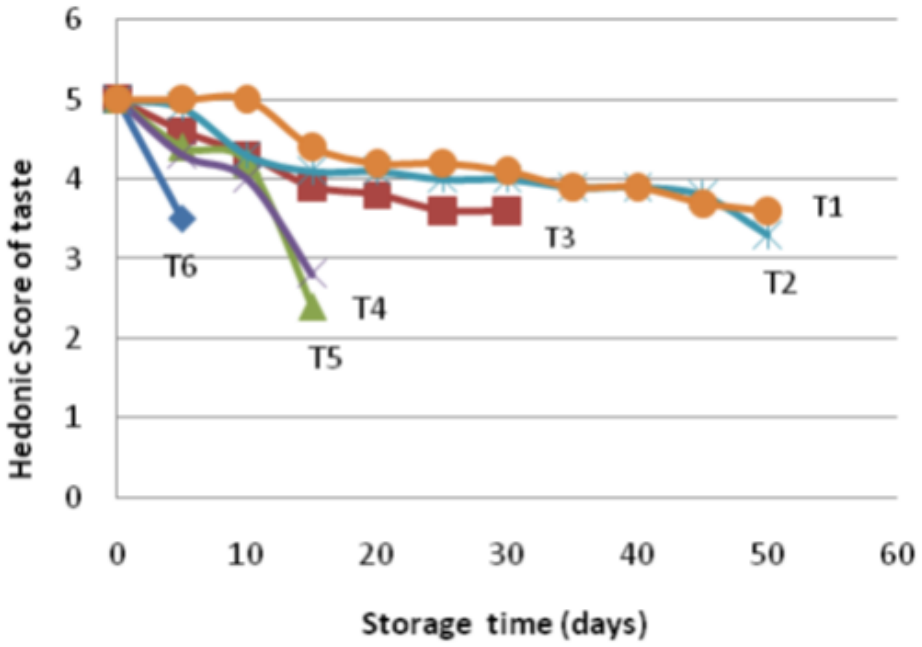

Figure 3. The effect of beeswax coating and storage temperatures on the taste of nectarine during storage period.

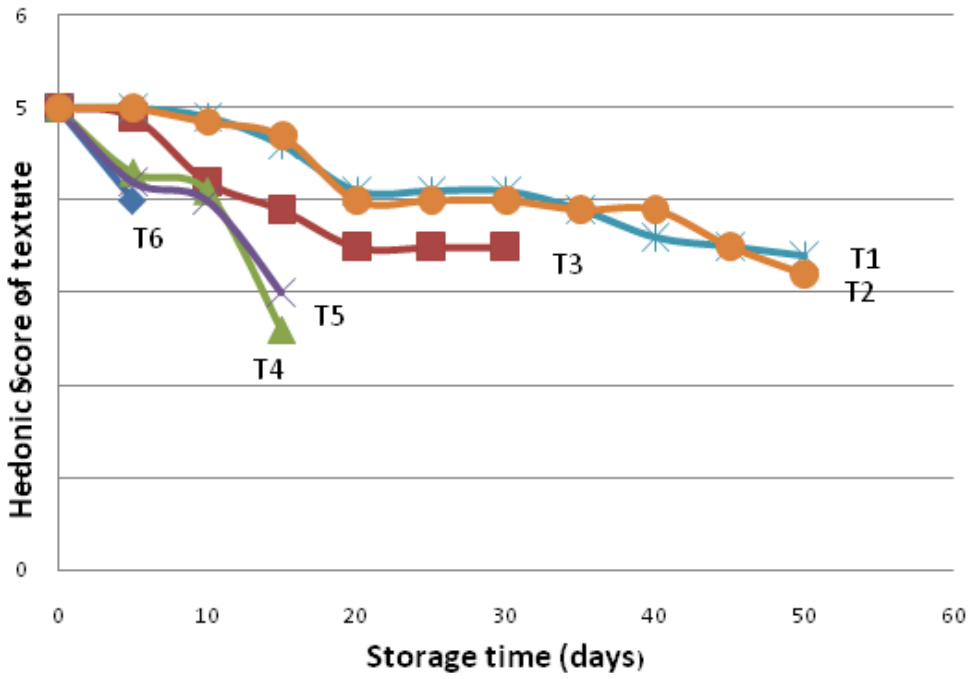

Figure 4. The effect of beeswax coating and storage temperatures on the texture of nectarine fruits during the storage period.

uncoated nectarine fruits while slowly decreasing exhibited values those fruits stored at 1 and 6°C temperatures. Hence, such a rapid decline in sourness and overall acceptability values may indicate shorter shelf life while slowly decreasing values show longer shelf life nectarine fruits. In line with this, it has been reported that

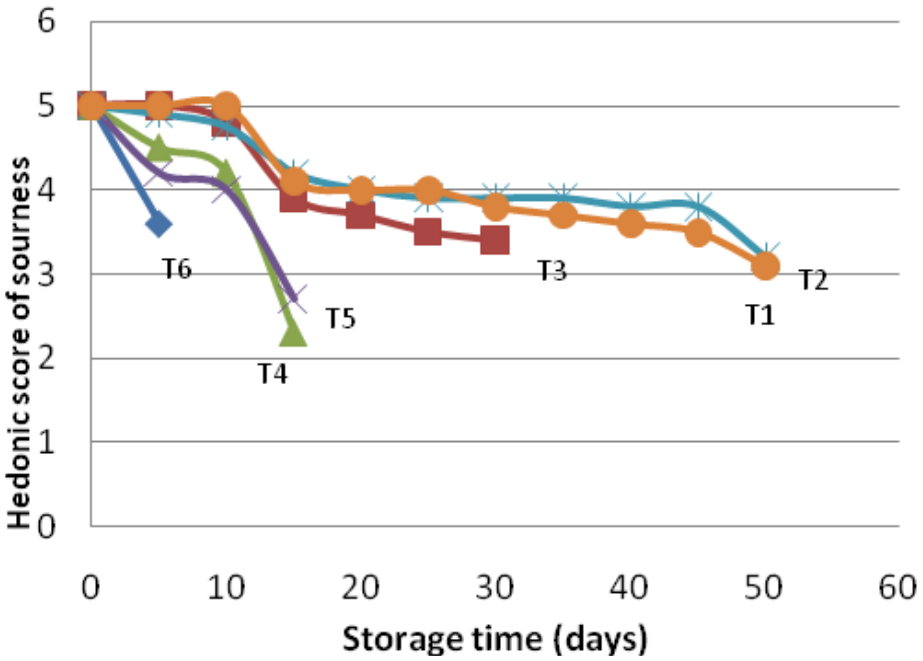

Figure 5. The effect of beeswax coating and storage temperatureson sourness of nectarine during storage period.
Note: T1=Coated fruits stored at 6°C, T2= Coated fruits at 1°C, T3=Coated fruits stored ambient temperature, T4=Uncoated fruits stored at 6°C T5=Uncoated fruits stored at 1°C and T6=Uncoated fruits stored at ambient temperature (22°C)

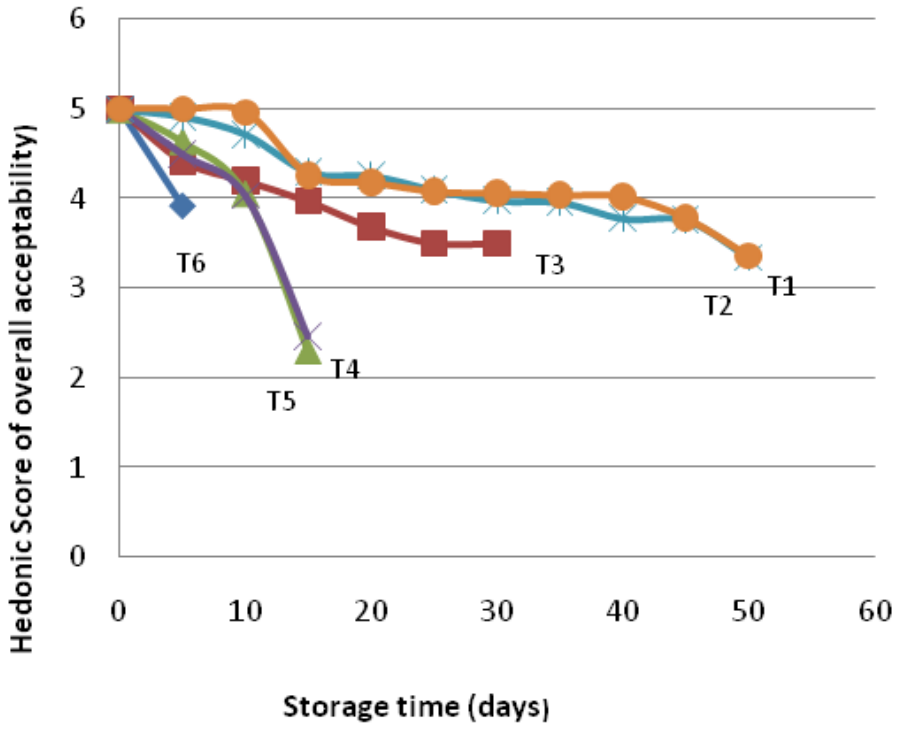

Figure 6. The effect of beeswax coating and storage temperatures on overall acceptability of nectarine during storage period.
Note: T1=Coated fruits stored at 6°C, T2= Coated fruits at 1°C, T3=Coated fruits stored ambient temperature, T4=Uncoated fruits stored at 6°C T5=Uncoated fruits stored at 1°C and T6=Uncoated fruits stored at ambient temperature (22°C)

combination of 12% wax coating and storage at 5°C was the best treatment for maintaining the quality and extending the shelf-life of the tangerine citrus var. Siam Banjar, as it exhibited a higher overall sensory acceptability value than did the other treatments or the control (Hassan et al., 2014). In general, the present study revealed that, waxing and storage under cold condition (1 and 6°C) was superior to the control and keeping nectarine fruits at room temperature in terms of all most all fruit quality attributes, including shelf life.

CONCLUSION AND RECOMMENDATION

Results of the present study showed that beeswax coating and storage temperature have a significant role to extend shelf life and maintain the quality of nectarine fruit. The highest percentage of titratable acidity and total soluble solids (TSS), as well as highest score values of sensory attributes were recorded for coated nectarine fruits stored at 6°C, while uncoated nectarine fruits stored at ambient temperature exhibited the highest percentage of weight loss and pH value and lowest mean score values of sensory parameters. Furthermore, the shelf life of the coated nectarine fruits stored at ambient temperature was extended to 30 days, compared to uncoated fruits stored at the same temperature which deteriorated within three to five days while life of coated nectarines fruits stored at 1 and 6°C was extended to 50 days. Hence, coating nectarine fruits by beeswax and storing at 6°C was found to be the most effective method in maintaining quality and extending shelf-life of the fruits. This finding also indicates waxing fruits and storing under cold conditions can be the option to reduce postharvest losses and increase the income of nectarine growing small scale farmers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Meat quality status and postharvest handling practices along the meat value chain in Kenya

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In Kenya, meat value chain (MVC) is an important component of the food supply chain serving as a source of nutrients and income. However, information regarding processing practices, hygiene and equipment use as affecting meat quality still remains unclear despite its relevance for data and for assessment for development of meat quality in the meat trade. Therefore, a cross sectional survey of selected slaughterhouses and butcheries in Eastern region of Kenya was carried out to assess the postharvest handling practices and meat quality. Forty meat samples were collected from rump, neck, stomach and hind legs cuts of the carcass and analyzed for total viable counts, *Staphylococcus aureus, Escherichia coli* and *Listeria monocytogenes*. The findings indicate that over 50% of the meat handlers in slaughterhouses and butcheries have not received any formal training in good hygiene practices for meat handling. Total viable counts ranged from 2.159 to 2.736 log CFU/g, *Staphylococcus aureus* ranged from 1.112 to 1.324 log CFU/g, *Escherichia coli* ranged from 1.211 to 1.320 log CFU/g and *Listeria monocytogenes* ranged from 0.101 to 0.193 log CFU/g in the meat cuts. In conclusion, the study showed poor handling of meat which poses risks to consumers.

Key words: Meat quality, post-harvest practices, meat value chain.

INTRODUCTION

Livestock production is an important economic activity in the developing world. Livestock products contain high value proteins, fats, vitamins and minerals (Mallhi et al., 2019). In Kenya, the livestock sector contributes about 12% of Kenya's Gross Domestic Product, 40 to 42% to the agricultural GDP and 50% employment to the agriculture sector (Shibia et al., 2017; Dabasso et al., 2018). Currently, there is a growing demand for meat products mainly in the urban set up and a number of small scale meat processors have entered the business during the past few years. In Nairobi city, the increase in population growth has seen total meat consumption increase by a factor of 2.2 cementing the importance of meat and meat products to city dwellers (Bosire et al., 2017).

The consumption of meat products demonstrates an upward trend and is envisaged to increase further in the future. In addition, the potential for growth of the livestock enterprises in rural communities and pastoral regions of Kenya is significantly high due to the improvement of the

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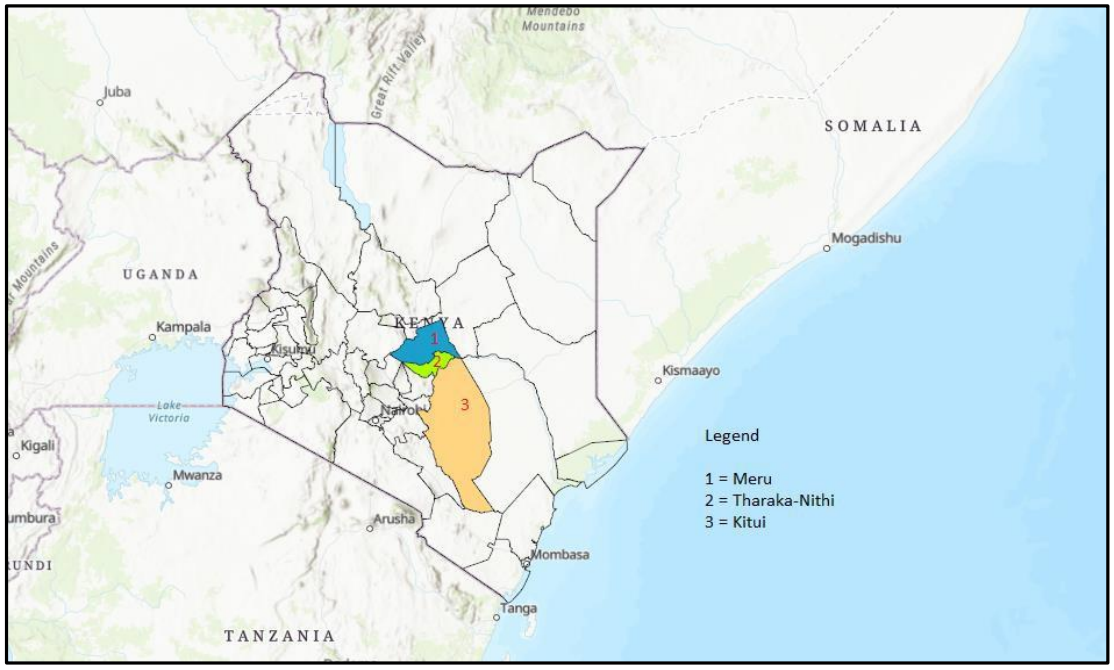

Figure 1. Map of Kenya showing the study areas.

market access, consumer perception and financial access by livestock owners. Red meat derived from cattle, goats, sheep and camel is highly consumed. Recent data puts the contribution of red meat in Kenya at 588693 tons with average carcass weight of 2273 hg/An (FAOSTAT, 2019).

The beef, value chain is a multi-sectoral system at various levels including primary meat producers, abattoirs, butcheries as well as traders, who buy, sell and transport livestock to and from primary and secondary markets (Dinku et al., 2019). Contamination of meat can occur at different stages of processing, distribution and retail (Mallhi et al., 2019). In this regard, it is imperative to process meat products in a safe and hygienic backdrop as they are essential for consumer protection and control of potential health risks (Wambui et al., 2017).

High levels of hygiene are necessary as meat is a perishable food and hence, food handlers in the meat value chain should have prerequisite food safety and hygiene knowledge (Tomasevic et al., 2016). Aspects of cleanliness of butcheries and personnel, hygiene of abattoirs is also very important since the feedback provides valuable insight that they can use to improve their businesses. A number of legislations and regulations that govern the management of livestock, their slaughter, handling and processing of meat as well as hygiene level have been developed in Kenya.

In Eastern regions of Kenya, the meat value chain serves as a source of nutrients and income enterprises to locals (Werikhe et al., 2019). However, the sector is hampered by poor meat quality, poor infrastructure, lack of capital, fluctuation in price of meat, weak extension services and low technical capabilities of processors (Gobena, 2017; Muzzo and Provenza, 2018). Price fluctuations where there is no government mechanism to reign in middlemen who reap a bigger chunk of cattle sales exacerbates the situation (Bunmee et al., 2018).

Addressing these challenges still remains unclear. This study was thus conducted to assess the different slaughterhouses/slaughter slabs, butcheries to understand how they work, identify gaps, and how to structure them for optimal performance.

MATERIALS AND METHODS

Study area

The study was carried out in Meru, Tharaka Nithi and Kitui counties (Figure 1). Meru County is situated in the former Eastern province. It has nine sub-counties, nine constituencies and forty five county assembly wards (County Government of Meru, 2018). It is found between latitudes 37° West and 38° East and between longitudes 0°6' North and 0°1' South. The county has an estimated total population of 1,535,635 (Kenya National Bureau of Statistics, 2019). Tharaka Nithi County is also located in the former Eastern province and divided into five administrative counties. It lies between latitude 00° 07' and 00° 26' South and between longitudes 37° 19' and 37° 46' East (County Government of Tharaka Nithi, 2018). The county comprises the highland and semi-arid zones. Based on the Kenya National Bureau of Statistics (KNBS, 2019) report, the population is estimated at 393,177. Kitui County is about 160 km from Nairobi city on the Eastern part of Kenya and divided into eight sub counties (County Government of Kitui, 2018). It is situated between latitudes 0°10 South and 3°0 South and

longitudes 37°50 East and 39°0 East. The population in the county is about 1,136,187 (Kenya National Bureau of Statistics, 2019).

Study design

The design was cross sectional involving a survey in the three counties namely Meru, Kitui and Tharaka Nithi. These areas were purposively selected due to large pastoral production and value addition of meat. A total of 26 main study sites in the counties were selected using the two-stage cluster sampling method. Out of the sites, a total of 100 respondents (31 slaughterhouses and 69 butcheries) were randomly sampled from the three counties. In each of the county, 23 butcheries were selected while for slaughterhouses 10, 11, and 10 were selected, respectively in Meru, Kitui and Tharaka Nithi.

Data collection

Primary data

Key informant interviews/individual interviews and Focus Group Discussions were conducted with value chain actors as well as the related service providers in the selected counties. The data collected was used to gain better understanding of the structure and dynamics of the meat value chains and the opportunities and barriers for private sector actors in the study region to improve availability of safe and quality meat products. This was meant to improve the understanding on the barriers to more effective integration between producers and private-sector food processors and vendors within the value chain. Simultaneously, observations of the slaughterhouses and butcheries were done during the study period.

Secondary data

The study also conducted review of relevant documents, including published literature, program reports, and county statistics.

Analytical methods

Sample collection and preparation

Meat and surface swab samples from equipment used for meat handling and personal protective equipment for the workers in the slaughterhouses were aseptically collected for further microbial analysis. Ten meat samples each weighing 1 g were cut from the rump, neck, stomach and hind leg of the carcass and thereafter aseptically transferred into tubes containing 10 ml buffered peptone water. For the meat handling equipment (weighing scale and wedging knife), an area of 100 cm^2 was swabbed for 50 s using sterile moistened cotton swab while for the steel file, an area of 10 cm^2 was swabbed for 50 s using sterile moistened cotton swabs and transferred into tubes containing 10 ml buffered peptone water. Samples were also collected from the gumboots, caps and dustcoats of the personnel. An area of 50 cm^2 was swabbed for 50 s using a sterile moistened cotton swab and transferred into 10 ml buffered peptone water.

Microbial analysis

Determination of total viable count

Total viable count was done by the pour plate method. 1 ml of the sample dilutions (10^{-4}, 10^{-5}, 10^{-6}) was poured on plates containing plate count agar. The plates were incubated at 35°C for 48 h and all grown colonies were enumerated thereafter.

Determination of Staphylococcus aureus

The *S. aureus* levels were determined as described by the ISO 6888-1 and ISO 6888-2 methods. 28 g of Baird parker selective media was mixed in 1 L of distilled water and autoclaved at 121°C for 15 min. It was then cooled to 45°C; thereafter 50 ml of egg yolk tellurite emulsion was added. 1 ml of each serial dilution of 10^{-4} to 10^{-6} was plated in duplicates using the spread plate method and the plates incubated at 37°C for 24 h. Coagulase positive black colonies on the selective media were indicative of *S aureus*.

Determination of Escherichia coli

Enumeration of *E. coli* was done as described in ISO 16649-1, ISO 16649-2 and ISO 16649-3. 28.1 g of Brilliance *E. coli*/coliform selective media was mixed in 1 L of distilled water and thereafter boiled to completely dissolve, and then cooled to 45°C. The molten media was then transferred to sterile plates. 1 ml of each serial dilution of 10^{-4} to 10^{-6} was plated in duplicates using the spread plate method and the plates incubated at 37°C for 24 h. A pink colony on the selective media was indicative of *E. coli*.

Determination of Listeria monocytogenes

The *L. monocytogenes* was determined by the ISO 11290-1:2004 method. 1 ml of the sample dilutions (10^{-4}, 10^{-5} and 10^{-6}) was spread on listeria selective agar plates which were inclusive of *L. monocytogenes* selective supplement. The plates were incubated at 35°C for 48 h and distinct *L. monocytogenes* colonies were counted after incubation.

Statistical data analysis

Field data obtained was entered in Microsoft excel 2013 spread sheet. Statistical computing of descriptive statistics of variables was done using STATA version 11. Microbial data was subjected to analysis of variance (ANOVA). The significance level was set at $p \leq 0.05$. Microbial counts were represented as log CFU/g.

RESULTS

Socio-demographic characteristics

The highest percentage of the meat handlers in both the slaughterhouses (68%) and butcheries (55%) was in the age bracket of 35 years and above (Tables 1 and 2), respectively. Most of the operators had attained basic education. Among the slaughterhouse operators, 39% had primary education, 32% secondary education and only 23% had tertiary education. On the other hand, most of the butchery operators (51%) had secondary level education, 39% primary level education, 9% tertiary level education and only 1% had no level of education. In terms of experience, majority of the respondents in the slaughterhouses (68%) and butcheries (46%) had an

Table 1. Socio-demographic characteristics of the operators in the slaughterhouses.

Demographics		Frequency	Percentage
Age (years)	Below 35	10	32
	Above 35	21	68
Education	No Education	2	6
	Primary	12	39
	Secondary	10	32
	Tertiary	7	23
Level of experience	Below 10	21	68
	11-15	5	16
	16-20	2	6
	Above 20	3	10

Table 2. Socio-demographic characteristics of the operators in the butcheries.

Demographics		Frequency	Percentage
Age (years)	Below 35	31	45
	Above 35	38	55
Education	No Education	1	1
	Primary	27	39
	Secondary	35	51
	Tertiary	6	9
Level of experience	Below 10	32	46
	11-15	23	34
	16-20	8	12
	Above 20	6	8

experience of below 10 years (Tables 1 and 2).

Hygiene practices during slaughter and butchery operations

In this study, postharvest handling begins immediately after stunning, where the animal is rendered unconscious before slaughter. After stunning, different actors including bleeders, flayers, carcass eviscerators and carcass dressers are involved in the follow up processes. Inspection is then done. Carcass is stamped if it meets the standard requirements as stipulated in the meat control act then transported to various butcheries. Tables 3 and 4 give the frequencies percentages of personnel hygiene practices by operators in the slaughterhouse and butcheries, respectively. All the meat handlers (100%) in the slaughterhouse and 97% in the butcheries possess a medical health certificate. Results of the study also show that over 50% of the respondents in the slaughterhouse and butcheries have not been trained in hygienic meat handling. From observations, in all the butcheries, the meat was hanged in open air for display and purchase by consumers. With regards to cleaning, most of the respondents in the slaughterhouse (93%) said that they cleaned the slaughterhouse after slaughter. On the other hand, all the meat handlers (100%) in the butcheries also indicated that they cleaned their facility after work. However, uncleaned ceilings and white walls with observable dirty spots were noticed. The findings also show that majority (90 and 84%) of the respondents in the slaughterhouses and butcheries, respectively wear protective clothing while working. However, from observations, most of the dust coats used had changed colour from white to brown and the gumboots were not nicely cleaned. Some of the operators were seen handling steel file used for sharpening the knives in their gumboots. When moving meat from the slaughter house

Table 3. Personnel hygiene practices by meat handlers in the slaughterhouses.

Attribute	Frequency	Percentage
Medical examination		
Yes	31	100
No	0	0
Training		
Yes	10	33
No	21	67
Cleaning schedule		
Yes	31	93
No	0	7
Personal protective clothing		
Yes	28	90
No	3	10

Table 4. Personnel hygiene practices by meat handlers in the butcheries.

Attribute	Frequency	Percentage
Medical examination		
Yes	67	97
No	2	3
Training		
Yes	21	31
No	48	69
Cleaning schedule		
Yes	69	100
No	0	0
Personal protective clothing		
Yes	58	84
No	11	16

to the vehicles they carry it on shoulders of their dirty coat. In addition, infrequent washing of hands was observed and standby hot water baths for sterilizing knives were also not available.

Meat handling and processing

With regards to processing, the findings showed a gap in meat value addition processing. All the slaughterhouses (100%) were not engaged in value addition of hides and skins, bones and horns. However, among the butcheries, 4% were engaged in value addition of hides and skins, 1% bones and horns and 3% deboning of meat for the market. In the slaughterhouses, most of the commonly used equipment included flaying knives, sharpening tools, handwashing basins, holding pen and hooks (Figure 2). In the butcheries, sharpening tools, soap and sanitizer dispenser, hooks and handwashing basins were the most commonly used equipment at retail level (Figure 3).

Transportation of meat and meat products to the meat enterprises

The results for mode of transport are shown in Table 5. In Meru and Kitui counties, 59.8 and 60.8%, respectively used motorbikes while in Tharaka Nithi County, 47.1% used pick-ups (Figure 4).

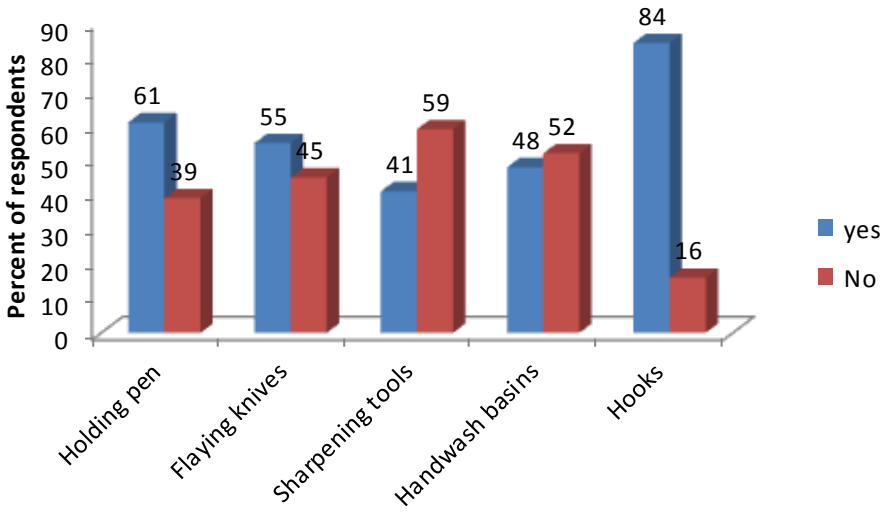

Figure 2. Equipment used in the slaughterhouse.

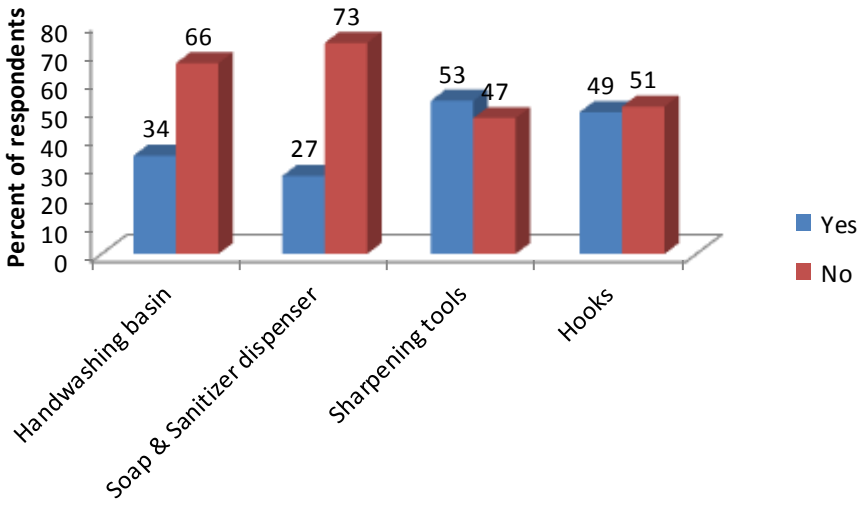

Figure 3. Equipment used in the butchery.

Microbial quality of meat samples obtained from slaughterhouses

Table 6 shows microbial counts of the different parts of the carcass: rump, neck, stomach and hind legs sampled. Total viable counts ranged from 2.159 to 2.736 log CFU/g, *S. aureus* ranged from 1.112 to 1.324 log CFU/g, *L. monocytogenes* ranged from 0.101 to 0.193 log CFU/g while *E. coli* ranged from 1.211 to 1.320 log CFU/g.

DISCUSSION

Postharvest handling of carcasses in the slaughterhouses begins immediately after stunning and proceeds during transport and then trading to consumers. Handling practices have been shown to affect the quality of meat (Bersisa et al., 2019). Several key aspects such as handling practices, personnel hygiene practices, mode of transportation, equipment usage and training were assessed in the present study. In addition, microbial quality of the carcasses at the slaughterhouses was quantified. The demographic results showed a variation in age, education and processing experience among the meat handlers. This indicates that the sample was indeed diverse. Good animal production practices on farm, good handling practices (GHPs), good hygienic practices (GHPs) and good manufacturing practices (GMPs) are crucial aspects in meat quality and lack of compliance

Table 5. Mode of transport used for meat and meat products.

Transportation mode	Meru	Kitui	Tharaka Nithi
Refrigerated vehicle	11.1±3.46[a]	11.1±2.56[a]	32.3±3.00 [b]
Metallic box mounted on pick up	11.1±2.09[b]	11.4±2.17	47.1±2.2 1[a]
Metallic box mounted on motor bike	59.8±1.39[a]	60.8±2.23[a]	17.6±2.04 [b]
Metallic box on a bicycle	12.3±1.75[a]	11.9±3.11[a]	2.4±1.68 [b]
Wooden box on bicycle	3.6±1.76[a]	3.00±1.90[a]	0.5±1.03 [b]
Wooden box on cart	2.1±1.02 [b]	1.8±1.15 [b]	0.1±1.25[a]

*Values=%means ± standard deviation; % means in the same row with different superscripts are significantly different p< 0.05.

Figure 4. Different modes of transport of meat from slaughterhouses to butcheries.

Table 6. Microbial counts (log CFU/g) of meat samples obtained from slaughterhouses.

Microbial Parameter	Carcass parts			
	Rump (n=10)	Neck (n=10)	Stomach (n=10)	Hindlegs (n=10)
TVC	2.736±0.033[a]	2.159±0.050[a]	2.360±0.076[a]	2.425±0.046[a]
Staphylococcus aureus	1.324±0.125[a]	1.241±0.104[a]	1.112±0.184[a]	1.235±0.177[a]
Listeria monocytogenes	0.138±0.123[a]	0.142±0.145[a]	0.101±0.821[a]	0.193±0.267[a]
Escherichia coli	1.214±0.103[a]	1.211±0.120[a]	1.320±0.120[a]	1.227±0.189[a]

*Values=means ± standard deviation, means in the same column with different superscripts are significantly different (p≤0.05). TVC=Total viable count, N=number of samples, CFU=colony forming unit.

can lead to meat contamination and spoilage (Idrees, 2016). However, most informal meat enterprises especially slaughterhouses and butcheries do not adhere to these good practices and standards; hence, a point of concern. Proper meat handling practices play a dominant role in ensuring meat quality and safety (Selepe and Mjoka, 2018). During meat processing and distribution, knowledge of meat hygienic handling practices are essential. Meat handlers can serve as a vehicle of cross contamination and spread of foodborne pathogens (Wambui et al., 2017).

In the present study, the results reveal both good and unhygienic practices in the slaughterhouses and butcheries. Wearing of dirty coats, infrequent washing of hands, lack of hot water baths for sterilizing of knives, keeping steel file in gumboots and carrying carcass on their dirty coats were unhygienic practices identified at the slaughterhouses and butchery retail shops. Similar results have been reported in a study conducted in slaughterhouses and butchery retail shops in Bishoftu, Ethiopia, where there was lack of hot water baths and infrequent handwashing by the meat handlers (Gutema et al., 2021).

In general, the observed unhygienic practices can be linked with lack of appropriate processing facilities, insufficient knowledge of basic hygienic practices and

poor compliance to standards of good handling practices of food. In the present study, proportion of the operators who needed training was considerably high. These findings agree with the results from previous studies conducted in small and medium enterprise butcheries in Nairobi and Isiolo counties in Kenya, where more than 50% of the operators had no training on meat handling hygiene (Chepkemoi et al., 2015). Improving the capacity of the meat handlers through training can translate into best practices in handling of meat and meat products (Akabanda et al., 2017).

In the present study, in terms of meat value addition processing, the gap identified was largely attributed by lack of equipment and skill. Additionally, the use of simple cutting tools indicates low level of professionalism of the enterprises. These findings relate to those reported by Asuming-Bediako et al. (2018) who found out those butchery operators in Accra, Ghana only use simple cutting tools such as axes and knives. Appropriate equipment for meat processing, storage and transportation are of uttermost importance for maintenance of quality and safety of meat (Kenya Market Trust, 2019). In addition, technical knowhow of the workers is also necessary. Limited use of appropriate equipment and lack of skills can result to poor quality of meat (Carron et al., 2017).

Most of the meat was transported under non-refrigerated conditions. This could be attributed by the low financial capacity of the butcheries which cannot afford to purchase refrigerated trucks, since most of them were small medium enterprises. Similar observations have been reported in various developing countries such as Uganda (Kyayesimira et al., 2018), where transportation of carcasses from slaughterhouses to butcheries was found to be carried using motorbikes fitted with an enclosed container.

The exposure of meat at ambient temperature in the butchery retail shop observed in the present study can also be explained by the low financial capacity of retailers to afford refrigeration facilities as well as insufficient knowledge in hygienic meat handling practices. These conditions in the butcher retail shops are comparable to the practices reported in Rwanda (Niyonzima et al., 2018), where they were associated with an increased risk of microbial contamination in the retailed meat.

Microbial quality of meat reflects the hygiene status and practice of workers (Teshome et al., 2020). In the present study, there was no significant difference in the mean microbial counts of the different carcass parts sampled. Among the isolated microbial pathogens, *Listeria monocytogenes* was above the acceptable limit compared to KEBS standards (KS 317-3: 2019) thus compromising on the quality and safety of the meat.

Detection of *L. monocytogenes* could be due to poor hygiene and sanitary practices through the value chain and indicates public health risk associated with the consumption of this meat. Using clean slaughter equipment, having trained personnel and following the right procedures in slaughtering can reduce the contamination (Maharjan et al., 2019).

Conclusion

Meat is an indispensable source of high-quality protein for most populations. Postharvest handling practices along the meat value chain are critical since they influence the quality and safety of meat. Hygiene practices identified in the slaughterhouses and butcheries were inadequate. High microbial contamination of meat was prevalent in most of the meat enterprises which increases public health risks. This necessitates the need for training in best practices along the meat value chain and implementation of stringent food safety management and quality control systems along the value chain.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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