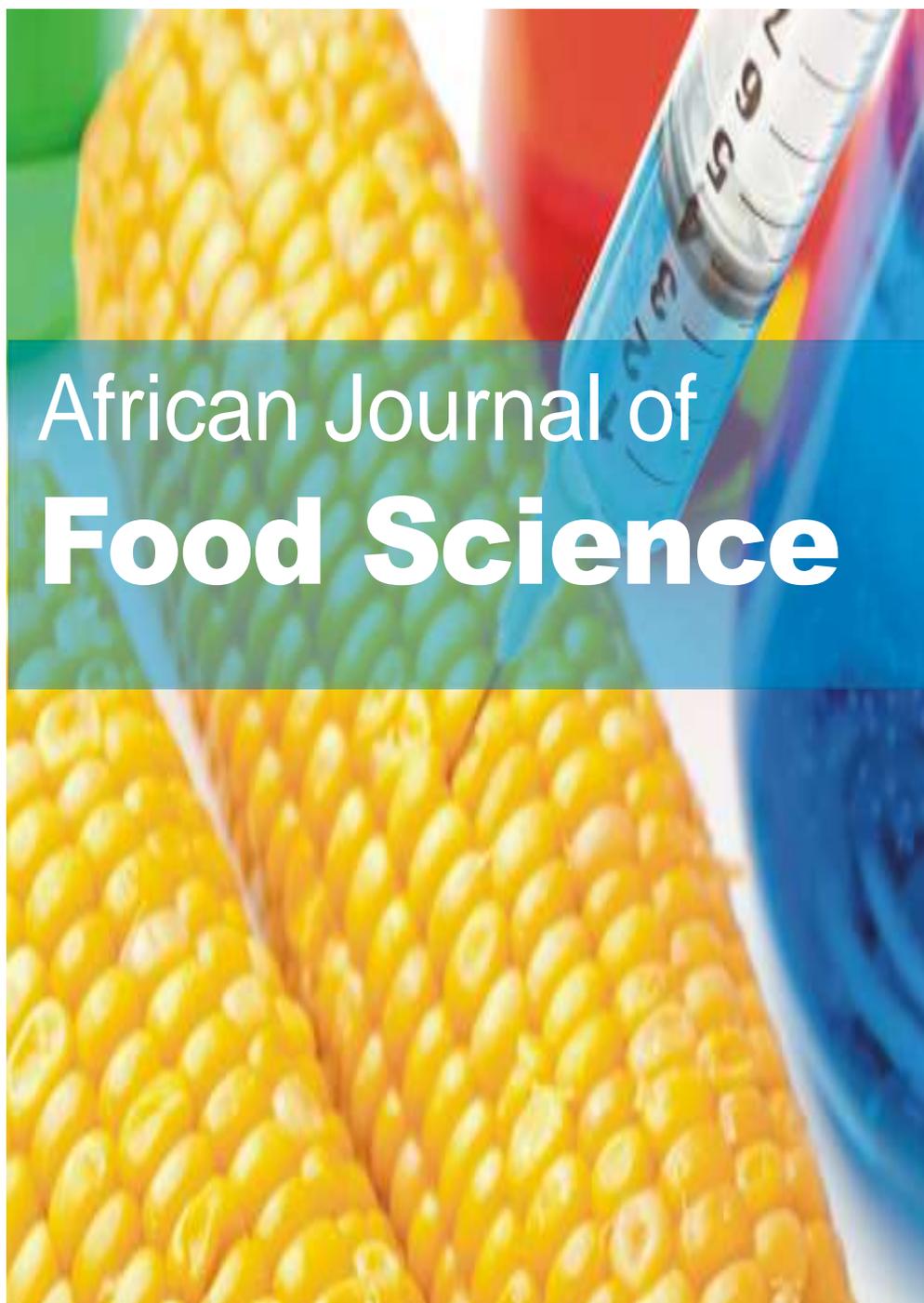


OPEN ACCESS



African Journal of **Food Science**

June 2018
ISSN 1996-0794
DOI: 10.5897/AJFS
www.academicjournals.org



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African Journal of Food Science

Table of Content: Volume 12 Number 6 June 2018 Issues

ARTICLES

- Chemical composition, functional and organoleptic properties of complementary foods formulated from millet, soybean and African locust bean fruit pulp flour blends** 126
Zakari U. M., Hassan A. and Fatima Kida
- Meat quality analysis of Djallonke lambs co-infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Trichostrongyloidea) and treated with two medicinal plants** 131
Ndah Germaine, Fonteh A. Florence, Tedonkeng Fernand, Mpoame Mbida, Lumofoet Jules, Temgoua I. F. Kengne , Kenmogne A.Tayou, Nzonang N. Douglas, Vemo B. Narcisse and Wabo J. Poné
- Comparison of traditional butter preservation techniques using microbial and organoleptic properties, West Shewa, Ethiopia** 140
Alganesh Tola, Debela Bayu, Lemma Fita, Bilatu Agza and Sara Birkie

Full Length Research Paper

Chemical composition, functional and organoleptic properties of complementary foods formulated from millet, soybean and African locust bean fruit pulp flour blends

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Received 30 January, 2018; Accepted 28 March, 2018

This study was aimed at producing and evaluating the quality of complementary foods from millet, soybean and African locust bean fruit pulp. The millet, soybean and African locust bean fruits were obtained locally in Kaduna, Nigeria. These materials were separately cleaned, dried, milled and sieved into flours. The flours were mixed as follows: Sample A (60% millet, 10% soybean, 30% African locust bean fruit pulp), sample B (60% millet, 20% soybean, 20% African locust bean fruit pulp), Sample C (50% millet, 40% soybean, 10% African locust bean fruit pulp), sample D (50% millet, 30% soybean, 20% African locust bean fruit pulp), sample E (50% millet, 20% soybean, 30% African locust bean fruit pulp). The chemical composition, functional properties and sensory attributes were determined using standard methods. The moisture content of the food samples varied from 7.5 to 11%, protein ranged from 28.9 to 37.0%, fat from 3.1 to 5.1%, Ash ranged from 1.4 to 2.51%, crude fibre ranged from 2.0 to 3.2%, carbohydrate varied ranged from 47.0 to 52.1%. The energy values ranged from 351 to 381.9 kcal/g and appreciable amount of minerals were recorded. Calcium ranged from 128.0 to 165.0 mg/100 g, iron ranged from 3.2 to 5.8 mg/100 g and zinc ranged from 2.6 to 3.2 mg/100 g. The results of functional properties showed that bulk density ranged from 0.61 to 0.66 g/ml, water absorption capacity ranged from 1.89 to 2.31 g/g, swelling capacity ranged from 0.67 to 2.45% and least gelation ranged from 9.00 to 11.62%. Sensory evaluation indicated that the samples were highly rated ($P < 0.05$) for all the parameters investigated. Sample E (50% millet, 20% soybean and 30% African locust bean pulp flour) was most acceptable to the panelists and should be encouraged in weaning food preparation.

Key words: African locust bean fruit pulp, complementary foods, functional properties, chemical composition.

INTRODUCTION

Protein-energy malnutrition among children is the major health challenges in developing countries, particularly Nigeria (FAO, 2001; Ijarotimi and Keshinro 2012). Malnutrition during early life leads to permanent stunting in growth (Nzeagwu and Nwaejike, 2008) and there may

also be irreversible sequence from micronutrient deficiencies that affect brain development and other functional outcomes (Martorell et al., 1995). This nutrition problem is associated with inappropriate complementary feeding practices, low nutritional quality of traditional

complementary foods and high cost of quality protein based commercial complementary foods (Nemer et al., 2001; Muller et al., 2003; Black et al., 2003; FAO, 2004; Alozie et al., 2009; Eka et al., 2010). The tragic consequences of malnutrition include death, disability, stunting, mental and physical growth and as a result, retarded national socio-economic development (Ijarotimi and Keshinro, 2012). It is evidence that high prevalence of deaths each year among children aged under five years in the developing world are associated with malnutrition (WHO, 2002). The interaction of poverty, poor health and poor complementary feeding practices has a multiplier effect on the general welfare of the children population and contributes significantly towards growth retardation, poor cognitive development, illness and death among children in developing countries, particularly Nigeria (Pollit, 1994; Duncan et al., 1994; Kretchmer et al., 1996; Bhattacharya et al., 2004; Anigo et al., 2007).

The complementary feeding which usually begins at 4 to 6 months continues up to the age of 24 months when the transition from exclusive breastfeeding to semi solid food begins. It is at this stage that the nutritional requirements of many infants are not met, thus leading to onset of malnutrition that is prevalent in children under 5 years of age (Anigo et al., 2009). The traditional complementary foods in Nigeria are cereal based (e.g. ogi) and other family diets (tuwo, cassava, yam, etc.) and these plant based complementary foods are not beneficial to the growth and development of children (Ijarotimi and Keshinro, 2012). Investigations have shown that ogi (corn gruel, a traditional complementary food) and other family diets often fail to meet the nutritional needs of the infants due to poor nutritive values (Fernandex et al., 2002; Solomon, 2005), hence they have been implicated in the etiology of protein energy malnutrition in the community where they are solely used as complementary foods (Okoye, 1992, Devlin, 1997).

In view of the nutritional problem associated with the traditional complementary foods, coupled with high cost of commercial baby foods, this study is therefore aimed at formulating complementary foods from millet, soybean and African locust bean fruit pulp (*Parkia biglobosa*) and to evaluate the quality of the formulated foods.

MATERIALS AND METHODS

Soybeans and millet grains were purchased from Monday Market in Kaduna South Local Government, Kaduna. Locust bean fruits were bought from Kasuwan Magani, a village in Kajuru Local

Government of Kaduna State. All the equipment used for this study were from the Department of Food Technology Kaduna Polytechnic, Kaduna. The chemicals used for analyses were of analytical grade and obtained from the laboratory of the Department of Food Technology, Kaduna Polytechnic, Kaduna. The chemicals were products of synth, Sao Paulo, Brazil.

Preparation of materials

Soybean seeds were cleaned, sorted and roasted at 100°C for 2 h. The roasted seeds were cooled and milled into flour using a laboratory hammer mill (Christy Hunt, UK). The flour was sieved using a 60 mm mesh sieve (British Standard). The flour was packed in a plastic container, sealed and stored at room temperature (25°C). The millet grains were cleaned and sorted. The sorted grains were then milled into powder using the laboratory hammer mill and sieved to fine powder using a 60 mm mesh sieve.

The locust bean fruit pulp flour was prepared using the method described by Zakari et al. (2013) and Zakari et al. (2015). The outer brown cover of the pods was manually striped open and the yellow pulp was separated from the seeds embedded within the pulp. The yellow pulp was dried in a hot air oven (model T121, Gen lab Widnes, UK) at 60°C for 9 h to moisture content of 10.5%. The dried powder was milled with a laboratory hammer mill and sieved using a 60 mm mesh sieve. The flour was also packed in a plastic container and stored at room temperature (25°C) prior to analyses.

Food formulation

Composites were formulated as indicated on table 1, from the processed flours in ratio of 60:10:30, 60:20:20, 60:30:10, 50:30:20, 50:20:30 of millet, soybean and locust bean in pulp, to obtain products A, B, C, D and E, respectively.

Analytical determinations

The nutrient composition of the flour samples was determined according to the standard assay methods of AOAC (2005). Moisture content was determined by oven method, crude protein by microkjedahl method using 6.25 as a conversion factor. Fat was determined by ether extraction using soxhlet extractor and ash was determined by drying ashing method. The carbohydrate content was determined by difference. Addition of all the percentages of moisture, fat, crude protein ash and crude fibre was subtracted from 100%. This gives the amount of nitrogen free extract otherwise known as carbohydrate. All the analyses were determined following the method of AOAC (2005):

$$\% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ fat} + \% \text{ Ash} + \% \text{ Crude fibre} + \% \text{ crude protein}).$$

The sample calorific value was estimated (in kcal/g) by multiplying the percentages of crude protein, fat and carbohydrate using Atwater conversion factor (4, 9, and 4, respectively) as proposed by Mahan and Escott-Stump (2008). Calcium, Iron and Zinc were determined by atomic absorption spectrophotometer.

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Table 1. Recipe formulation.

Component	Samples				
	A	B	C	D	E
Millet flour	60	60	50	50	50
Soybean flour	10	20	40	30	20
African locust bean fruit pulp	30	20	10	20	30

Functional properties of the flour samples were also determined. Bulk density, water absorption capacity were determined by Okaka and Porter (1979). The gelation properties were determined by the method of Coffman and Garcia (1977). The swelling capacity was determined by Coffman and Garcia, (1977) with slight modifications. One gram of the flour sample was mixed with 10ml distilled water in a centrifuge tube and heated at 80°C for 30 min. This was continually shaken during the heating period. After heating, the suspension was centrifuged at 1000 × g for 15 min. The supernatant was decanted and the weight of the paste taken. Swelling capacity was calculated as weight of the paste/weight of dry flour.

Sensory evaluation

Porridges were prepared from each of the composite flours. Briefly, 20 g of each of the samples were homogenized in 60 ml of water and the slurry was heated slowly with constant stirring for 15 min, one teaspoon of sugar was added to each sample. The porridges were kept separately in thermos flask for sensory evaluation. Sensory evaluation was conducted on the reconstituted samples which were coded and presented to 15 untrained panelists. The sensory evaluation was conducted in a well standard sensory evaluation room, where each of the panelists was positioned in a separate cubicle to avoid interference. The samples were rated on the following sensory characteristics, that is, colour, taste, mouthfeel and overall acceptability using 9-point hedonic scale, with 9 as like extremely and 1 as dislike extremely (Ihenkoronye and Ngodi, 1985).

Statistical analysis

The data obtained from the study were analyzed using means and standard deviation. Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test (DNMT) (Ihenkoronye, 1985) were used to test the significance between the means ($p < 0.05$) of sensory scores.

RESULTS AND DISCUSSION

Table 2 presents the nutrient composition of millet, soybean and African locust bean fruit pulp flour blends. The moisture content of blends ranged from 7.5 to 11.0%. The moisture content of the blends (7.5 to 11.0%) was within the range for flour (11 to 12%) as reported by Ihenkoronye and Ngoddy, (1985). This moisture level is also in agreement with the work of Nzeagwu and Nwaejike (2008). The low moisture content is desirable as it enhances the keeping quality of the samples since

water for microbial activity is low. This report agreed with similar work of Ijarotimi and Keshinro (2012).

The protein content ranged from 28.9 to 37%. The high levels of protein could be a result of supplementation with soybean. The protein levels increased with increasing addition of soybean flour. Nnam (2001) made a similar observation when the sorghum traditional complementary food was supplemented with bambara groundnut and sweet potato. Similarly, Nzeagwu and Nwaejike (2008) reported increases in protein when sorghum traditional complementary food was supplemented with groundnut and crayfish. The content also follows the same trend of increases with increasing addition of soybean. The fat varied from 3.00 to 5.1%. The high fat content of soybean resulted in the high fat level of sample C (50:40:10). The ash content varied from 1.4 to 2.51%. The carbohydrate content ranged between 47.0 to 52.1%. The energy value ranged from 351 to 381.9 kcal with sample C having the highest energy value. The energy values of the formulated samples met the FAO/WHO (1991) specification guidelines for young children complementary food formulations. The mineral composition of the formulated food samples shown on Table 2, indicated that calcium ranged from 128 to 165 mg/100g, with sample A having the highest calcium level. The high calcium levels in sample A may be due to high proportion of millet and African locust bean fruit pulp flour. The iron and zinc content were also higher in sample A (60:10:30), with the lowest percentage of soybean.

The functional properties of the formulated food materials are shown in Table 3. The results showed that bulk density ranged from 0.61 to 0.66 g/ml, water absorption capacity ranged from 1.89 to 2.31% swelling capacity ranged from 0.67 to 2.45%, least gelation ranged from 9.0 to 11.62%. The result showed that the samples possess low bulk density, which is an advantage in the preparation of complementary food, because high bulk limits the caloric and nutrient intake per feed of a child (Onimawo and Egbekun, 1998; Omueti et al., 2009). The low bulk density is economical in food packaging. The water absorption capacity is also at lower level for all the samples. The water absorption capacity is an index of the maximum amount of water that a food product would absorb and retain (Ijarotimi and Keshinro, 2012). With respect to water absorption capacity, Giami and Bekehand (1992) reported that microbial activities of food

Table 2. Proximate and mineral composition of millet, soybean and locust bean fruit pulp blends.

Parameter	Sample A (60:10:30)	Sample B (60:20:20)	Sample C (50:40:10)	Sample D (50:30:20)	Sample E (50:20:30)
Moisture (%)	10.5 ± 0.14 ^a	10.5 ± 0.14 ^a	7.5 ± 0.21 ^c	8.0 ± 0.14 ^b	11.0 ± 0.49 ^a
Protein (%)	28.9 ± 0.14 ^d	32.7 ± 0.14 ^c	37 ± 0.20 ^a	35 ± 0.02 ^b	31.6 ± 0.21 ^c
Fat (%)	3.00 ± 0.20 ^d	4.5 ± 0.02 ^b	5.1 ± 0.02 ^a	4.8 ± 0.20 ^c	4.46 ± 0.20 ^c
Ash %	2.5 ± 0.02 ^a	2.0 ± 0.02 ^b	1.4 ± 0.14 ^c	2.1 ± 0.14 ^b	2.51 ± 0.02 ^a
Crude Fibre (%)	3.00 ± 0.02 ^a	2.5 ± 0.02 ^b	2.0 ± 0.02 ^c	2.51 ± 0.02 ^b	3.2 ± 0.02 ^a
Carbohydrate (%)	52.10 ± 0.22 ^a	47.80 ± 2.00 ^b	47.0 ± 0.02 ^e	47.59 ± 0.02 ^c	47.2 ± 0.02 ^e
Energy (Kcal/g)	351 ^c	362.5 ^c	381.9 ^a	373.56 ^b	355.61 ^d
Calcium (mg/100g)	165 ± 0.2 ^a	160 ± 0.2 ^c	128 ± 0.4 ^e	159 ± 0.1	163 ± 0.2 ^b
Iron (mg/100g)	5.8 ± 0.2 ^a	4.8 ± 0.1 ^b	3.2 ± 0.2 ^d	4.7 ± 0.2 ^b	4.7 ± 0.2 ^c
Zinc (mg/100g)	3.2 ± 0.01 ^a	2.6 ± 0.02 ^d	2.7 ± 0.01 ^c	2.9 ± 0.2 ^b	2.93 ± 0.2 ^b

Means with different superscripts on horizontal line are significantly (P<0.05) different.

Table 3. Functional properties of millet, soybean and locust bean fruit pulp blends.

Parameter	Sample A (60:10:30)	Sample B (60:20:20)	Sample C (50:40:10)	Sample D (50:30:20)	Sample E (50:20:30)
Bulk Density (g/ml)	0.65 ± 0.05 ^a	0.60 ± 0.05 ^c	0.63 ± 0.02 ^b	0.6 ± 0.02 ^c	0.65 ± 0.05 ^a
Water absorption capacity (g/g)	2.19 ± 0.2 ^c	1.96 ± 0.02 ^d	2.04 ± 0.02 ^b	1.89 ± 0.2 ^e	2.31 ± 0.02 ^a
Swelling capacity (%)	0.67 ± 0.04 ^c	2.22 ± 0.03 ^d	2.45 ± 0.02 ^a	2.42 ± 0.03 ^b	2.41 ± 0.02 ^c
Least Gelation (%)	9.00 ± 0.02 ^d	11.50 ± 0.04 ^c	11.62 ± 0.01 ^b	11.61 ± 0.03 ^b	11.51 ± 0.02 ^c

Means with different superscripts on horizontal line are significantly (P<0.05) different.

Table 4. Sensory attributes of complementary foods from millet, soybean and African locust bean fruit pulp (P=0.05).

Parameter	Sample A (60:10:30)	Sample B (60:20:20)	Sample C (50:40:10)	Sample D (50:30:20)	Sample E (50:20:30)
Taste	6.87 ^c	7.23 ^c	6.73 ^c	7.03 ^c	7.93 ^a
Mouthfeel	7.00 ^c	7.46 ^b	6.86 ^c	7.46 ^b	7.93 ^a
Colour	6.40 ^a	6.0 ^a	7.00 ^c	7.60 ^a	7.80 ^a
Overall acc	6.93 ^a	7.33 ^c	6.80 ^d	7.60 ^b	7.93 ^a

Means with different superscripts on horizontal line are significantly (P<0.05) different.

products with low water absorption capacity would be reduced. Hence the shelf life of such product would be extended. The swelling capacity is used in the determination of the amount of water that food samples can absorb and the degree of swelling within a given time. The gelling properties indicated that the products can be used as complementary food formulation.

The results of sensory evaluation are shown in table 4. The results showed that there are significant (<0.05) differences in all the attributes tested for the samples. However sample E (50:20:30) with 50% millet, 20%

soybean and 30% African locust bean fruit pulp was more acceptable to the panelists on all the attributes tested.

Conclusions

The study has shown that acceptable complementary food can be produced from composites of millet, soybean and African locust bean fruit pulp. This study has also shown that composites of millet, soybean and African locust bean fruit pulp are nutritionally adequate and

possess good functional and sensory properties, which are required for the preparation of complementary foods for infants and children.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Meat quality analysis of Djallonke lambs co-infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Trichostrongyloidea) and treated with two medicinal plants

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Received 19 January, 2018; Accepted 20 March, 2018

Gastrointestinal nematode infection adversely affects small ruminant productivity all over the world, especially in tropical countries. A study was carried out to evaluate the extent to which concurrent infection with these nematodes may influence lamb meat quality, and how phytotherapy might improve these parameters. During the trial, 24 male Djallonke lambs (Age: 3 to 5 months) were experimentally infected with *Teladorsagia circumcincta* (4000 infective larvae) and *Trichostrongylus colubriformis* (10000 infective larvae) and treated from day 26 post infection with methanol extracts of *Harungana madagascariensis* and *Momordica foetida* twice daily. Lambs were divided into 8 groups: An untreated (Group 1), Albendazole 7.5 mg/kg (Group 2), *H. madagascariensis* extract at 125 mg/kg (Group 3), *H. madagascariensis* extract at 250 mg/kg (Group 4), *H. madagascariensis* at 500 mg/kg (Group 5), *M. foetida* extract at 125 mg/kg (Group 6), *M. foetida* 250 mg/kg (Group 7), and *M. foetida* at 500 mg/kg (Group 8). Meat quality attributes of lambs from different treatment groups were evaluated. Treatment with different doses of plant extracts led to differences in the level of parasitic load at necropsy. This effect was accompanied by an elevated pH₂₄ in meat from the most severely infected lambs and a concomitant decrease in their chemical composition. This high pH₂₄ equally had significant influence on meat flavour, overall acceptability and water retention of lamb meat. The present study suggested that nematode infection may influence pH of resulting carcass, nutritional composition and some sensory parameters of meat while treatment using *M. foetida* at 500 mg/kg maybe important in reducing parasite burden while enhancing quality of subsequent lamb carcass.

Key words: Phytotherapy, meat quality, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, *Harungana madagascariensis*, *Momordica foetida*, Djallonke lambs.

INTRODUCTION

Infection with gastrointestinal nematodes has a worldwide distribution, occurring in both tropical and subtropical areas of the world (Biu et al., 2012b; Mohamed et al., 2016). In Cameroon, gastrointestinal helminth infections are very prevalent, especially in the Western High Lands of the country where the rainfall pattern and ambient temperature favour the growth and multiplication of parasite (Ndamukong et al., 1989). Field trials carried out by Mbafor et al. (2014) in West Region of Cameroon indicated that *Trichostrongylus* and *Teladorsagia* species are among the most common nematodes of sheep in the Western Highlands, with a high prevalence of 93.3 and 53.3%, respectively. During primary infection with *Teladorsagia* spp., dysplasia of the abomasal glands and a reduction in acid-secreting cells occur as the nematodes colonise the stomach mucosa (Fox, 1997; Rinaldi et al., 2011). Severe villus atrophy (decreased villous: crypt ratios), goblet cell hyperplasia as well as sloughing of enterocytes into the intestinal lumen occur with establishment of *Trichostrongylus colubriformis* larvae (Roy et al., 2004). Characteristic dark foul smelling diarrhoea, anorexia, diminished absorptive capacity or poor food conversion efficiency are the consequences of the destruction of the mucosal architecture (Williams and Palme, 2012; Rajib et al., 2014). It has been reported that co-infections with *Teladorsagia* and *Trichostrongylus* spp. has a synergistic effect (Steel et al., 1982; Sykes et al., 1988). The combined effect of anorexia, malabsorption and diarrhoea may result in loss of quantity and quality of resulting carcass (Mushi et al., 2007).

Nowadays, consumers are highly interested in the quality of the products they eat, especially when this refers to meat (Guerrero et al., 2013). Furthermore, meat quality is of significant importance for consumers' health (Ivanov et al., 2017). It has been reported that infections with gastrointestinal nematodes affect some meat characteristics, including the meat color stability, composition and conformation of small ruminants (Arsenos et al., 2009; Zhong et al., 2015).

Although, chemical anthelmintics have long been used in combating helminthiasis, their use is increasingly discredited due to the increasing resistance of these nematode to most classes of anthelmintics available, drug residues in animal products and loss of productivity in host animals (Zhong et al., 2015., Jackson and Coop, 2000; Tsiboukis et al., 2013). The general swing towards the use of natural compounds has stimulated research into their use as anthelmintic alternatives. Thus, many studies have explored the use of plant-derived phenolic

compounds, especially tannins, which show direct and indirect anthelmintic activity for gastrointestinal nematode control in ruminants (Vargas-Magaña et al., 2014; Zhong et al., 2015).

Momordica foetida (Cucurbitaceae) and *Harungana madagascariensis* (Hypericaceae) are two medicinal plants widely known for their anthelmintic and appetite stimulating potentials (Pavan et al., 2013; Olukayode et al., 2008). Boronkini et al. (2012) carried out a phytochemical analysis of both plants and reported that they are rich in secondary metabolites such as tannins, flavonoids and alkaloids. In addition, *M. foetida* contains lipids and lipophilic compounds which have been used in fattening domestic herbivores (Oloyede and Aluko, 2012). Furthermore, giving ruminants polyphenolic plants could improve meat quality by increasing antioxidants into the circulatory system, which when assimilated and retained in the meat, they will improve sensory characteristics such as flavor and juiciness scores of meat (Qwele et al., 2013; Moyo, et al., 2014; Zhong et al., 2015).

It is clear that infection with *T. circumcincta* and *T. colubriformis* may depress appetite, reduce nutrient utilization and deplete glycogen reserves, thus influencing meat quality. However, the extent to which these nematodes can influence meat quality remains doubtful (Zhong et al., 2015). Moreover, if this is true, can administration of plant extracts improve nutrient utilization and the resultant meat quality after slaughter? With these questions in mind, the present study was designed to evaluate changes in the nutritional value of meat from lambs co-infected with *T. circumcincta* and *T. colubriformis*, and how treatment use with methanol extracts of *M. foetida* and *H. madagascariensis* may improve these parameters.

METHODOLOGY

Study animals

A total of 24 male Djallonke sheep were used in this study. Ages of the animals ranged between 4 and 5 months. Dentition pattern as described by Samad (2008) was used to determine ages of experimental animals. These animals were acquired from two sheep farmers in Fongo Tongo village, 40 km away from Dschang town. Two weeks before arrival, all the animals in the two farms were vaccinated against Small Ruminant Pests (SRP) using Capri-pestovax and were also treated with anthelmintics (levamisole injection 10 mg/kg and Albendazole tablets 10 mg/kg). Two days after arrival of animals in the experimental animal house of the Faculty of Agronomy and Agricultural Sciences of the University of

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Dschang, experimental animals were given antibiotics (Oxytetracycline 1 ml/10 kg body weight), anticoccidia (Toltrazuril, 10 mg/kg), multivitamin and anti-stress (Stress vita 2.5 ml/10 kg body weight). They were allowed to acclimatize for 14 days and their faecal samples examined daily within this period to confirm that animals were helminth free by the use of the concentrated salt flotation technique as described by Thienpont et al. (1986) and Baker (2007). After this adaptation period, all animals were inoculated with the same dose of 14000 L₃ larvae as follows: day 1: 5.000 L₃ of *T. colubriformis* per animal using a syringe via the oral route. Day 2: 5.000 L₃ of *T. colubriformis* and day 3: 4.000 L₃ of *T. circumcincta*. Animals were randomly allocated to eight sub groups of 3 individuals on day 26 post inoculation and treated as follows: Group 1, Untreated group (received the vehicle 3.2% dimethyl sulphoxide); Group 2, Positive control group (Albendazole 7.5 mg/kg); Group 3, *H. madagascariensis* 125 mg/kg; Group 4, *H. madagascariensis* 250 mg/kg; Group 5, *H. madagascariensis* 500 mg/kg; Group 6, *M. foetida* 125 mg/kg; Group 7: *M. foetida* 250 mg/kg; Group 8, *M. foetida* 500 mg/kg body weight. Each group was placed in separate pens of dimension 7.5 m² (3 m × 2.5) on slatted floors. A solid partition separated adjacent pens and care was taken to avoid contamination of pens with nematode larvae from outside sources. All animal groups were served 6 kg of the same fresh forage (mainly *Tripsacum laxum* and *Leucaena leucocephala*) harvested 24 h before feeding, washed in clean water, dried in the sun for 2 h and chopped before serving to the animals. Water was provided *ad libitum*. The weight of the animals was taken weekly and weight of food consumed by each group was recorded daily.

Selection of plants

An ethnobotanic study permitted the selection of two medicinal plants with anthelmintic and appetite stimulating potentials. They were harvested in April 2016, identified and authenticated at the National Herbarium of Cameroon in Yaounde by voucher specimen N° 46105/HNC for *H. madagascariensis* and N° 24393/HNC for *M. foetida*. The bark of *H. madagascariensis* and whole aerial parts of *M. foetida* were shade dried at room temperature. The dried plant parts were powdered mechanically using a commercial electrical blender and stored in airtight plastic bags at 4°C until preparation of extracts.

Preparation of methanol extracts

Four hundred and fifty grams of each plant powder were macerated in 3 L of methanol. The mixture was stirred for 10 min and stored for 72 h at room temperature. This solution was sieved through Whatman No. 1 filter paper, divided into portions of 200 ml each then evaporated in a rotavapor at 50°C. The dry extracts were stored at 4°C until used for *in vivo* assays.

Treatment of experimental lambs

Treatment with plant extracts was administered on day 26 post inoculation and subsequently twice daily for 4 days. Albendazole 7.5 mg/kg was administered on the last day of treatment. On day 56 post inoculation (30 days post treatment), all 24 animals were humanely sacrificed, the slaughtering was carried out by severing the jugular vein and the carotid arteries to ensure proper bleeding (Kefyalew et al., 2013). The lambs were bled within 3 min after slaughter and suspended to remove their skin, head (at the occipito-atlantal joint), forefeet (at the carpal-metacarpal joint), and

hind feet (at the tarsal-metatarsal joint). The viscera including the gastrointestinal tract were removed.

Meat quality analysis

Meat samples from *longissimus dorsi* muscle were taken between 6 and 11th ribs of each animal and used for physical and technological evaluation while meat from the left hind leg was used for chemical and sensory evaluation.

Physical evaluation

Determination of pH₂₄

To determine pH₂₄, 10 g of *I. dorsi* muscle from each carcass was weighed using an electronic balance (max 6000 g, d = 0.1 g) and refrigerated at 0°C for 24 h. The ultimate pH₂₄ was evaluated after thawing by inserting the sharp tip of a portable pH meter (Testo 205, Testo AG, Lenzkirch, Germany) to a depth of about 2 cm into each meat sample. The pH value was then read directly on the pH meter.

Evaluation of technological properties of meat

Drip loss

The drip loss was evaluated following the procedure described by Honikel (1998). One hour after slaughter, 100 g of muscle was weighed and put in nylon net bags. Each bag was hung over a beaker at 4°C for 24 h, after which the samples were removed, dabbed dry on a serviette and reweighed. Percentage drip loss was calculated using the formula:

$$\text{Drip loss (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100 \quad (2)$$

Cook-out loss

The cook-out-loss was evaluated as described by Paisentier et al. (2003). To this effect, 50 g of *I. dorsi* muscle was weighed and put inside weighed Ziploc bags. These were immersed in a thermostatic water bath at 75°C for 15 min, after which the muscles were removed, allowed to cool to room temperature for about 1 h, dabbed on a paper serviette and re-weighed. The percentage cook-out-loss from each sample was calculated as follows:

$$\text{Cook-out-loss (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100 \quad (3)$$

Freezing loss

This was determined based on the procedure described by Paisentier et al. (2003). To this effect, 33 g of *I. dorsi* muscle from each lamb carcass was weighed into zip lock plastic bags. These were frozen at -20°C for 14 days. They were then removed and allowed to thaw at room temperature, after which each sample was dabbed dry and re-weighed. Percentage freezing loss was calculated as follows:

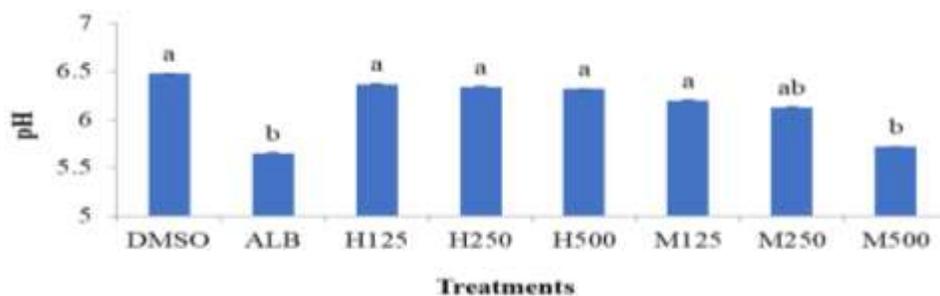


Figure 1. Influence of treatment using methanol extracts of *Harungana madagascariensis* and *Momordica foetida* on pH₂₄ of meat from lambs co-infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. ^{ab}Bars with different letters are significantly different. DMSO: Dimethyl sulphoxide (3.2%); Alb: Albendazole 7.5 mg/kg; H125: *Harungana madagascariensis* 125 mg/kg; H250: *Harungana madagascariensis* 250 mg/kg; H500: *Harungana madagascariensis* 500 mg/kg; M125: *Momordica foetida* 125 mg/kg; M250: *Momordica foetida* 250 mg/kg; M500: *Momordica foetida* 500 mg/kg.

$$\text{Freezing loss (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Evaluation of chemical composition of lamb meat

Meat samples were minced and dried in a ventilated oven at 105°C for 20 to 24 h when a constant weight was attained. Moisture content was calculated as the difference in weight before and after drying. Crude protein, crude lipid and ash contents were estimated on dry matter basis as according to the AOAC (2005). The results were expressed as percentages.

Minerals analysis

Calcium (Ca), magnesium (Mg), and iron (Fe), were determined using Atomic Absorption Spectrophotometer (GBC atomic absorption spectrophotometer, Australia) according to Pauwels et al. (1992) and AOAC (2005). The values of Mg, Ca, and P were determined as mg/g dry weight, while the value of Fe was measured as mg/kg dry weight.

Evaluation of sensory properties

Sensory characteristics of meat were determined after slaughter of animals using 20 test panellists. Test panellist was selected among students of the Department of Animal Production, Faculty of Agronomy and Agricultural Sciences (FASA), University of Dschang. Screening exercises were administered prior to panel selection in order to diagnose the most qualified panel members. These included taste identification and taste intensity exercises, using score charts adapted from Abd El-aal and Suliman (2008) and Dimple and Rohanie (2014). After selection of the most qualified test panellists, samples of meat were sliced into 2.0 cm steaks, put in zip lock bags based on different treatment groups and boiled for 45 min using an electric cooker. The panellists were served with samples of meat in different trays. To evaluate these sensory characteristics, a 5-point scale adapted from Abd El-aal and Suliman (2008) and Dimple and Rohanie (2014) was used.

Sensory attributes assessed included tenderness, juiciness, flavour intensity, and overall acceptability as follows: (tenderness: 5 = tender, 1 = very tough; juiciness: 5 = juicy, 1 = very dry; aroma: 5 = very good flavour, 1 = off flavour; general acceptability: 5 = highly desirable, 1 = extremely poor).

Statistical analysis

Statistical analysis was performed using SPSS version 20.0. To test the effects of treatment on studied parameters, one-way analysis of variance (ANOVA), General Linear Model approach, was used. Pearson correlation coefficient was used to establish the relationship among adult worm burden and pH₂₄ of lamb meat. The post hoc test employed for all analysis was Duncan's Multiple Range Test. Results obtained were expressed as mean ± standard deviation. Probability values P<0.05, was considered significant.

RESULTS

Results showing the variation in adult worm burden recovered at necropsy from the abomasums and intestines of lambs co-infected with *T. circumcincta* and *T. colubriformis* and treated with albendazole and methanol extracts of *H. madagascariensis* and *M. foetida* in Germaine et al. (2017).

Figure 1 summarizes the influence of treatment using methanol extracts of *H. madagascariensis* and *M. foetida* on meat ultimate pH (pH₂₄).

Ultimate pH₂₄ in lamb was observed to be significantly lower (P<0.05) in albendazole treated lambs and those treated with *M. foetida* at dose rate 500 mg/kg when compared with untreated group. Treatment with *H. madagascariensis* extracts at all tested doses and *M. foetida* at doses less than 500 mg/kg had no significant influence (P>0.05) on meat pH₂₄ when compared with untreated lambs. There was a strong positive correlation

Table 1. Influence of treatment using methanol extracts of *Harungana madagascariensis* and *Momordica foetida* on technological properties of lamb meat co-infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*.

Treatment	Cooking loss (%)	Freezing loss (%)	Drip loss (%)
Vehicle (DMSO 3.2%)	11.14 ± 1.44	9.66 ± 0.04	7.17 ± 0.50 ^b
Alb7.5 mg/kg	11.33 ± 2.32	10.31 ± 1.26	9.84 ± 2.82 ^a
H125 mg/kg	11.31 ± 1.50	9.31 ± 1.43	7.32 ± 1.71 ^b
H250 mg/kg	12.16 ± 2.25	9.16 ± 0.68	7.55 ± 0.39 ^b
H500 mg/kg	11.18 ± 1.15	9.46 ± 1.02	7.69 ± 3.39 ^b
M125 mg/kg	11.70 ± 1.65	9.85 ± 0.79	7.80 ± 2.21 ^b
M250 mg/kg	10.51 ± 1.85	9.44 ± 1.88	7.73 ± 0.75
M500 Mg/kg	11.51 ± 0.43	10.05 ± 1.89	8.70 ± 1.74 ^{ab}
P-value	0.56	0.21	0.01

^{abc}Mean values followed by the different letters on the same columns are significantly different ($P < 0.05$). DMSO: Dimethyl sulphoxide (3.2%); Alb: Albendazole 7.5 mg/kg; H125: *Harungana madagascariensis* 125 mg/kg; H250: *Harungana madagascariensis* 250 mg/kg; H500: *Harungana madagascariensis* 500 mg/kg; M125: *Momordica foetida* 125 mg/kg; M250: *Momordica foetida* 250 mg/kg; M500: *Momordica foetida* 500 mg/kg.

Table 1 shows the effects of treatment on technological properties of lamb carcasses. There was no significant difference between all groups of animals concerning cooking loss and freezing loss. However, significant differences ($P \leq 0.05$) in drip loss was found among the different animal groups, with those treated with *M. foetida* 500 mg/kg and albendazole allowing drip significantly more ($P < 0.05$) than all other experimental groups.

Influence of treatment using methanol extracts of *H. madagascariensis* and *M. foetida* on chemical properties of meat is summarized in Table 2. The lipid content showed no significant variation ($P > 0.05$) between the different animal groups. Protein and ash contents showed a reverse trend, being significantly higher in albendazole 7.5 mg/kg, *H. madagascariensis* 500 mg/kg, *M. foetida* 250 mg/kg and *M. foetida* 500 mg/kg treatment groups. This significant difference observed in ash content was more pronounced in the results of individual mineral analysis. Meat from lambs treated with albendazole 7.5 mg/kg, *H. madagascariensis* 500 mg/kg, *M. foetida* 250 mg/kg and *M. foetida* 500 mg/kg recorded significantly higher, calcium, magnesium and phosphorus contents when compared with the untreated lambs. However, no significant difference ($P > 0.05$) in iron content across the different groups was recorded during this study.

Influence of treatment using methanol extracts of *H. madagascariensis* and *M. foetida* on sensory characteristics of lambs co-infected with *T. circumcincta* and *T. colubriformis* is summarized in Table 3. There was a significantly higher flavour liking of meat from lambs treated with albendazole and *M. foetida* 500 mg/kg than all other groups. The overall acceptability result closely matched that of flavour liking, with meat from albendazole and *M. foetida* 500 mg/kg treated lambs being the most acceptable. It was noticed in this experiment that meat from all animal groups was equally tender and juicy.

DISCUSSION

In this study, methanol was used for extraction because of its ability to extract almost all the chemical components of plants due to its high polarity index (Eloff, 1998). Since almost all identified components like tannin, phenol and alkaloids that are active against helminthes are aromatic or saturated organic compounds, they are often obtained through initial extraction with methanol or ethanol (Vilegs et al., 1997; Khadijah, 2015).

The pH of muscle at post mortem is the main indicator of meat quality at a commercial level (Cvrtila Fleck et al., 2015). The present results revealed that pH₂₄ differed significantly between treatment groups. It was observed that apart from lambs that received albendazole and *M. foetida* 500 mg/kg, lambs in all other treatment groups (*M. foetida* 125 mg/kg, *M. foetida* 250 mg/kg, *H. madagascariensis* 125 mg/kg, *H. madagascariensis* 250 mg/kg, *H. madagascariensis* 500 mg/kg) and the untreated group had significantly higher pH₂₄ (pH ≥ 6). Phillips and Wheeler (2008) reported that ultimate pH₂₄ (that is after the post mortem biochemical changes involving the depletion of muscle glycogen are completed) in lamb tends to be higher in animals subjected to chronic stress. Reference has already been made to the fact that the pH₂₄ of the best quality lamb meat tends to fall in the pH range of 5.3 to 5.8 (Beriaïn et al., 2000). The higher pH₂₄ could thus be attributed to depleted glycogen reserves, owing to the fact that sheep severely infected with *T. circumcincta* and *T. colubriformis* have reduced feed intake and significantly lower food utilization ability (Steel et al., 1982; Stear et al., 2003; Rinaldi et al., 2011; Germaine et al., 2017). Abd El-aal and Suliman (2008) had indicated that ultimate pH is affected by factors such as diet and prolonged hunger before slaughter. Stress has a marked negative impact

Table 2. Influence of treatment using methanol extracts of *H. madagascariensis* and *Momordica foetida* on chemical properties of lamb meat, co-infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*.

Chemical composition	Treatment								P
	DMSO (Vehicle)	Albendazole	Har 125	Har 250	Har 500	Momo 125	Momo 250	Momo 500	
Proteins (g)	19.70 ± 0.59 ^b	21.76 ± 0.18 ^a	19.30 ± 0.23 ^b	20.38 ± 0.14 ^{ab}	21.33 ± 0.10 ^a	20.70 ± 0.35 ^{ab}	21.27 ± 0.49 ^a	21.43 ± 0.65 ^a	0.04
Ash(g)	2.08 ± 0.06 ^b	2.90 ± 0.14 ^a	2.04 ± 0.02 ^b	2.17 ± 0.19 ^b	2.82 ± 0.11 ^a	2.11 ± 0.11 ^b	2.69 ± 0.42 ^a	2.75 ± 0.15 ^a	0.00
Lipids (g)	3.82 ± 0.33	3.94 ± 0.72	3.36 ± 0.75	3.97 ± 1.11	3.32 ± 0.67	3.72 ± 0.34	3.80 ± 0.44	3.84 ± 0.67	0.08
Moisture (g)	75.38 ± 0.65 ^a	73.52 ± 0.92 ^b	75.66 ± 0.33 ^a	75.57 ± 0.27 ^a	74.48 ± 0.34 ^{ab}	76.28 ± 0.44 ^a	75.14 ± 0.28 ^a	73.61 ± 0.33 ^b	0.03
Ca (mg)	6.73 ± 0.05 ^b	7.28 ± 0.1 ^a	6.8 ± 0.1 ^b	6.73 ± 0.05 ^b	7.33 ± 0.05 ^a	6.76 ± 0.05 ^b	7.06 ± 0.15 ^a	7.16 ± 0.05 ^a	0.05
Mg (mg)	20.33 ± 3.21 ^b	27.33 ± 0.30 ^a	23.13 ± 1.15 ^b	23.00 ± 2.64 ^b	26.13 ± 0.81 ^a	23.60 ± 2.62 ^b	25.73 ± 0.25 ^a	27.93 ± 0.47 ^a	0.00
Fe (mg)	1.72 ± 0.05	1.93 ± 0.25	1.72 ± 0.06	1.73 ± 0.15	1.83 ± 0.09	1.70 ± 0.10	1.76 ± 0.05	1.88 ± 0.10	0.09
Ph (mg)	175.67 ± 13.05 ^b	197.67 ± 2.51 ^a	183.67 ± 22.54 ^{ab}	192.33 ± 11.06 ^a	200.00 ± 5.29 ^a	171.00 ± 3.00 ^b	200.00 ± 1.00 ^a	204.00 ± 1.00 ^a	0.04

^{a,b,c}Means with the same letters on the same columns are not significantly different ($P > 0.05$). DMSO: Dimethyl sulphoxide (3.2%); Alb: Albendazole 7.5 mg/kg; H125: *Harungana madagascariensis* 125 mg/kg; H250: *Harungana madagascariensis* 250 mg/kg; H500: *Harungana madagascariensis* 500 mg/kg; M125: *Momordica foetida* 125 mg/kg; M250: *Momordica foetida* 250 mg/kg; M500: *Momordica foetida* 500 mg/kg.

not only on meat pH, but concomitantly on technological properties of resulting carcass. High pH₂₄ affects the water binding nature of the proteins and therefore directly affects the water holding capacity of meat (Huff-Lonergan et al., 2010). During this study, it was observed that meat from lambs in the untreated group had an unusually higher water holding capacity, this being evident by their lower drip loss than albendazole and *M. foetida* 500 mg/kg treated lambs. These results are in agreement with the report of Cheng et al. (2008) who indicated that high pH meat does not readily allow drip, thus resulting to dry, firm and dark meat. Again, Phillips and Wheeler (2008) recorded high water holding capacity corresponding to carcass with the highest pH.

This high water holding capacity has a major disadvantage of retaining more water than necessary, leading to more rapid deterioration due to microbial growth; hence, such meat will have a shorter shelf life (Huff-Lonergan et al., 2010). It

was observed during our investigations that there was no significant difference between all groups of animals concerning cooking loss and freezing loss. Our results are in conformity with those obtained by Abd El-aal and Suliman (2008) and Fonteh et al. (2015) who equally recorded no significant difference in cooking loss and freezing loss of meat in groups of animals exposed to different stress levels. It was observed in this trial that carcass from different treatment groups with different levels of gastrointestinal adult worm burden had significantly different levels of moisture and crude protein while the lipid content showed no significant variation. Meat from lambs treated with plant extract at doses less than 500 mg/kg had a higher water content (>75 g) and significantly lower protein content (<20 g) per 100 g of meat. These values deviated from the standards reported by Sinclair et al. (1990) and Williams et al. (2006). These authors indicated that the protein content of 100 g of lamb meat is

between 21.5 and 22.5% and moisture content is approximately 73.5%. The high moisture content could be attributed to increase level of stress due to gastrointestinal parasitaemia. Page et al. (2001) reported that stress promotes accumulation of intracellular water which reflects less light and causes the muscle to appear dark. On the other hand, the lower protein content could be attributed to their significantly lower ability of feed utilization when compared with healthy animals. Anorexia and and/or reduction in food conversion efficiency has been observed in sheep co-infected with *T. colubriformis* and *T. circumcincta* (Steel et al., 1982; Stear et al., 2003; Rinaldi et al., 2011; Germaine et al., 2017).

Few studies have compared the chemical composition of meat from lambs with different levels of gastrointestinal nematode parasitism. However, the present study report agrees with Valieva et al. (2014) who observed significantly higher protein and fat content in healthy

Table 3. Effect of treatment using methanol extracts of *Momordica foetida* and *Harungana madagascariensis* on sensory characteristics of lamb co-infection with *Teladorsagia circumcincta* and *Harungana madagascariensis*.

Treatment	Tenderness	Juiciness	Flavour	General acceptability
Vehicle (DMSO)	2.67 ± 1.55	3.88 ± 0.90	2.68 ± 0.58 ^b	3.00 ± 0.78 ^c
Alb 7.5mg/kg	3.3 ± 1.02	3.67 ± 0.58	4.33 ± 0.00 ^a	4.50 ± 0.00 ^a
H125 mg/kg	2.67 ± 0.58	3.0 ± 1.73	2.67 ± 0.58 ^b	3.00 ± 1.00 ^c
H250 mg/kg	2.67 ± 0.57	4.00 ± 1.00	2.67 ± 0.58 ^b	3.33 ± 0.58 ^c
H500 mg/kg	3.33 ± 1.15	4.33 ± 0.58	3.0 ± 1.0 ^{ab}	3.67 ± 0.58 ^{ab}
M125 mg/kg	2.68 ± 1.52	3.33 ± 0.57	2.33 ± 0.58 ^b	3.33 ± 0.58 ^c
M250 mg/kg	3.0 ± 0.00	4.33 ± 0.57	3.67 ± 0.58 ^a	3.33 ± 1.15 ^c
M500 mg/kg	4.33 ± 0.58	4.33 ± 0.58	4.00 ± 0.00 ^a	4.33 ± 0.58 ^a
P-value	0.48	0.53	0.02	0.03

^{a,b,c}Means with the same letters on the same columns are not significantly different ($P > 0.05$). DMSO: Dimethyl sulphoxide (3.2%); Alb: Albendazole 7.5 mg/kg; H125: *Harungana madagascariensis* 125 mg/kg; H250: *Harungana madagascariensis* 250 mg/kg; H500: *Harungana madagascariensis* 500 mg/kg; M125: *Momordica foetida* 125 mg/kg; M250: *Momordica foetida* 250 mg/kg; M500: *Momordica foetida* 500 mg/kg.

sheep as compared to those suffering from echinococcosis. Again, Sykes et al. (1988) observed that infection with nematode parasites have an adverse effect upon nitrogen retention, a feature that has been confirmed by the lower protein content of carcass from sheep co-infected with *T. colubriformis* or *T. circumcincta* than in pair-fed controls. This reduced digestibility contributes to the poorer overall economy of such carcasses with lower protein content. The latter is primarily a reflection of the lower efficiency with which they utilize apparently digested nitrogen. Furthermore, Bermingham et al. (2007) had reported that more skeletal muscle protein may need to be degraded and mobilized to supply increased amino acid demands of the intestine and liver in sheep infected with *T. colubriformis*.

The ash and mineral contents varied between the different treatment groups. Lambs treated with albendazole, *H. madagascariensis* 500 mg/kg, *M. foetida* 500 mg/kg, and *M. foetida* 250 mg/kg (with lower worm burden) had significantly higher calcium, magnesium and phosphorus hence overall ash content. Knox et al. (2006) suggested that the metabolism of mineral ions is modified by the presence of gastrointestinal nematodes. Sykes and Coop (1976) supported this fact by indicating that skeletal calcium and phosphorus in meat from severely infected lambs may be 15 to 45% lower than in uninfected animals.

Many consumers judge meat quality from its sensory characteristics. In this study, co-infection with *T. circumcincta* and *T. colubriformis* had no significant effect on carcass tenderness and juiciness. However, significant differences were recorded for flavor and overall acceptability. The most preferred carcasses were from albendazole and *M. foetida* 500 mg/kg treated lambs. This could be attributed to pH differences after slaughter. Carcass pH has a significant effect on flavor and overall

acceptability, with high pH carcass producing undesirable meat with slightly soapy off flavor (Miller, 2007). The most dominant sulphur (S) compound in cooking meat volatiles is H_2S (Young et al., 1993). These authors further explained that as meat pH increases, more H_2S is evolved during cooking. Their data indicate that a pH increase from 5.6 to 6.6, for example, would increase the evolution of H_2S by about 60%. H_2S has its own odour and is also involved in a reaction that generates the volatile and very odorous compound thiophenol (benzene thiol). Ha and Lindsay (1991) and Lopez and Lindsay (1993a) showed that thiophenol is the source of unpleasant flavour in cooking sheep meat, because thiophenol's precursor, phenylphosphate, is almost unique to sheep. Thus, either directly or indirectly, high pH meat might result in enhanced off flavours, hence less desirable by test panelists.

Conclusion

Parasitism due to co-infection with *T. circumcincta* and *T. colubriformis* had a significant influence on meat pH₂₄ with meat from most severely infected lambs (lambs with the highest worm burden) showing the highest pH₂₄. This high pH₂₄ had significant influence on meat flavour, overall acceptability and water retention ability of lamb meat. Furthermore, severely infected lambs were inferior in chemical composition, having lower protein and mineral content than healthy lambs, while treatment with methanol extracts of *M. foetida* at 250 and 500 mg/kg and *H. madagascariensis* at 500 mg/kg led to an improvement in meat chemical composition. However, further studies are needed to evaluate the muscle energy status or lactic acid concentration in the muscles of lambs with different levels of gastrointestinal parasitism in order

to confirm these results.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to all the sheep farmers and traditional healers in Menoua Division who gave them valuable information concerning the plant species they use as traditional anthelmintic and the methods of preparing and administration of each plant species. Further appreciation goes to the Laboratory of Animal Biology and Applied Ecology, Laboratory of Animal Production and Animal Health, Laboratory of Soil Sciences, all second and third year students of the Department of Animal Production in the University of Dschang for providing the environment, resources and expertise that made this work successful.

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Full Length Research Paper

Comparison of traditional butter preservation techniques using microbial and organoleptic properties, West Shewa, Ethiopia

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Received 16 March, 2018; Accepted 26 April, 2018

The study was conducted in Dire Inchini and Ambo districts of West Shewa, Oromia, Ethiopia to assess traditional butter preservation methods and compare their efficiency. Semi-structured questionnaire was prepared, pretested and used to interview 120 women respondents having experience in butter making. Butter preservation methods identified were ghee making (100%), spicing (98.33%), melted butter (29.17%) and salting (11.67%). Commonly used spices were *Trachyspermum ammi*, *Nigella sativa*, *Aframomum angusti-folium*, *Trigonella foenum*, *Zingiber officinale* and *Allium sativum*. Based on the survey, 7 kg of fresh butter samples were purchased from open market in the two districts and taken to Holeta Dairy laboratory and randomly allocated to each treatment namely traditional ghee, untreated, salted, spiced, melted, frozen (-20°C) and refrigerated butter (4°C). Microbial and organoleptic qualities of butter were analyzed at one month interval for 3 consecutive months. Microbial qualities of the samples were substandard; but, traditional ghee and salting were more efficient. Optimization of utilization of spices and comprehensive evaluation including oxidative deterioration is vital.

Key words: Traditional, butter, preservation, microbial, organoleptic quality.

INTRODUCTION

Like most sub Saharan Africa countries, Ethiopia is unable to meet the increasing demand for dairy products for its increasing population (Azage et al., 2000; Tsehay, 2001). Smallholder farmers and pastoralists produce and supply 98% of the total annual milk production (Yonad, 2009). The majority of milk produced in rural areas of

Ethiopia is processed into milk products at household level using traditional technologies (Muriuki and Thorpe, 2001). In rural areas, 40% of milk produced is spontaneously fermented for three to four days without addition of specific starter culture and is churned to make butter, butter milk and whey as a byproduct. Rural

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producers are forced to produce butter due to limited market outlet, shorter shelf life of milk, lower price for whole milk, ease of handling of butter and for product diversification (Ayantu, 2006; Kassahun, 2008). Traditional butter ferments slowly at ambient temperature, offering rural consumers a relatively shelf stable dairy product (LMP, 2007). According to Getachew (2003), of the total butter production, 80% is used as food ingredient and the remaining is used for hair dressing and other purposes. The same study revealed that 70% of butter produced in the country is used in rural areas; while 30% is channeled to the Addis Ababa market. Therefore, butter contributes much to the dietary requirements of the society and saves milk from spoilage and diversifies its use (FAO, 1990). Workneh and Ulfina (2011) observed the significant importance of butter in Ambo area of West Shoa Zone and indicated that 75% of dairy producers in Ambo area process milk into butter. However, in Ethiopia, the climate is hot and humid; leading dairy products to spoil easily during storage unless cooled or treated with preservatives. Moreover, commercial preservatives are not readily available in rural areas. Cooling systems are also not feasible because of lack of infrastructure (O'Mahoney and Peters, 1987). In rural areas of Ethiopia, producers use different traditional preservation methods to increase shelf life of butter (Lemma, 2004; Mekdes, 2008). The local preservatives are used as a principle of acidification and moisture reduction and can make butter of good storage stability (O'Mahony and Peters, 1987). However, traditional butter preservation techniques and their efficiency in these districts were not studied. Therefore, the study was initiated to assess traditional butter preservation techniques and to compare their efficiency through evaluation of microbial and organoleptic properties.

MATERIALS AND METHODS

Description of the study areas

The study was conducted in Ambo and Dire Inchini districts of West Shoa Zone of Oromia, Ethiopia which is located at 105 km to the west of Addis Ababa, Ethiopia.

Ambo district

It is located between 80 47'N -90 21'N and 370 32'E and located at 115 km to the West of Addis Ababa (ADADO unpublished data, 2014). It has a mean annual temperature and rain fall of 23 to 25°C and 1300 to 1700 mm, respectively. The altitude ranges between 500 and 3,200 m above sea level (ADADO Unpublished Data, 2014).

Dire Inchini district

It is located at 162 km to the West of Addis Ababa and 50 km from Ambo (Tamrat, 2007). The average annual rainfall ranges between 1000 and 1400 mm. Average minimum and maximum temperatures are 8.8 and 21.6°C, respectively (Tamrat, 2007).

Sampling techniques

The survey was conducted in three major ecologies of the districts as follows: Ya'i cabo (high altitude), Gosu qora (mid altitude), Sankele and Farisi '(low land) and, Waldo hindhe (high land), Nano Jidu (mid altitude) and Toke Abuye (low land) from Ambo and Dire Inchini district, respectively.

Data collection

Survey

One hundred twenty respondents who own at least one lactating cow and having experience in traditional butter preservation techniques were purposively selected. Semi structured questionnaire was prepared, pretested and used for individual interview by trained enumerators. The questionnaire focused on butter preservatives and preservation methods. The collected data was entered into SPSS computer program.

Laboratory analysis

Collection of butter samples

Seven kilograms of butter was bought from open markets of the two districts to assess the microbial and organoleptic qualities. The purchased butter samples were kept in an icebox and transported to Holeta dairy laboratory within 4 h of collection. The samples were thoroughly mixed to form composite sample which was divided into seven equal weights each treatment receiving one kilogram of butter. The samples were randomly allocated to seven treatments namely traditional ghee, spiced, salted, melted, untreated, frozen (-20°C) and butter stored at 4°C. All analysis was done using duplicate samples.

Preparation of treatments

The samples were tested for their microbial and organoleptic qualities at 0, 1, 2 and 3 months of preservation. The treatments were prepared as follows.

Traditional ghee/'Nitir Kibe'

One kilogram butter was placed in a sauce pan and melted over a slow heating stove. White cumin, fenugreek, korerima, ginger, garlic, turmeric, black cumin and other herbs such as basil and other herbs of desirable aroma such as rue (*Ruta graveolence*), basil (*Ocimum spp.*) and 'Kussayyee' (*Ocimum hardiense*) aroma were added to the boiling butter fat based on the respondents' experience. The melting butter fat and spices were stirred while boiling until foaming had stopped. Finally, the sauce pan was removed from the stove and left aside until it was settled. The butter fat was filtered through metal sieve into high density poly ethylene bucket and kept at room temperature for a period of 3 months.

Spiced butter

One kilogram of butter was removed from composite butter sample and thoroughly mixed with about 45 g of fenugreek and black cumin powders, respectively. Based on the recommendation of the respondents, spiced butter sample was placed in high density poly ethylene bucket and kept at room temperature for 3 months.

Salted butter

One kilogram butter was thoroughly mixed with 30 g of NaCl and kept in high density poly ethylene bucket and was placed at room temperature for 3 months.

Melted butter/'Nigur kibe'

To make melted butter one kilogram of butter was placed in sauce pan and kept on a slow heating stove and stirred until the butter was completely melted. The melted butter was removed from the heat source and kept in cool place to settle down and left there for overnight until it has completely solidified. Then, impurities that were settled at the bottom of the sauce pan were decanted off by opening the solidified butter in sauce pan from one side. Then the sample was placed in high density poly ethylene bucket and kept at room temperature for 3 months.

Untreated butter

One kilogram of fresh butter sample was kept in high density poly ethylene bucket and was placed at room temperature for 3 months.

Frozen butter

One kilogram of butter sample was kept in high density poly ethylene bucket and placed in deep freeze at -20°C for 3 months.

Refrigerated butter

One kilogram of composite butter sample was kept in high density poly ethylene bucket and placed in refrigerator at 4°C for 3 months.

Each time at 0, 1, 2 and 3 months of preservation, required amount of sample was removed for analysis of aerobic mesophilic bacteria, total coliforms, total lactic acid bacteria, Enterobacteriaceae and yeast and mold counts and color, texture, odor and overall acceptability. The analysis of each treatment was performed in duplicates.

Microbial analysis

Aerobic mesophilic bacterial count: The butter samples were homogenized and aseptically transferred to stomacher bag and held for melting on a water bath adjusted at 46°C. Then, the samples were immediately serially diluted by adding 1 ml of butter into 9 ml of peptone water. One milliliter of the sample from a chosen dilution was placed on the Petri dish using pour plating technique. Then, plate count agar media of 15 to 20 ml was poured onto the petri-dish and thoroughly mixed with the sample and allowed to solidify for 15 min and incubated for 48±2 h at 35°C. Finally, the colonies were manually counted. The plate counts were calculated by multiplying the count on the dish by 10^n , in which n stands for the number of consecutive dilutions of the original sample (FAO, 1997; Michael and Joseph, 2004; FSSAI, 2012).

Total coli forms count: Samples were decimally diluted and plated with violet red bile agar media /VRBA into Petri dishes for enumeration of total coliforms bacteria as coliforms colony forming units per milliliter. Plates were incubated at $t 32\pm 1^\circ\text{C}$ for 24±2 h. One milliliter of melted sample was serially diluted using peptone water and transferred into sterile Petri-dishes. 10 to 15 ml of violet red bile agar media tempered to a temperature of 44 to 46°C was added to the milk sample and thoroughly mixed and allowed to solidify for 5 to 10 min. The mixture was then overlaid with the same

plating agar media of 3 to 4 ml to inhibit surface colony formation. The medium were allowed to solidify. The plates were inverted and incubated at $32 \pm 1^\circ\text{C}$ for 24±2 h. Counts were made manually. Finally, the plate counts were calculated as N, the number of colony forming units of coliforms per milliliter of milk sample using the formula $N = \sum c / (n_1 + n_2) d$ where $\sum c$ = Sum of all colonies on all plates counted were conducted according to the standard procedures of FAO (1997), Michael and Joseph (2004) and FSSAI (2012).

Lactic acid bacteria counts: 0.1 ml of appropriate decimal dilutions of butter sample was poured on Petri dishes in duplicates and mixed with MRS agar media. Then, after incubating the plates anaerobically at 30°C for 48 h, lactic acid bacteria were counted (FAO, 1997; Michael and Joseph, 2004; FSSAI, 2012).

Yeast and mold counts: Potato dextrose agar (PDA) media was autoclaved for 15 min at 121°C and tempered in water bath adjusted at 45°C. Appropriate decimal dilutions of butter sample (0.1 ml) were poured into a 15 x 90 ml Petri dishes and mixed with 20 ml of PDA containing antibiotic; chloramphenicol solution. Then, after incubation at 25°C for 5 days, yeast and mould were counted with plates containing 10 to 150 colonies (FAO, 1997; Michael, 2004; FSSAI, 2012).

Enterobacteriaceae count: One milliliter of homogenized melted butter sample was added into 9 ml peptone water to yield a dilution of 1:10 cfu/ml. Violet red bile glucose agar (VRBGA) medium was used to enumerate Enterobacteriaceae. The mixture was then overlaid with the same plating agar media of 3 to 4 ml. Plates were aerobically incubated for 24 h at 37°C and inspected for purple-red colonies surrounded by a purplish circle of light or halo color. The plate counts were calculated by multiplying the count on the dishes by 10^n , where n stands for the number of consecutive dilutions of the original sample (ILSI, 2011, FSSAI, 2012).

Organoleptic quality of butter: Organoleptic quality were evaluated by 10 semi-trained sensory panelists using 5 point hedonic rating scale where 1=dislike extremely, 2= dislike slightly, 3= neither like nor dislike, 4= like moderately and 5 = like extremely. The samples were coded for identification purpose.

Data analysis

Survey and organoleptic quality data were analyzed using descriptive statistics using SPSS (2011). Microbiological counts were transformed to log₁₀ cfu/g and analyzed using the General Linear Model of SAS version 9.1 (SAS, 2009). Least significant difference was used to test the differences between treatment means and time of preservation.

RESULTS AND DISCUSSION**Traditional butter preservation techniques in the study areas**

Traditional methods of butter preservation in Dire Inchini and Ambo districts were presented in Table 1. In the study areas, 100, 98.33, 29.17 and 11.67% of interviewed smallholder producers commonly preserve butter in the form of traditional ghee, spiced, melted and salted butter, respectively. This finding is in agreement with previous reports in Arsi Negele, Oromia, Walayita, Southern Ethiopia, North Western Ethiopia and East Wollega, Ethiopia by Lemma et al. (2004), Mekdes (2008), Eyassu (2014) and Alganesh and Yetenayet (2016), respectively.

Table 1. Traditional methods of butter preservation in the study areas.

Preservation method	Dire Inchini (N=60)		Ambo (N=60)		Overall mean (N=120)	
	N	%	N	%	N	%
Traditional ghee	N	%	N	%	N	%
Mixing with spices	60	100	60	100	120	100
Melting	58	96.67	60	100	118	98.33
Salting	20	33.33	15	25	35	29.17

N = Number of respondents.

Table 2. Spices used for butter preservation techniques in the study areas.

Local name	Common name	Scientific name	N (%)
Habasuuda adii	White cumin	<i>Trachyspermum ammi</i>	120 (100)
Ogiyoo	Kororima	<i>Aframomum angusti-folium</i>	98(81.67)
Abishii	Fenugreek	<i>Trigonella foenum</i>	85(70.83)
Jinjibila	Ginger	<i>Zingiber officinale</i>	71(59.17)
Qullubbii adii	Garlic	<i>Allium sativum</i>	61(50.83)
Irdii	Turmeric	<i>Curcuma domestica</i>	3(2.5)
Qarafaa		<i>Cinnamomum verum</i>	3(2.5)
Dimbilaala	Basil	<i>Cordiandrum sativum</i>	1(0.83)
Qullubbii diimaa	Onion	<i>Allium cepa</i>	3(2.5)
Qundeberbere	Black peper	<i>Piper nigrum</i>	2(1.67)
Habasuuda gurraacha	Black cumin	<i>Nigella sativa</i>	104(86.67)

N= number of respondents.

In the study areas in wet season and during Ethiopian Orthodox fasting time, demand for butter is low and during such occasions, surplus butter is preserved using traditional preservation techniques. In the present study, spiced, melted and salted butter are used as raw materials for traditional ghee making and they are optional butter preservation techniques.

Spices used for ghee making and spicing butter (spiced butter)

The result for the spices used for spiced butter and traditional ghee making in the study areas were indicated in Table 2. For traditional ghee making smallholder producers in the study areas mainly use *Trachyspermum ammi*, *Aframomum angusti-folium*, *Nigella sativa*, *Trigonella foenum*, *Zingiber officinale*, *Allium sativum* and *Curcuma domestica* is used for coloring purpose. While for making spiced butter, they use *Trigonella foenum* and *Nigella sativa*. Similar study by Joe et al. (2009) stated that spices are used to enhance aroma, flavor and for preservation of food substances.

Microbial properties of butter preserved using traditional preservation methods

Aerobic mesophilic bacterial count

The mean total bacterial count (log cfu/g) for the

treatments and preservation time is presented in Table 3. The aerobic mesophilic bacterial counts of butter preserved using traditional ghee, salted and spiced butter at 0, ends of 1, 2 and 3 months of preservation did not show significant difference at $P < 0.05$. While aerobic mesophilic bacterial counts of melted, untreated, frozen and butter stored in refrigerator at 4°C showed significant difference among treatments at 0, ends of 1, 2 and 3 months at $P < 0.05$ level of significance. The total aerobic mesophilic bacterial counts for traditional ghee, salting, spicing, melted, untreated, frozen and refrigerated butter (4°C) showed significant difference among the treatment means at $P < 0.05$ level of significance. At initial preservation time, greater counts of aerobic mesophilic bacterial counts was observed in salted (9.58 log cfu/g) and melted butter (9.65 log cfu/g), which were significantly different ($P < 0.05$) from other treatments. This might be due to the poor hygiene of the salt used in preserving the butter. In the case of melted butter, the increase in the aerobic mesophilic bacterial count might probably be as a result of post melting contamination. At initial preservation period, the mean aerobic mesophilic bacteria in untreated butter were 8.71 log cfu/g of butter samples. The current result is far beyond the maximum tolerable limit of 6 log cfu/g of aerobic mesophilic bacteria counts set by Standards Authority of Ethiopia (QSAE, 2009). The sensory attributes used to evaluate butter samples in this experiment were odor, texture, color and overall acceptability. So there was no risk on the health

Table 3. Mean total aerobic mesophilic bacterial counts (log cfu/g) of traditionally preserved butter.

Variable	Time (month)	Traditional ghee	Salted butter	Spiced butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter	Maxlimit set by ESA
Aerobic mesophilic bacterial	0	9.48±0.08 ^{ab}	9.39±0.09 ^{ab}	9.58±0.05 ^{ab}	9.65±0.02 ^{ab}	8.71±0.03 ^{bc}	8.71±0.02 ^{acd}	8.71±0.02 ^{bd}	6
	1	9.62±0.03 ^{aA}	9.37±0.06 ^{ab}	9.62±0.04 ^{ab}	9.72±0.05 ^{ab}	9.61±0.03 ^{ab}	9.59±0.09 ^{ac}	9.59±0.09 ^{ab}	
	2	9.64±0.04 ^{aA}	9.66±0.06 ^{aA}	9.84±0.04 ^{aA}	9.23±0.06 ^{bc}	9.68±0.04 ^{ab}	9.61±0.04 ^{ab}	9.63±0.05 ^{ab}	
	3	9.73±0.06 ^{aA}	9.78±0.03 ^{ac}	9.89±0.06 ^{aA}	9.93±0.03 ^{aA}	9.98±0.04 ^{aA}	9.24±0.04 ^{bd}	9.97±0.03 ^{ab}	

Means followed by similar lower case letters in a column are not significantly different ($P<0.05$), Means followed by similar upper case letters in a row are not significantly different ($P<0.05$). ESA: Ethiopian Standard Authority.

Table 4. Mean total lactic acid bacteria counts (log cfu/g) of traditionally preserved butter.

Variable	Time (month)	Traditional ghee	Salted butter	Spiced butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter
Total lactic acid bacteria	0	6.65±0.09 ^{aA}	6.55±0.05 ^{aA}	6.72±0.06 ^{aA}	6.80±0.28 ^{aA}	6.77±0.03 ^{aA}	6.77±0.08 ^{aA}	6.77±0.07 ^{aA}
	1	6.06±0.06 ^{bc}	6.31±0.06 ^{abc}	6.09±0.13 ^{bd}	6.40±0.05 ^{bc}	6.65±0.09 ^{ab}	6.23±0.04 ^{ab}	6.59±0.04 ^{ab}
	2	5.56±0.06 ^{cc}	5.19±0.09 ^{cc}	5.72±0.05 ^{cc}	5.26±0.07 ^{cc}	6.60±0.07 ^{ab}	6.28±0.08 ^{ab}	6.12±0.10 ^{ac}
	3	5.09±0.11 ^{cd}	5.01±0.17 ^{cd}	5.53±0.04 ^{bd}	5.01±0.14 ^{cd}	6.32±0.07 ^{ac}	6.04±0.10 ^{ac}	6.06±0.10 ^{ad}

Means followed by similar lower case letters in a column are not significantly different ($P<0.05$), Means followed by similar upper case letters in a row are not significantly different ($P<0.05$).

and safety of the panelists since they evaluated the butter by smelling to check for the odor, checked the texture using smoothness, solidity and appropriate degree of firmness by their hands and visual observation of the color of the butter samples. The current result is also similar to a report from Wolayita in Southern Ethiopia of 8.10 log cfu/g of butter samples of total bacterial count (Mekdes, 2008). After one month of preservation, aerobic mesophilic bacterial counts of 9.37 log cfu/g was observed and this was significantly different ($P<0.05$) from the other treatments. Relatively lower mean aerobic mesophilic bacterial counts were observed in frozen butter, traditional ghee and spiced butter. This might be due to the antagonistic effects of active ingredients

of spices, heat treatment and low temperatures in spiced butter, traditional ghee, melted butter, butter kept at 4 and -20°C , respectively on bacterial growth and survival. Similar studied by Kilcast and Subramaniam (2000) confirmed that shelf life of products can be extended by the use of processing treatments such as heat and radiation which kills the microorganisms or control of microbial growth by chilling, freezing, reducing the water content and addition of preservatives.

Lactic acid bacterial count

The mean total lactic acid bacteria counts (log cfu/g) of treatments and preservation time are

described in Table 4. There were significant differences ($P<0.05$) between total lactic acid bacterial counts in traditional ghee, salted, spiced and melted butter at 0, ends of 1, 2 and 3 months of preservation. While the total lactic acid bacterial counts of butter preserved using untreated, frozen (-20°C) and refrigerated (4°C) butter showed no significant difference at $P<0.05$ among the means at 0, 1, 2 and 3 months of preservation, respectively. Total lactic acid bacterial counts at 0 and 1 month of preservation showed no significant ($P<0.05$) difference for traditional ghee, salted, spiced, melted, untreated, frozen and refrigerated (4°C) butter samples. While, the total lactic acid bacterial counts at the end of the second month of preservation did not show significant ($P<0.05$)

Table 5. Mean yeast and mold counts (log cfu/g) of traditionally preserved butter.

Variable	Time (month)	Traditional ghee	Salted butter	Spiced butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter	Max. limit set by ESA
Yeast and molds	0	5.70±0.08 ^{aB}	6.54±0.03 ^{bC}	5.74±0.05 ^{aC}	5.56±0.03 ^{dD}	6.70±0.05 ^{bD}	6.70±0.04 ^{aA}	6.70±0.05 ^{aB}	1
	1	5.70±0.04 ^{aB}	6.67±0.03 ^{aB}	6.36±0.05 ^{aA}	5.78±0.09 ^{cC}	6.74±0.03 ^{cC}	6.46±0.05 ^{bB}	6.72±0.06 ^{aB}	
	2	6.16±0.08 ^{aA}	6.69±0.04 ^{aB}	6.28±0.08 ^{aB}	6.28±0.07 ^{bB}	6.78±0.03 ^{aB}	6.14±0.10 ^{bC}	6.76±0.05 ^{aA}	
	3	6.19±0.08 ^{aA}	6.78±0.05 ^{aA}	6.37±0.06 ^{aA}	6.71±0.07 ^{aA}	6.84±0.05 ^{aA}	6.42±0.14 ^{bC}	6.79±0.04 ^{aA}	

Means followed by similar lower case letters in a column are not significantly different from each other ($P < 0.05$), Means followed by similar upper case letters in a row are not significantly different from each other ($P < 0.05$). ESA: Ethiopian Standard Authority

difference among traditional ghee, salted, spiced, melted and refrigerated butter (4°C) except for untreated and frozen butter (-20°C). Whereas, at the end of the third month of preservation, the mean total lactic acid bacterial counts in traditional ghee, salted, spiced, melted and refrigerated butter samples did not show significant ($P < 0.05$) difference among their mean counts except for untreated and frozen butter which did not significantly ($P < 0.05$) differ from each other. The mean total lactic acid bacterial counts of the treatments at initial time of preservation ranged from 6.55 to 6.80 log cfu/g. This could be due to prior fermentation of composite sample as local butter is usually made of spontaneously fermented whole milk. At initial time of preservation, the mean lactic acid bacterial count in traditional ghee was 6.06 log cfu/g and it significantly differed at $P < 0.05$ from other treatments; except for salted butter which was 6.09 log cfu/g of butter samples. The current result is also similar with the finding of Mekdes (2008) who reported mean lactic acid bacterial count of 7.51 log cfu/g for butter sample collected from Wolayita in Southern region. From the second to third months of preservation time, mean lactic acid bacterial count of spiced, melted butter and traditional ghee were relatively lower and significantly differed ($P < 0.05$) from other

treatments. This might be due to heat treatment and antimicrobial effects of spices used in melted butter, traditional ghee and spiced butter, respectively.

Yeast and mold counts

The result for the mean yeast and mold counts (log cfu/g) of treatments and preservation time is indicated in Table 5. The mean yeast and mold counts during initial 0, 1, 2 and 3 months of preservation for traditional ghee, spiced and butter refrigerated at 4°C did not significantly ($P < 0.05$) differ from each other. The mean yeast and mold counts (log cfu/g) of salted, spiced, melted, untreated and frozen butter samples significantly differed ($P < 0.05$) from each other. The mean yeast and mold counts (log cfu/g) of traditional ghee, salted, spiced, melted, untreated, frozen and refrigerated butter samples did not show significant difference ($P < 0.05$) between the treatment means. Mean yeast and mold counts of butter at initial preservation time for traditional ghee (5.70 log cfu/g), salted butter (5.74 log cfu/g) and melted butter (5.56 log cfu/g) significantly differed ($P < 0.05$) from spiced, untreated, frozen (-20°C) and refrigerated (4°C) butter. This might be

attributable to the effect of heat treatment; antimicrobial properties of spices used to treat butter samples and reduced water activity in salted butter. The temperature range for yeast and mold growth is 0 to 47°C, out of which, the growth of yeast and mold can be hampered. This is in agreement with Seriler (2003) who revealed the possibility of reducing mold growth on the surface of butter by salting. The mean yeast and mold count of untreated butter sample at initial time of preservation was 6.70 log cfu/g. The current finding is beyond the maximum tolerable limit of 1 log cfu/g of yeast and mold count of butter sample recommended by the Ethiopian Standards Authority (QSAE, 2009). The present result is also higher than the mean yeast and mould count of 5.58 log cfu/g of butter samples reported in Wolayita Southern Ethiopia (Mekdes, 2008). Increasing trends of yeast and mold counts have been observed from one month of preservation period to the end in untreated, refrigerated (at 4°C), spiced and melted butter and these results were significantly different ($P < 0.05$) from other treatments. In the case of spiced butter poor hygiene of spices purchased from open market might have contributed to the high rate of contamination. In untreated and refrigerated butter samples water activity might have been high and

Table 6. Mean total coliform counts (log cfu/g) of traditionally preserved butter.

Variable	Time (month)	Traditional ghee	Salted butter	Spiced butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter	ESA
Total coliform	0	5.70±0.08 ^{aA}	6.54±0.03 ^{aB}	5.74±0.05 ^{aC}	5.56±0.03 ^{aD}	6.70±0.05 ^{aB}	6.70±0.04 ^{aA}	6.70±0.05 ^{aA}	Absent
	1	5.70±0.04 ^{aA}	6.67±0.03 ^{aB}	6.36±0.05 ^{aB}	5.78±0.09 ^{aC}	6.74±0.03 ^{bC}	6.46±0.05 ^{aA}	6.72±0.06 ^{aA}	
	2	6.16±0.08 ^{aA}	6.69±0.04 ^{aB}	6.28±0.08 ^{aB}	6.28±0.07 ^{aB}	6.78±0.03 ^{aB}	6.14±0.10 ^{aA}	6.76±0.05 ^{aA}	
	3	6.37±0.08 ^{aA}	6.96±0.11 ^{aA}	6.54±0.05 ^{aC}	6.86±0.09 ^{aB}	6.98±0.09 ^{aA}	6.34±0.07 ^{aA}	6.62±0.05 ^{aA}	

Means followed by similar letters in a column are not significantly different from each other ($P<0.05$); means followed by similar bold letters in a row are not significantly different from each other ($P<0.05$). ESA: Ethiopian standard Authority.

have created favorable environment for the growth of yeast and molds.

Total coliform counts

Mean total coliforms count (log cfu/g) for the treatments and time of preservation is presented in Table 6. There were no significant difference ($P<0.05$) between the mean total coliforms bacterial counts of butter preserved using traditional ghee, salted, frozen and refrigerated butter at 0, 1, 2 and 3 months of preservation, while, the total coliforms bacterial counts of spiced, melted and untreated butter showed significant ($P<0.05$) difference among treatments at 0, 1, 2 and 3 months of preservation, respectively. Mean total coliform counts at initial time of preservation for traditional ghee, spiced, melted, untreated, frozen and refrigerated butter samples did not differ ($P<0.05$) significantly except for salted butter. The mean total coliforms counts at the end of one month of preservation for traditional ghee, spiced, melted, frozen and refrigerated butter samples were not significantly different at $P<0.05$ except for salted and untreated butter. While the mean total coliform counts at the end of one month of preservation for salted and untreated butter samples did not significantly

($P<0.05$) differ from each other. At the end of second month of preservation, the mean total coliforms counts of traditional ghee, spiced, melted, frozen and refrigerated butter samples did not significantly ($P<0.05$) differ from each other except for salted and untreated butter. At the end of the third month of preservation, the mean total coliforms counts of traditional ghee, spiced, melted, frozen, refrigerated, salted and untreated butter samples did not significantly ($P<0.05$) differ from each other. Mean total coliform count of untreated butter at initial preservation period was 5.62 log cfu/g of butter sample. The current result is by far beyond the mean total coliform count of 2 log cfu/g of butter samples collected from Wolayita zone (Mekdes, 2008). From two months of preservation period and onwards, relatively decreasing trends of total coliform count were observed in traditional ghee, frozen and salted butter compared to other treatments. This might be associated with the inhibitory effects of heat treatment, low storage temperature and addition of salt in ghee, butter kept at -20°C and in salted butter, respectively.

Enterobacteriaceae count

Mean Enterobacteriaceae count (log cfu/g) for the

treatments and preservation time is presented in Table 7. There were no significant difference ($P<0.05$) between mean Enterobacteriaceae counts of traditional ghee, salted, spiced, melted, frozen and refrigerated butter except for untreated butter at 0, 1, 2 and 3 months of preservation, respectively. The mean Enterobacteriaceae counts of butter samples for traditional ghee, spiced, melted and frozen butter at 0 month of preservation did not significantly ($P<0.05$) differ from each other. While the mean Enterobacteriaceae counts of butter samples for salted, untreated and refrigerated butter at initial preservation time significantly ($P<0.05$) differed from other treatments but the mean counts for untreated and refrigerated butter did not significantly differ from each other. The mean Enterobacteriaceae counts of samples for traditional ghee, spiced, melted and frozen butter at the end of one month did not significantly ($P<0.05$) differ from each other. While, the mean Enterobacteriaceae counts of samples for salted, untreated and refrigerated butter at initial preservation time significantly ($P<0.05$) differed from other treatments. But the mean counts for untreated and refrigerated butter did not significantly differ from each other. The mean Enterobacteriaceae counts of butter samples at the end of second months of preservation for

Table 7. Mean Enterobacteriaceae counts (log cfu/g) of traditionally preserved butter.

Variable	Time (month)	Traditional ghee	Salted butter	Spiced butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter	EUS limit
Enterobacteriaceae	0	4.26±0.04 ^{aA}	6.70±0.07 ^{aB}	5.83±0.06 ^{aA}	6.27±0.03 ^{aA}	5.07±0.08 ^{cC}	5.07±0.33 ^{aA}	5.07±0.31 ^{aC}	3
	1	5.79±0.08 ^{aB}	6.84±0.03 ^{aA}	5.94±0.06 ^{aB}	6.42±0.03 ^{aB}	5.64±0.04 ^{aC}	5.57±0.04 ^{aB}	5.67±0.05 ^{aC}	
	2	5.84±0.06 ^{aA}	6.52±0.03 ^{aB}	5.97±0.06 ^{aA}	6.57±0.07 ^{aB}	6.23±0.09 ^{aB}	5.69±0.04 ^{aB}	5.96±0.11 ^{aA}	
	3	6.12±0.17 ^{aA}	6.43±0.03 ^{aB}	6.23±0.10 ^{aA}	6.61±0.06 ^{aA}	6.71±0.06 ^{aA}	5.69±0.09 ^{aA}	6.36±0.08 ^{aA}	

Means followed by similar lowercase letters in a column are not significantly different from each other ($P < 0.05$); Means followed by similar uppercase letters in a row are not significantly different from each other ($P < 0.05$). EUS: European Standard.

traditional ghee, spiced, untreated and refrigerated butter did not significantly ($P < 0.05$) differ from each other. While, the mean Enterobacteriaceae counts of butter samples for salted, melted and frozen butter at the end of second months of preservation significantly ($P < 0.05$) differed from other treatments. However, at the end of third months of preservation, the mean Enterobacteriaceae counts of traditional ghee, spiced, untreated, melted, refrigerated and frozen samples did not significantly differ ($P < 0.05$) except for salted butter. During the initial preservation time, relatively smaller mean Enterobacteriaceae count of 4.26 log cfu/g of butter was observed in traditional ghee compared to other treatments. This might be ascribed due to the heat treatment and moisture removal from butter during ghee making. A report by Mattick et al. (2001) stated that some thermo tolerant Enterobacteriaceae comprising a sub-group of mesophiles are capable of growth at up to 44°C, with an optimum growth temperature of 37°C. Fellows (2008) also reported that ghee is preserved by a combination of heat which destroys enzymes and contaminant microorganisms by removing moisture from the butter oil to prevent microorganisms growing during storage. Samaraweera et al. (2001) confirmed that lowering moisture content substantially reduces the growth rate of some Enterobacteriaceae. During the initial preservation

time, relatively higher counts of Enterobacteriaceae of 6.70 log cfu/g was observed in spiced butter than in other treatments. This might be attributable to poor hygienic status of spices purchased from open market. Throughout the preservation period, relatively smaller mean Enterobacteriaceae count was observed in frozen, refrigerated butter and traditional ghee compared to the other treatments. This could be explained in terms of the inhibitory effects of low storage temperatures in refrigerated and frozen butter and heat treatment in traditional ghee. Mattick et al. (2001) confirmed that cooling of food to normal refrigeration temperatures of 0 to 8°C inhibits Enterobacteriaceae growth in storage facilitates. Rhea (2009) also reported that deep freeze retards the growth of undesirable microorganisms and proper salting of butter removes moisture droplets and negatively affects the growth of undesirable microorganisms.

Organoleptic quality of butter preserved using traditional preservation techniques

Organoleptic quality of butter at the beginning of preservation

The hedonic rating scale at the end of one month of preservation for color, texture and odor is

presented in Table 8. The hedonic rating scale of color, texture and odor of butter samples preserved using traditional ghee; salted, spiced, melted, untreated, frozen and refrigerated butter is presented in Figure 1. At the initial time of preservation, color, texture and odor of traditional ghee, spiced, salted, melted, untreated and butter stored in deep freeze and in refrigerator at 4°C were in acceptable range except for spiced butter which was relatively lower compared to others. This might be attributable to darkening of the color of spiced butter due to mixing with spices such as black cumin. At initial time of preservation, traditional ghee and salted butter were extremely liked compared to untreated, butter stored in refrigerator at 4°C and in deep freezer which were liked moderately. The color and odor of traditional ghee and salted butter were extremely liked. Except in spiced butter the color of butter in the other treatments were extremely liked.

Organoleptic quality of butter at the end of one month of preservation

The hedonic rating scale at the end of one month of preservation for color, texture and odor is presented in Table 8. At the end of one month of preservation, the color, texture and odor of butter samples for traditional ghee, spiced, salted,

Table 8. Mean scores for descriptive sensory attribute of butter treated under different preservation methods.

Sensory attribute	Duration (months)	Preservation technique						
		Traditional ghee	Spiced butter	Salted butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter
Odor	0	4.70±0.15 ^a	3.50±0.40 ^b	4.70±0.15 ^a	4.00±0.39 ^{ab}	4.20±0.25 ^{ab}	4.20±0.25 ^{ab}	4.20±0.25 ^{ab}
	1	4.50±0.22 ^a	3.10±0.23 ^b	4.80±0.13 ^a	3.70±0.34 ^b	3.10±0.23 ^b	4.50±0.23 ^a	3.70 ±0.34 ^b
	2	4.43±0.20 ^a	2.71±0.36 ^b	3.71±0.36 ^a	2.14±.34 ^{bc}	1.57±0.30 ^c	4.14±0.26 ^a	3.71 ±0.18 ^a
	3	4.85±0.14 ^a	1.71±0.42 ^c	3.43±0.30 ^b	1.71±0.36 ^c	1.43±0.30 ^c	3.86±0.40 ^b	3.57 ±0.20 ^b
Texture	0	4.30±0.26 ^a	3.10±0.23 ^b	4.40±0.22 ^a	4.50±0.17 ^a	4.30±0.15 ^a	4.30±0.15 ^a	4.30 ± 0.15 ^a
	1	4.50±0.17 ^a	3.50±0.40 ^b	4.70±0.21 ^a	3.50±0.40 ^b	4.50±0.17 ^a	4.50±0.17 ^a	3.50 ± 0.40 ^b
	2	4.43±0.20 ^a	3.00±0.38 ^{bc}	3.71±0.42 ^{ab}	2.71±0.29 ^{bc}	2.29±0.42 ^c	3.14±0.26 ^b	3.00±0.31 ^b
	3	4.71±0.18 ^a	2.57±0.53 ^b	3.43±0.37 ^b	2.43±0.43 ^b	2.29±0.42 ^b	2.86±0.40 ^b	2.86 ±0.34 ^b
Color	0	4.70±0.21 ^a	2.40±0.27 ^c	4.70±0.21 ^a	3.70±0.34 ^b	4.50±0.22 ^a	4.50±0.22 ^a	4.50 ±0.22 ^a
	1	4.70±0.15 ^a	2.40±0.27 ^c	4.50±0.22 ^a	3.50±0.40 ^b	3.50±0.40 ^b	4.70±0.21 ^a	4.70 ±0.21 ^a
	2	4.86±0.14 ^a	1.71±0.36 ^c	4.14±0.14 ^{ab}	4.14±0.14 ^{ab}	1.86±0.34 ^c	1.71±0.29 ^c	3.57 ±0.20 ^b
	3	4.86±0.14 ^a	1.29±0.18 ^c	3.71±0.47 ^b	1.57±0.30 ^c	1.29±0.18 ^c	3.71±0.52 ^b	3.43 ±0.30 ^b

Means followed by similar letters in a row are not significantly different from each other ($P < 0.05$)

melted, untreated and butter stored in deep freeze and in refrigerator at 4°C were in acceptable range except for the color of spiced butter which ranged between dislike slightly to neither like nor dislike. At the end of one month of preservation, the sensory panelists extremely liked traditional ghee, salted, refrigerated and butter kept in deep freeze. While, spiced butter was neither liked nor disliked by the sensory panelists. Melted and untreated butter samples were moderately liked.

Organoleptic quality of butter at the end of two months of preservation

The hedonic rating scale result of color, texture and aroma of the treatments is presented in Table 8. At the end of two months of preservation, the sensory acceptability of spiced, melted and untreated butter highly deteriorated compared to others. Traditional ghee and salted butter were rated as extremely liked for their color, aroma and texture. This is in agreement with a report of Illingworth et al. (2009) that revealed that application of heat during preparation of ghee and removal of moisture and solid non-fat contribute to a product of unique color, flavor and texture.

Organoleptic quality of butter at the end of three months of preservation

The hedonic rating scale of the butter samples at the end of three months of preservation based on color, texture and odor is presented in Table 8. The color, texture and

odor of traditional ghee and salted butter were extreme and moderate likeness, respectively. While frozen and refrigerated butter were moderately liked by the sensory panelists. Whereas, the odor and texture of spiced butter is rated as neither like nor disliked. The color and aroma of melted and untreated butter were slightly disliked except for their texture.

Overall acceptance of traditionally preserved butter at the end of three months

The hedonic rating scale on the overall acceptance of color, texture and odor of butter samples at the end of three months of preservation is presented in Figure 1. Among all the treatments traditional ghee was extremely liked followed by salted butter which was rated between moderate and extreme likeness. At the end of three months, refrigerated and frozen butter were moderately liked by the sensory panels. The relative reduction in likeness in the overall acceptability of refrigerated and frozen might be attributable to the change in odor as a result of the metabolic and enzymatic activities of psychrophilic bacteria that can multiply under low temperature.

CONCLUSION AND RECOMMENDATION

Major traditional butter preservation methods in the present study are traditional ghee making, spicing, melting and salting. Major spices used in traditional ghee making are *Trachyspermum ammi*, *Aframomum angustifolium*

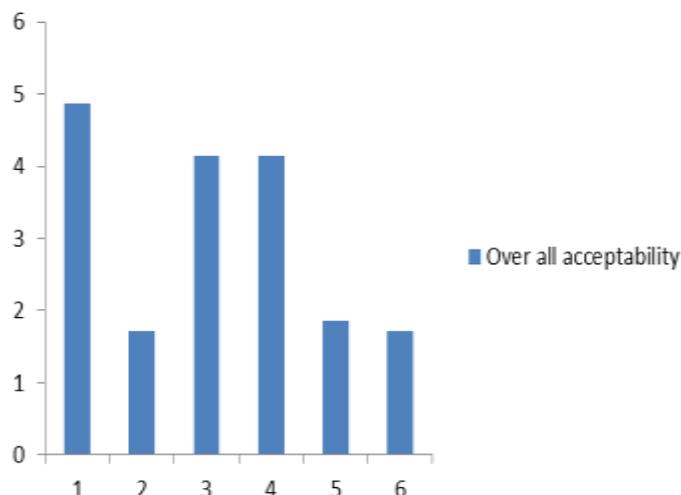


Figure 1. Overall acceptability of traditionally preserved butter at the end of three months of preservation.

folium, *Nigella sativa*, *Trigonella foenum*, *Zingiber officinale*, *Allium sativum* and *Curcuma domestica*. For spicing butter *Trigonella foenum* and *Nigella sativa* are mainly used. Microbial quality of the butter samples were substandard starting from initial preservation time. Moreover microbial and organoleptic properties of the samples deteriorated as the storage time elapsed except for traditional ghee and salting. Hygiene of spices, herbs and salt used to preserve butter should be kept to reduce contamination. Appropriate application level of spices and plants materials used per unit weight of butter need to be optimized. Moreover, comprehensive evaluation including oxidative deterioration of traditionally preserved butter is vital.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors would like to acknowledge the unreserved efforts made by Holeta dairy laboratory research team during the microbiological analysis and sensory evaluation.

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