ADAN J. Agric. 2023, 4: 119-134 DOI:10.36108/adanja/3202.40.0121

ISSN 2736-0385

Phytogenic effect of garlic (Allium sativum), ginger (Zingiber officinale) and roselle (Hibiscus sabdariffa) on the keeping quality of shredded meat

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Abstract
The qualities of meat during storage are deteriorated by microbial growth, oxidation and enzymatic autolysis. Garlic and ginger are recipes used in meat product preparation as antimicrobial and antioxidant substances. .A total of sixty 6-week old broiler chickens were sacrificed and meat removed from the breast muscle where meat chunks were randomly distributed into six treatments. Treatments comprised of shredded meat prepared with a standard recipe containing ginger and garlic (SR-GaG), standard recipe without ginger and garlic (mGaG), standard recipe with ginger (pGin), standard recipe with roselle (pRos), standard recipe with garlic (pGar) and standard recipe with vitamin E (pvE) replacing ginger and garlic respectively. Data were obtained for Moisture, Crude Protein, CP, Crude Fat, CF, Ash, Thiobarbituric Reactive Substances, TBARS) for 0, 7 and 14days refrigerated ($4^{o}C$) storage including Total Bacterial Count (TBC), Total Fungi Count (TFC) and Total Coliform Count(TCC) for 7, 14 and 21 days refrigerated storage. Data were subjected to analysis of variance using IBM SPSS version 21.. Reduced ($p < 0.05$) moisture content was observed in group mGaG followed closely by pGin group over 14 days of storage. Significantly higher ($p < 0.05$) CP was recorded in pGin and pvE groups. Reduced ($p < 0.05$) CF was observed in pvE group at 7^{th}day while pRos group showed reduced CF among the phytogenics and through the storage periods. The TBARS of pGin, pRos, and pGar reduced ($p <0.05$) along storage periods. The TBC, TCC, and TFC significantly reduced for shredded meat (mGaG and pGin) while elevated values were observed for other treatments across the storage periods. Listeria salivarius and Micrococcus varians were isolated in SR-GaG throughout the storage periods. Therefore, single addition of ginger and garlic to recipe of shredded meat is recommended for oxidative stability, improved meat quality and meat preservation.

Keywords: *phytogenics, coliform count, fungi count, bacteria count, shredded meat*

Introduction

Meat is a biological tissue consisting of water, amino acids, proteins, sugars, minerals, vitamins, peptides and nucleotides which provided a suitable medium for the growth of microorganisms (Henchion *et al*., 2014; Bohrer, 2017). The spoilage of meat could be attributed to microbial growth, oxidation and enzymatic autolysis (Seemat, 2015). Microbial infections are identified through mould, yeast and bacteria invasion of food materials causing undesirable odour and flavour. Enzymes' activities lead to destruction of polymers in some foods while oxidation and rancidity (chemical reaction) causes decomposition in others (Geng *et al*., 2023). Consumers' rejection or acceptance of meat product is determined by the product shelf-life (period of time during which food retains its qualitative attributes) which could be shortened by spoilage organisms (Dousset *et al*., 2016). However, the said spoiled meat may be safe to eat, without causing illness if there are no pathogens or presence of toxins (Seemat, 2015).

Dried meat products are the result of the simple dehydration or drying of lean meat in natural condition or in an artificially created environment. Shredded meat or meat floss, a traditional meat product originated from China is becoming a household name in Nigeria and can be categorized as an intermediate meat product. It is identified with different names viz Rousong in China, Abon in Indonesia, Moo yong in Thailand and Danbunama in northern part of Nigeria (Ogunsola and Omojola, 2008). It is processed by cooking, pounding and pan frying with addition of spices (Ogunsola and Omojola, 2008).Shredded meat is usually processed from the thigh muscle of cattle in the northern part of Nigeria, and also from different meat types (Omojola *et al*., 2014). Hence, it can be produced from broiler chicken (danbukaji). Shredded meat can be kept for several days at room temperature at no extra cost to the farmers thus; extra cost of feeding broiler after six weeks can be saved.

Phytochemical in natural spices have shown their free metal binding capacity and synthesis of microbial nucleotides in order to stop the growth and proliferation of microbes. Several researchers had stated the antioxidant and antimicrobial properties of some herbs and spices like clove, ginger, cinnamon, roselle, garlic and green tea contributing to the keeping quality and preservation of meat and meat products (Shah *et al*., 2014; Sirag *et al*., 2014; Atanasov *et al*., 2018; Horbanczuk *et al*., 2019, Saeed *et al*. (2019). To the best of our knowledge, work on garlic and ginger as spice additives in processed meat have been used in binary application in raw meat and meat products Omojola *et al*., 2014; Omojola and Adediran, 2015; Okorie, 2018; Kassim and Omojola, 2020). Specifically, roselle calyces have not been used as spice in meat products and for preservation of broiler meat. Roselle (*Hibiscus sabdariffa*) calyces contain phenolic and polyphenolic compounds like anthocyanins and gossypetin which conferred on it antioxidant, antiseptic, hypocholesterolemic and antimutagenic properties (Nizar*et al*., 2014). Allicin, alliin and some organo-sulphur compounds in garlic (*Allium sativum*) have antioxidant, antifungal and antibacterial properties (Shang *et al*., 2019). Gingerol and zingerone in ginger (*Zingiber officinale*) possess antimicrobial and antioxidant activities (Rahmani *et al*., 2014).

There have been no acceptable national standards for preparation, production and spice addition of meat floss as it varied from region to region (Ogunsola and Omojola, 2008; Huda *et al*., 2012; Sukisman *et al*., 2014). The purposes of spices addition are to improve organoleptic properties and serve as preservative of the end products. Some of the spices used include garlic, coriander, red onions, ginger, clove and turmeric, most of which contains antioxidant and antimicrobial components and preservative agents. Therefore, this study was designed to investigate the potentials of garlic, ginger and roselle as antioxidants and antimicrobials on the keeping quality of shredded meat (meat floss).

Materials and Methods

Experimental site

The cooking and preparation of shredded meatwere carried out at the Meat Laboratory, Department of Animal Nutrition and Biotechnology, Faculty of Agricultural Sciences, Ogbomoso. Nigeria with geographical coordinate Latitude80 81 0[[North and Longitude 40 16[0[[East of the Greenwich meridian (Ogunkan and Jelili, 2010).

Test ingredients, meat floss preparation and experimental design

Fresh garlic bulbs and ginger rhizomes were obtained from commercial market in Ogbomoso, Nigeria. The rhizomes and bulbs were washed, peeled, sliced into smaller pieces and then sundried until crisped. Dried roselle calyces were purchased from the market. The spice (test ingredients) were then finely ground and the coarse particles removed using a sieve apertures size of 1.00mm and were kept in an air-tightplastic container until when required for use.

A total of sixty 6-week old broiler chickens were sacrificed, scalded manually and dissected as described by Abdullah *et al*. (2010). Meat was removed from the carcass of animal after 1hour postmortem and transferred to the laboratory for product development. Meat samples were cleaned of all visible dirt, connective tissue, ligament and fat. Meats from the breast muscle were cut into thirty chunks of 100 g average weight. The chunks of meat were randomly distributed into six treatments with three replicates each in a completely randomized design.

The cooking and shredding recipe composed of spice mix containing ginger and garlic according to Omojola *et al.* (2014) representing standard recipe (SR), the standard recipe excluding ginger and garlic (mGaR), standard recipe excluding garlic (pGin), standard recipe excluding ginger and garlic and replaced by roselle (pRos), standard recipe excluding ginger (pGar) and standard recipe excluding ginger and garlic but replaced by vitamin E, a synthetic antioxidant (pvE); totaling to six treatment groups.

Product development

Cooking – the meat samples were placed inside a pot and heated on an adjustable Pifco Japan Electric Hot plate (Model Number P15001). Water was added in the ratio of 1.0 liters to 1.0kg of meat. The cooking recipe was added to meat samples in the ratio 1g of spice to 100g of meat and 500g of onions was thinly sliced and added as described by Omojola*et al*. (2014). The meat samples were cooked to an internal temperature of 72oC and the broth allowed drying with the meat after which it was removed and allowed to cool at room temperature and then weighed.

Shredding – the cooked samples were shredded with a local pestle and mortal and the shredding recipe added in the ratio of 50g of spice to 1kg of meat. The spices were added little at a time as pounding progress for uniform mixing. Twelve gram of onions was added to every 100g of spice used. The pounding continued until the meat was beaten into shreds.

Frying and draining- Groundnut oil was pre-heated to 180oC (1liter of oil to 100g of meat), and the meat samples were deep fried for 15minutes with 72 strokes per minute. This continues until a golden brown colour of meat was obtained. The product was drained with a sieve cloth with application of pressure to remove excess oil and prevent final product from sticking. The meat floss were kept in an acrylic bottle in a refrigerator (4oC), because acrylic bottles was reported to be best packaging material that reduces microbial spoilage and as lipid stability maintenance kit tool as compared to polyethylene and polyamide (Kassim and Omojola, 2020).

Chemical analyses

Chemical composition of meat

The chemical composition of meat floss samples were measured according to standard methods of AOAC (2000) at 0, 7 and 14days of storage period. Shredded meat samples were dried in an oven at 100oC until a constant weight was achieved. Further analyses for crude protein content (N x 6.25) was determined using Kjeldah method (No. 978.04 of AOAC, 2005). Soxhlet extraction was used to determine the crude fat (No. 930.09 of AOAC, 2005). According to method No 930.05 (AOAC, 2005), the ash content was evaluated for by sample incineration into a muffle furnace at 550oC. Thiobarbituric Acid Reactive Substances was determined by homogenizing 1g of meat sample with 2mL of distilled water. Two and half millilitres trichloroacetic acid (TCA) was added and then filtered. Two millilitres of filtrate was pipetted and 2mL of TBA was added. A blank was prepared containing TBA, TCA and distilled water. The absorbance was read at 530nm using spectrophotometer (Rosiminiet *al*., 1996).

Microbiological Identification and Characterization of Meat Floss

Total microbial count was determined according to the method of Adegoke (2004) at intervals of 7, 14 and 21 days of storage. About 1g of finely ground sample was weighed into a sterilized 10mL McCartney Bottle and 9mL of sterilized distilled water added, shaking vigorously on a vortex mixer to give a homogenous suspension which was serially diluted. Ten-fold serial dilution of water samples was prepared aseptically in sterile physiological saline water up to 10-4 according to Bergery (1994). About 0.1mL aliquot of each dilution was inoculated on dried nutrient agar and sabouraud dextrose agar plates, in triplicates using the spread plate technique for enumeration of total bacterial count and fungi count respectively.The Salmonella Shigella Agar (SSA) was prepared according to the manufacturer's instruction and 0.1mL aliquot of each water sample was transferred into the surface of dried sterilized SSA plate. The plates were inoculated in triplicate and incubated at 370C for 24-48h. Thereafter, pure cultures were obtained by sub culturing into freshly prepared SSA plates and pure calories were identified using biochemical reactions.

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using General Linear Model in a factorial arrangement using SPSS (IBM SPSS, 2000) version 21. Means were separated by Duncan New Multiple Range Test of the same package at 5% level of significance.

Results and Discussion

Phytogenic effect of garlic, ginger and roselle on the chemical analysis of shredded meat

The phytogenic addition effect on the chemical analysis of shredded meat is shown in Table1. All the parameters except ash content were affected ($p<0.05$) by phytogenics addition. Increased ($p<0.05$) moisture content was observed in vitamin E (pvE) treated sample (22.94%) and roselle (pRos) treated sample (22.28%)while reduced value was recorded in sample without the test ingredients (mGaG) (11.60%). Reduced ($p<0.05$) moisture content (below 20%) was also observed in ginger treated group (pGin), garlic treated group (pGar) and standard recipe containing ginger and garlic (SR-GaG) groups. Low moisture content (< 20%) of shredded meat prepared with ginger (pGin), garlic (pGar) and ginger and garlic (SR-GaG) observed in this study could depict increased preservative capacity of the phytogenic substances. Water activity play important role in fungal spoilage; 20% lean meat moisture content deters most bacteria, yeast and mould while 15% moisture content impedes some species of fungi (Okorie, 2018).

Table 1: Main effects of spice addition on chemical composition of shredded meat

Parameters	SR-GaG	mGaG	pGin	pRos	pGar	pvE	SEM	p-value
Moisture (%)	17.59^b	11.60^d	15.48^c	22.28^a	17.55^b	22.94^a	0.136	<0.0001
Crude Protein (%)	47.91^c	43.24^d	52.05^a	48.83^c	50.34^b	52.89^a	0.136	<0.0001
Crude Fat (%)	28.51^b	40.67^a	28.03^b	23.10^d	26.74^c	16.32^e	0.136	<0.0001
Ash (%)	4.10	4.30	4.13	3.95	4.01	4.07	0.134	<0.9820
TBARS (nmMDA/g)	23.74^b	13.33^d	20.37^c	20.53^c	23.80^b	30.07^a	0.086	<0.0001

^{abcd}Means on the same row with different superscripts are significantly different (p <0.05). SR-GaG- standard recipe containing ginger and garlic, mGaG- standard recipe minus ginger and garlic, pGin- recipe with ginger, pRos- recipe with roselle, pGar- recipe with garlic, pvE- recipe with vitamin E, SEM- Standard Error of Mean, TBARS- Thiobarbituric Reactive Acid Substances

Table 2: Effects of storage period on the chemical composition of shredded meat

Parameters	Day 0	Day 7	Day 14	SEM	p-value
Moisture (%)	18.80^a	18.39^a	16.53^b	0.236	0.000
Crude Protein (%)	48.93	49.16	49.55	0.236	0.188
Crude Fat (%)	26.43^c	27.22^b	28.04^a	0.236	0.000
Ash (%)	4.30	4.10	3.87	0.231	0.434

^{abcd}Means on the same row with different superscripts are significantly different (p <0.05)

Shredded meat prepared with garlic (pGar) and standard recipe (SR-GaG) could inhibit bacteria, yeast and mould while sample without the test ingredients (mGaG) might deter some species of fungi. Contrary to the result obtained in the present study, Huda *et al*. (2012) observed 8.60-13.56% in shredded chicken sample; Okorie, (2018) observed 10.3% MC in kilishi, while Sukisman *et al*. (2014) observed a range of 4.20 - 9.02 %. The results of this study showed that moisture content reduced as storage time increased. This could imply that the test spices used have aided moisture reduction at refrigeration temperature. The result disagrees with the earlier findings of Akinwumi and Olagoke (2019) who reported that MC of chicken sausage treated with antioxidants were not affected through 30days.Shredded meat without the test spices (mGaG) and that of ginger might not be a medium for microbial growth because of reduced MC at 15days of storage. Moreover, ginger and garlic (SR-GaG) and single treatment of garlic-prepared shredded meat might also not accommodate microbial deterioration as it also showed reduced MC at 14days of storage time.

The crude protein (CP)of shredded meat in pGin(52.05%) and pvE (52.89%) groups had significantly (p <0.05) elevated values while the lowest (p< 0.05) was noted at them GaGgroup (43.24%). Significantly (p < 0.05) increased crude fat (CF) was observed in mGaG group (40.67%) while the lowest value was recorded in pvE. The protein contents observed in this study were higher than what were observed in previous studies which include 30.64, 36.39, 41.21, 42.90 and 47.91 % (Huda *et al*., 2012; Okorie, 2018; Sukisman *et al*. 2014; Ockerman and Li, 1999; Akinwumi and Olagoke, 2019).

Table 3: Interaction effects of spice addition and storage period on shredded meat

Parameters	SR-GaG	mGaG	pGin	pRos	pGar	pvE	SR-GaG	SEM
Moisture (%)	0	17.91^{by}	13.00^{dx}	16.66^{cx}	23.30^{ax}	18.72^{bx}	23.21^{ay}	0.570
	7	18.41^{cx}	11.65^{ey}	15.92^{dy}	22.54^{by}	17.56^{cy}	24.27^{ax}	0.570
	14	16.46^{bz}	10.15^{dz}	13.87^{cz}	20.99^{az}	16.36^{bz}	21.34^{az}	0.570
	SEM	0.136	0.136	0.136	0.136	0.136	0.136	
CrudeProtein(%)	0	47.48a^{by}	42.83^{bz}	51.66	48.74^{aby}	50.16^{aby}	52.70^{ay}	0.577
	7	48.06^{abx}	43.16^{by}	51.96^{ay}	48.81^{abx}	50.10^{abz}	52.85^{ax}	0.577
	14	48.18^{cx}	43.74^{dx}	52.53^{ax}	48.94^{cx}	50.75^{bx}	53.13^{ax}	0.577
	SEM	0.136	0.136	0.136	0.136	0.136	0.136	
Crude Fat (%)	0	27.63^{bz}	38.00^{az}	25.91^{cz}	22.24^{dz}	25.83^{cz}	18.95^{ex}	0.577
	7	28.59^{by}	41.35^{ay}	28.74^{by}	23.11^{dy}	26.60^{cy}	14.95^{ey}	0.577
	14	29.31^{bx}	42.60^{ax}	29.43^{bx}	23.96^{dx}	27.79^{cx}	15.07^{ez}	0.577
	SEM	0.136	0.136	0.136	0.136	0.136	0.136	
Ash (%)	0	4.26	4.77	4.41^{x}	4.06	4.12	4.18	0.567
	7	4.08	4.16	4.27^{x}	3.97	4.04	4.10	0.567
	14	3.96	3.90	3.70^{y}	3.81	3.86	3.93	0.567
	SEM	0.134	0.134	0.134	0.134	0.134	0.134	

^{abcd}Means on the same row with different superscripts are significantly different (p <0.05).^{xyz} Means on the same column with different superscripts are significantly different (p <0.05). SR-GaG-standard recipe containing ginger and garlic, mGaG- standard recipe minus ginger and garlic, pGin- recipe with ginger, pRos- recipe with roselle, pGar- recipe with garlic, pvE- recipe with vitamin E,
SEM- Standard Error of Mean

Table 4: Main effect of spice addition on the microbial load of shredded meat

Parameters	SR-GaG	mGaG	pGin	pRos	pGar	pvE	SEM	p-value
Total Bacteria Count($x10^3$/cfu/g)	4.31^{a}	3.36^{ab}	2.55^{b}	3.63^{a}	3.47^{ab}	4.06^{a}	0.14	0.018
Total Coliform Count($x10^2$/cfu/g)	3.00^{a}	1.10^{b}	1.13^{b}	2.22^{a}	2.33^{a}	2.72^{a}	0.14	0.001
Total Fungi Count($x10^2$/cfu/g)	2.84^{a}	1.01^{b}	1.05^{b}	2.13^{a}	2.12^{a}	2.53^{a}	0.13	0.001

^{ab}Means on the same row with different superscripts are significantly different (p <0.05). SR-GaG-standard recipe containing ginger and garlic, mGaG- standard recipe minus ginger and garlic, pGin-recipe with ginger, pRos- recipe with roselle, pGar- recipe with garlic, pvE- recipe with vitamin E, SEM- Standard Error of Mean

Moreso, Fachrudding (1997) reported that Indonesian standard for abon requires that the protein content of shredded meat product should be more than 15%. In the present study, the best CP was recorded in shredded meat prepared with ginger among the spices used. This implies that ginger had no deleterious effect on the protein content of the treated shredded meat. The non-significant of the storage effects of crude protein agreed with report of Akinwumi and Olagoke (2019) which imply no negative effect of the phytogenics and their ability to maintain the product chemical composition

The thiobarbituric reactive substances (TBARS) significantly increased (p<0.05) in pvEgroup (30.07nmMDA/g), compared to reduced (p<0.05) values in other treatment groups, however, the lowest value (13.33nmMDA/g) was observed in mGaG. The TBARS recorded for pGin (20.37 nmMDA/g) and pRos (20.53nmMDA/g) was lower than the other groups. The antioxidant potency of the natural antioxidants especially ginger and roselle could be an indication of the test ingredient reducing the lipid oxidation in the added shredded meat. This result is in accordance with the report of Omojola and Adediran (2015) that ginger extract reduced oxidation in raw chicken meat patties during cold storage (14 days) compared to garlic and roselle. The authors also observed oxidative stability in meat patties treated with ginger, garlic and roselle extracts. Natural antioxidants extended the shelf life of shredded meat in this study contrary to the report of Omojola *et al*. (2014) who observed increased TBARS in beef, chevon and pork as the storage lengthened (up till 21 days).

The storage effect of phytogenics addition onto shredded meat is shown in Table 2. The moisture content significantly (p< 0.05) reduced through the storage periods (from 18.39 to 16.53%). The CP and ash were not significantly (p> 0.05) different across the storage periods. The CF significantly (p <0.05) increased as the storage time increased (26.43-28.04%). Thiobarbituric acid reactive substances decreased (p<0.05) along the storage periods (29.73-17.47 %)

The interaction effect of phytogenics addition upon storage period on chemical composition of shredded meat is displayed in Table 3. All the parameters were significantly different (p< 0.05) across the treatment and storage period. The MC of all the treatments was affected (p < 0.05) along spice addition and the storage periods. Ginger treatments (pGin) had lower value (13.87%) compared to other treatments except for mGaG (10.15%) at 14^{th}day of storage. Increased (p < 0.05) CP was also recorded for all the treatments and storage periods. The ginger treated groups (pGin) and synthetic antioxidant (pvE) had significantly (p < 0.05) elevated values (52.53 % and 53.13 % respectively). The pvE group had significantly (p < 0.05) reduced crude fat (14.95%) at 7^{th} day of storage. Meanwhile, reduced (p < 0.05) crude fat was noticed in pRos treated group compared with other treatment groups throughout the storage periods. Ginger treated group had reduced (p < 0.05) ash content alongside the storage periods from 4.41 % to 3.70%. The TBARS of shredded meat prepared with standard recipe (SR-GaG) significantly (p < 0.05) increased across the treatments and at fresh state (day 0) but significantly reduced (p < 0.05) across the treatments and storage day 14. Other natural antioxidant significantly lowered the TBARS over the storage period compared to pvE.

Table 5: Effect of spice addition on the microbial load of shredded meat over storage periods

Parameters	7day	14days	21days	SEM	p-value
Total Bacteria Count($x10^3$/cfu/g)	4.16^a	3.54^{ab}	3.00^b	0.24	0.010
Total Coliform Count($x10^2$/cfu/g)	2.72^a	2.13^a	1.41^b	0.24	0.037
Total Fungi Count($x10^2$/cfu/g)	1.76	2.00	2.08	0.23	0.488

^{ab}Means on the same row with different superscripts are significantly different (p<0.05).

The interaction effects of storage periods and phytogenics addition showed that ginger (a natural antioxidant) could perform as much as the vitamin E (synthetic antioxidant) in improving the crude protein content of shredded meat over the storage periods. Plants herbs and spices have been reported to possess relatively high macronutrients (protein, fat and carbohydrate) and micronutrients (minerals and vitamins) which improved nutritional quality of meat products (Horbanczuk *et al*., 2019; Akinleye *et al*., 2020; Jaworska *et al*., 2021). This contradicted the results reported by Jimoh *et al*. (2018) who recorded significant reduction in protein, fat and ash of meat samples marinated with ginger, onion and garlic, fried and stored as the periods of storage advanced.

Shredded meat prepared without the addition of ginger, garlic or roselle showed highest crude fat. According to Fachrudding (1997) the Indonesia standard for fat content of abon shredded meat should be less than 30%. All the shredded meat samples comply with this percentage except those without any of the spices used as test ingredient. Shredded meat prepared with roselle had the lowest fat content, followed by garlic, ginger and standard recipe. This confirms the hypocholesterolemic activity of anthocyanins and ã-tocopherol in roselle calyces. Previous studies also reported 6.11% (Huda *et al*. 2012) 22.33% in kilishi (Okorie, 2018), 39.00% (Sukisman *et al*., 2014) and 31.14% in fish (Ockerman and Li, 1999)

Although the ash content did not show any significance, however, all the values obtained were higher. Akinwumi and Olagoke (2019) had earlier reported 2.79% ash content in sausage. However, Indonesia's standard for abon opined that the ash content of abonserundiing should be less than 7%. The spices and other non-meat ingredient used in preparation of meat floss might be responsible for the differences observed in this study and other ones. It could be deduced that standard recipe reduced crude fat as the storage time increased (up till 14days) with concentrated CP. On the other hand the crude fat content of all the samples except those without the test ingredient (mGaG) correlate with the standard fat content for abon shredded meat product by Indonesia (below 30%) and protein above 15% (Fachrudding, 1997).

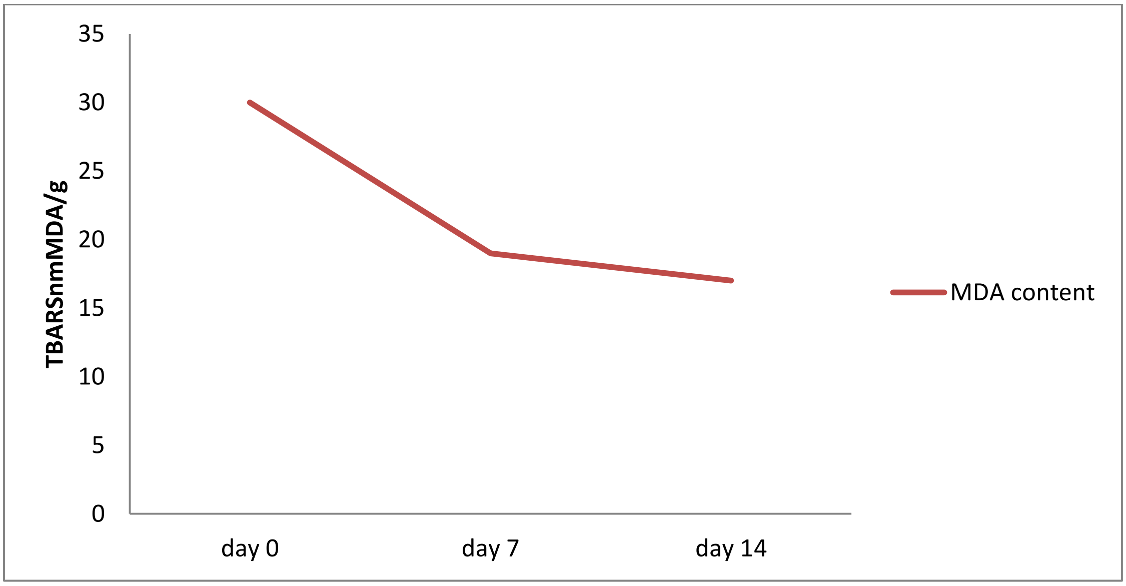

Figure 1: Effects of storage on the lipid peroxidation of shredded meat

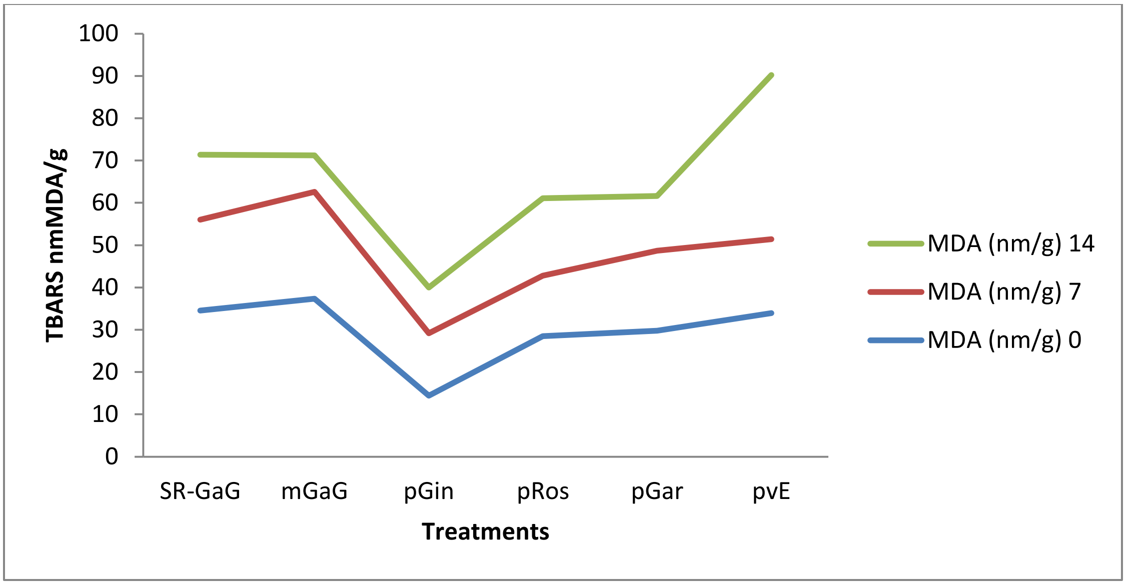

Figure 2: Effects of storage and spice addition on the lipid peroxidation of shredded meat

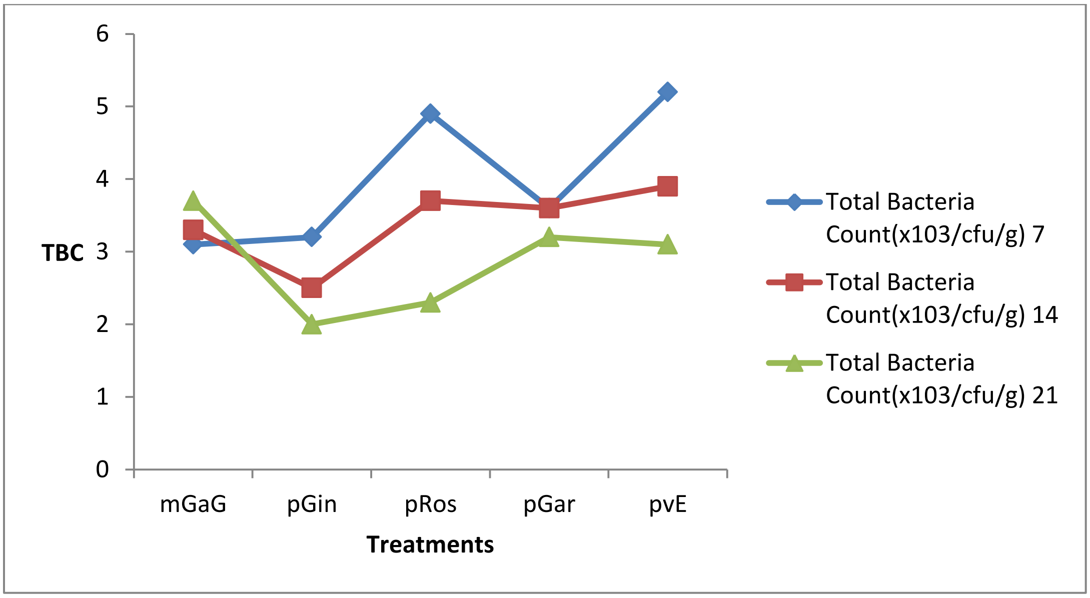

Figure 3: Interaction effect of spice addition over storage periods on the total bacteria count of meat floss

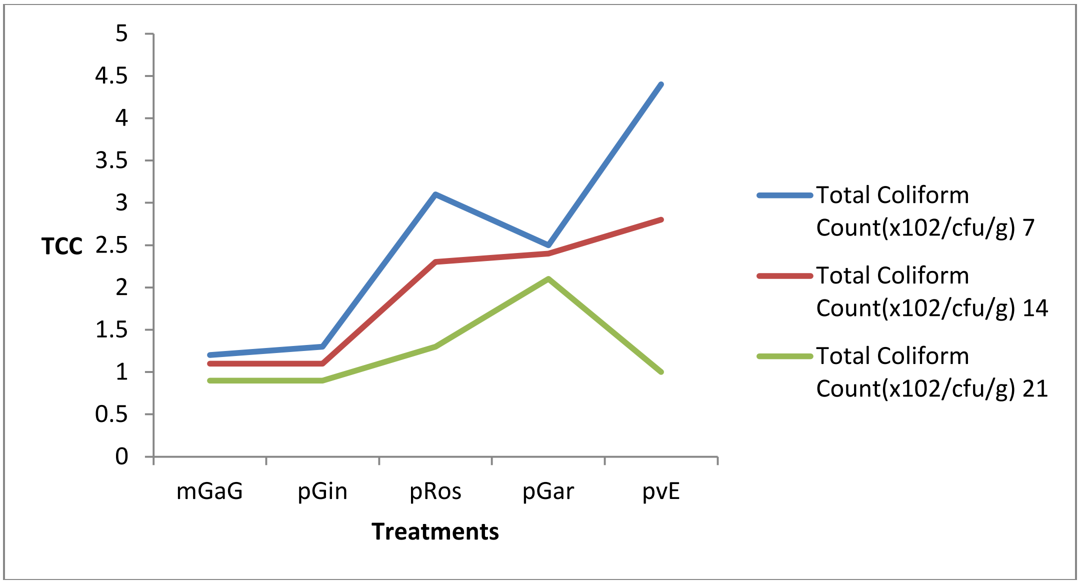

Figure 4: Interaction effect of spice addition over storage periods on the TCC of shredded meat

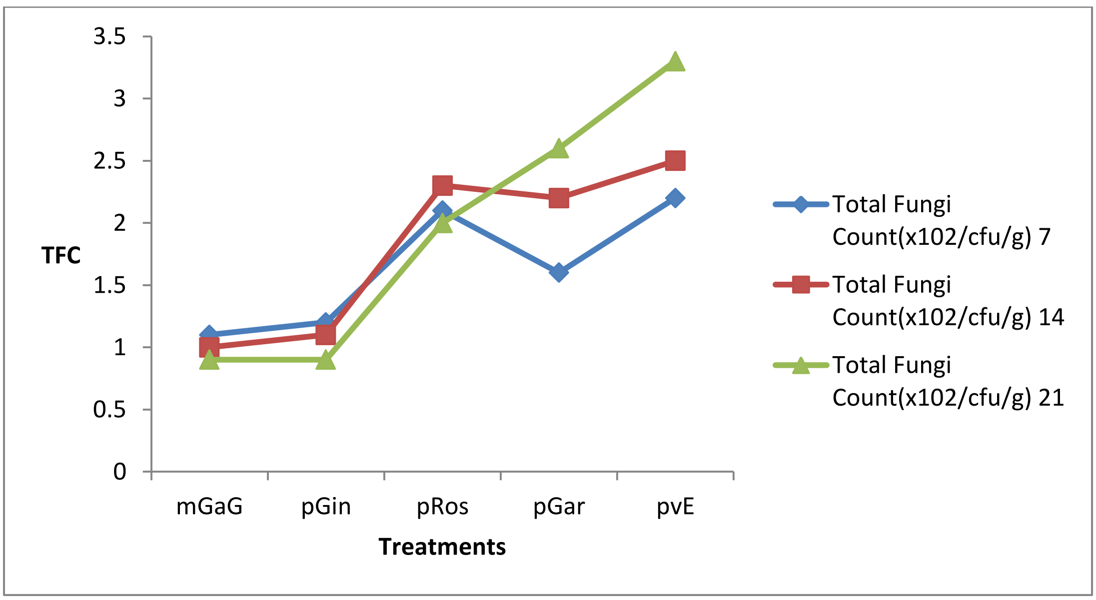

Figure 4: Interaction effect of spice addition over storage periods on the TFC of shredded meat

Table 6: Effect of spice addition on the microbial identification of microorganism in shredded meat over storage

Bacteria Isolates at 7 and 14 days storage			Coliform and Fungi Counts at 7 and 14 days storage							
Treatments			*Escherichia coli*		*Aspergillus spp*		*Sacharomycess pp*		*Penicillims pp*	
	7	14	7	14	7	14	7	14	7	14
SR-GaG	*Listeria salivarius Micrococcus varians Bacillus varians*	*Listeria salivarius Micrococcus varians Bacillus varians*	+	+	+					+
mGaG	*Staphylococcus aureus Bacillus megatarium*	*Staphylococcus aureus Bacillus megatarium Enterobacter*	+	+	-	-	+	+	+	-
pGin	*Micrococcus varians Bacillus varians Pseudomonas aureginosa*	*Micrococcus varians Bacillus varians*	+	+	+	-	-	-	+	-
pRos	*Streptococcus faecium Bacillus pumilus Rumicoccus albicans*	*Streptococcus faecium Bacillus pumilus Rumicoccus albicans*	+	+	+	-	-	-	-	+
pGar	*Micrococcus luteus Staphylococcus aureus Enterobacter*	*Micrococcus luteus Staphylococcus aureus*	+	+	-	+	-	-	+	
pvE	*Proteus mirabilis Staphylococcus aureus Bacillus megatarium*	*Streptococcus faecium Staphylococcus aureus, Bacillus megatarium*	+	+	-	+	+	-	-	-

Phytogenic effects of garlic, ginger and roselle on the microbial characterization and identification of shredded meat

The total bacteria count, TBC, total coliform counts, TCC and total fungi counts, TFC, as affected by phytogenic addition in shredded meat stored for 21 days are as shown in Table 4. There were significant ($p < 0.05$) differences across the treatments in the microbial load of shredded meat. The TBC of shredded meat treated with ginger (pGin) had significantly ($p < 0.05$) reduced value ($2.55x10^3$cfu/g) which was not statistically different from garlic treated group (pGar) and mGaG(3.47 and 3.3610^3cfu/g respectively). Elevated ($p< 0.05$) TBC was observed in meat floss prepared with standard recipe ($4.31x10^3$cfu/g), which was significantly higher ($p < 0.05$) than $2.55x10^3$cfu/g obtained in shredded meat treated with ginger (pGin) followed closely by vitamin E ($4.06x10^3$cfu/g). The same trend was observed for TCC and TFC. Reduced ($p< 0.05$) TCC ($1.10x10^2$cfu/g and $1.13x10^2$cfu/g) and TFC (1.01 $x10^2$cfu/g and 1.05 $x10^2$cfu/g) were noticed for pGin and mGaG groups.

The effect of phytogenic addition on the microbial load of shredded meat over the storage periods is as shown in Table 5. There were significant ($p< 0.05$) differences across the storage periods (TBC: 4.16-3.00 and TCC:2.72-1.41 $x10^3$cfu/g) while a numerical increased was observed in TFC (1.76-2.08 $x10^2$cfu/g). The interaction effect of phytogenic addition and storage periods on meat floss is as shown in Table 6. All the parameters were affected by the phytogenics addition through the storage periods. The TBC of shredded meat without the test ingredient increased (mGaG) (3.12, 3.30, 3.65) along the storage periods. At fresh state the TBC of pGin, pRos, pGar and pvE increased ($p < 0.05$) and then reduced across the storage periods. Shredded meat in pGin significantly reduced over the storage time compared to other treatments. Garlic, ginger and roselle singly reduced the TCC across the storage periods. Meanwhile, shredded meat in pGin and mGaG had significantly ($p < 0.05$) reduced TCC (0.9810^3cfu/g) up to 14^{th} day. Elevated ($p < 0.05$) values were observed at fresh state of pvE group (3.7510^3cfu/g). The TFC of shredded meat in pGar, SR-GaG, pGin and pvE had increased values over the storage periods. Other treatment groups however, reduced. Significantly lowered TFC was noticed in pGin and mGaG(0.89) groups.

Meat is regarded as suitable medium of microbes because of concentration of nutrients in it (Shamsudden and Ameh, 2008; ICMSF, 2011). Processing methods and spice addition during processing lowers microbial count (Okorie, 2018). Addition of ginger onto preparation of shredded meat have been shown in this study to perform better than other antioxidant sources (garlic, roselle, ginger+garlic) in terms of reducing the microbial load. The TCC permissible limit by Directive 88/657/European Economic Community of Nepal is $\log 10^2$. The TCC of all the shredded meat were within the permissible limit ($\log 10^6$ CFU/g) according to EOS (2005) except for standard spice and vitamin E (synthetic antioxidant) and therefore classified as acceptable. It therefore implies that addition of ginger alone to other spice mix in preparation of shredded meat could better preserve the meat by reducing the microbial loads up to 21 days stored at refrigerator temperature (4^0C). The inhibitory effects of single addition of ginger and garlic could be attributed to the observed changes. Ginger and garlic addition effectively inhibited the growth of microbes compared to others. The strongest TBC lowering effects was found in ginger while the weakest effect was observed in standard spice group (Ginger (pGin) > other spice (mGaG) > Garlic (mGAr) > vitamin E (pvE) > Standard spice (SR-GaG).

The result of the identification of microorganisms present in the stored shredded meat is presented in Table 7. The bacteria isolated from shredded meat in SR-GaG group include *Listeria salivarius*, *Micrococcus varians* and *Bacillus varians* through the storage periods. *Staphylococcus aureus* was identified in garlic (pGar), pvE and mGaG. *Bacillus megatarium*were isolated in pvE, SR-GaGand mGaG groups of shredded meat. *Streptococcus faecium* was isolated in pRos treatment. Coliform and Fungi isolated includes *Escherichia coli*in all the treatments and storage days. *Aspergillus* sppwas isolated in SR-GaG, pGinand pRosat day 7 of storage, with isolations in SR-GaG, pGar and pvEat 14th day of storage. *Sacharomyces* and *Penicillium* spp were also isolated at 7thand 14th day of storage mGaG, and at 7th day of storage for pGar. It was also isolated at 14th day of storage in pRos and SR-GaG treatments.

It was observed that microbes identified in ginger treatment group reduced across the storage periods. Jaworska *et al.* (2021) also observed inhibitory effects of spices and herbs on psychrotrophic bacteria as compared to the control and their extracts. The same author reported lower limit of *Bacillus* spp bacteria in the ready-to-eat product. Gantner *et al.* (2018) reported controlled microbial growth during refrigerated storage of pre-cooked turkey meatballs. *Bacillus* specie is heat resistant while *Pseudomonas* proliferates so easily at temperature close to 0°C especially in processed products, which could be resulted to their presence in the final product. Garlic treated shredded meat had reduced microbes isolated as the storage periods lengthened. On the other hand, the combination of ginger and garlic (standard recipe) in the shredded meat could not prevent *Listeria salivarius* which are serious food borne microbes, therefore might not be preferred as they are of public health significance.

It has been reported that meats and meat products are sources of *Listeriamono cytogens* (Borch and Arinder, 2002). Enroll and Sireli (1999) identified 3% of *L. monocytogens* in chicken carcasses. *Salmonella* and *Campylobacter* have been classified to be most serious food borne pathogens. Nortje *et al*. (1999) reported that *B. cereus* is found to be higher in cooked and processed meat than in raw meat samples. Edema *et al.* (2009) isolated *B. cereus* from suya samples. This might be an indicator of animals' condition prior, during and after slaughter which critically affect the finished product quality (Satin, 2002). *Bacillus cereus* form spores and are heat resistant which are found in air and even spice. *Pseudomonas spp* caused spoilage (change in smell or taste and unpleasant texture and colour) in meat and meat products but not to the extent of *E. coli* or *Salmonella*. *Pseudomonas* are also found widely spread in nature most especially soil, water on plants or even spices. Meat can be contaminated by *Staphylococcus aureus* through nasal mucosa and animal or human skin (Corner *et al.*, 2001). Hargis *et al.* (2001) observed that *S. aureus* are not activated completely either by cooking or pasteurization.

Conclusion and Recommendation

All the shredded meat prepared with natural antioxidants had increased crude protein with the best in sample with addition of ginger. Fat content of shredded meat prepared with roselle addition better decreased compared to garlic and mixture of ginger+garlic addition. Meat floss prepared with addition of ginger and the one without the test ingredient would not to be exposed to microbial spoilage because of reduced moisture content at 14days of storage. Addition of ginger+garlic reduced crude fat with concentrated crude protein in meat floss up to 14days. Shredded meat containing ginger reduced microbial load compared to other treatment groups. The total coliform counts were within the permissible limits for all, except vitamin E (synthetic) and ginger + garlic combination (standard spice). Therefore, meat floss prepared with ginger, stored at 4°C could be preserved up to 21days of storage with reduced microbial load and less serious microorganisms. Meat floss with garlic also had reduced microbes along the storage periods while ginger+garlic combination could have serious food borne as it contains *Listeria salivarius.* Hence, addition of ginger alone in the preparation of meat floss will enhance crude protein content, reduced microbial load and preservation of meat during storage periods. Single garlic addition in preparation of meat floss is encouraged for reduced microbial load.

References

Abdullah, A. Y., Al-beitawi, N. A., Rjoup, M. M. S., Qudsieh, R. I. and Ishmais, M. A. A. (2010). Growth performance, carcass and meat quality characteristics of different commercial crosses of broiler strains of chicken. *Japan Poultry Science*, 47:13-21

Adegoke, G. O. (2004). Understanding food microbiology, Alleluia Ventures Ltd, Ibadan, Nig. Pp 99-100

Akinleye, S. B., Afolabi, D. K., Luka, S. J., Ayanniyi, N. N. and Adeyemi, S. A. (2020). Nutritional properties and microbiological loads of meat floss prepared from mutton of four breeds of ram. *European Journal of Science and Technology*, 8 (3): 23-33

Akinwumi, A. O. and Olagoke, O. C. (2019). Effect of different antioxidant and the storage time on nutritional and microbiological qualities of chicken sausage. *International Journal of Science and Research*, 8(9): 1700-1705

AOAC. Official Methods of Analysis (2000). 19th Edition AOAC International, Inc. Washington, DC.;1219

AOAC, (2005). Association of Official Analytical Chemist. Official Methods of Analysis 18th Edition AOAC Inc. Arlington, Virginia USA. 1094pp

Atanasov, A. G., Sabharanjak, S. M., Zengin, G., Mollica, A., Szostak, A. and Simirogiotis, M. (2018). Pecan nuts: A review of reported bioactivities and health effects. *Trends Food Science and Technology*, 71: 246-257

Bergery, G., Knaape, C., Balline, G. and Seidel, D. (1994). Biological control *Verticillium dahlia kelp*. By natural occurring rhizophere bacteria. *Archieve of Pathology and Plant Protection*, 29(30), 249-262

Bohrer, B. M. (2017). Review: Nutrient density and nutritional value of meat products and non-meat foods high in protein. *Trends Food Science and Technology*, 65; 103-112

Borch, E. and Arinder, P. (2002). Bacteriological safety issues in red meat and ready-to-eat meat products as well as control measures. *Meat Science*, 62**:**381-390

Corner, D. E., Davis M. A. and Zang, L. (2001). Poultry-Borne Pathogens II: Plant Considerations. In: Poultry Meat Processing, Sams, A.R. (Ed.). Chapter 9, CRC Press LCC., Boca Raton, FL., USA., ISBN-13: 9781420042177, pp: 137-158

Dousset, X., Jaffres, E. and Zagorec, M. (2016). Spoilage:Bacterial spoilage, 1st ed., Elsevier Ltd: Amsterdam, the Netherlands, vol. 5 pp. 106-112

Edema, M. O. Temitope, O. A. and Innocent, D. C (2009). Evaluation of microbial hazards associated with the processing of suya (a grilled meat product). *Scientific Research and Essays*, **3**(12):621-626

EOS, (2005). Egyptian Organization for standardization and Quality Control. No. 1522 for meat.

Enrol, I. and Sireli U. T. (1999). Incidence and serotype distribution of Listeria monocytogenes in frozen broiler carcasses. *Turkey Journal Veterinary Animal Science*,23:765-770.

Fachruddin, L. (1997). Appropriate Technology in Production of Different Kind of Abon. Kanisius, Yogyakarta, pp: 1-72

Gantner, M., Brodowska, M., Gorska-Horczyczak, E., Wojtasik-Kalinowska, I., Najda, A., Pogorzelska, E. and Godziszewska, J.(2018). Antioxidant effect of sage (*Salvial officinalis* L.) extract on turkey meatballs packed in cold modified atmosphere. *Journal of Food*, 16:628-636

Grigson, J.(1985), World Atlas of Food, Bookthrift Company, ISBN 978-0-671-07211-7

Hargis, B. M., Caldwell, D. J., Byrd, J. A. (2001). Microbiological pathogens: live poultry considerations. In: Sams A.R., Ed., Poultry Meat Processing, CRC Press, USA, pp. 121–137

Henchion, M. McCarthy, M., Resconi, V. C. and Troy, D. (2014). Meat consumption: Trends and quality matters. *Meat Science*, 98(3):561-568 https://doi.org/10.1016/j.meatsci.2014.06.007

Horbanczuk, O. K., Kurek, M. A., Atanasov, A. G., Brncic, M. and Brncic, S. R. (2019). The effects of natural antioxidants on quality and shelf life of beef and beef products. *Food Technology and Biotechnology*, 57(4):439-447

Huda, N., Fatma, Y., Fazillah, A. and Adzitey, F. (2012). Chemical composition, colour and sensory characteristics of commercial serunding (Shredded Meat) in Malaysia. *Pakistan Journal of Nutrition*, 11 (1): 1-4

ICMSF (2011). Microorganisms in food. Use of Data for Assessing Process Control and Product Acceptance. New York: Springer. 400 S

Jaworska, D., Rosiak, E,,Kostyra, E., Jaszczyk, K., Wroniszewska, M. and Przyrylski , W. (2021). Effect of herbal addition on the microbiological oxidative stability and sensory quality of minced meat. *Journal of Foods*, 10:1537-1553

Jimoh, M. O., Olurin, O. T. and Salako, O. O. (2018). Effect of particle size of selected composite spices on storability of fried meat. *African Journal of Food Science*, 13(1): 1-9 DOI: 10.5897/AJFS2018.1756

Kassim, O. R. and Omojola, A.B. (2020). Effect of cooking oil and packaging media on quality of meat floss. *Nigerian Journal of Animal Production*, 47**(**3): 323-335

Nizar, S., Elhadi, M. M., Algali, A. M. Hassan, H. M. and Ohaj, M. (2014). Determination of phenolic contents and antioxidant activity of roselle calyx ethanolic extract. *Standard Research Journal of Pharmacy and Pharmacology,* 2: 034-039

Nortje, G. L., Vorster, S. M., Greebe, R. P. and Steyn, P. L. (1999). Occurrence of Bacillus cereus and Yersinia enterocolitica in South African retail meats. *Food Microbiology*, 16: 213–217

Ockerman, H. W. and Li, C. T. (1999). The Evaluation of the Palability of a Dehydrated Meat Product- Meat Floss. The Ohio State University, Bulletin Research and Reviews: Meat, Special Circular, pp: 172-199

Ogunsola, O. O. and Omojola, A. B. (2008). Qualitative Evaluation of Kilishi Prepared from Beef and Pork. *African Journal of Biotechnology*, 7(11), 1753-1758

Okorie, A. P. (2018). Processing effect on the microbial and proximate composition of kilishi at South East Nigeria. *International Journal of Research-Granthaalayah*, 6(5):340-345. https//doi.org/10.5281/zenodo.1283430

Omojola, A. B., O. R., Kassim, O. O., Olusola P. O., Adeniji and Aremo J. O. (2014). Development and Quality Evaluation of Danbunama (Meat Floss)- A Nigerian Shredded Meat Product. *British Journal of Appled Sciences and Technology*, 4(26):3862-3873.

Omojola, A.B. and Adewumi A.O. (2015). Effect of Ethanolic Extract of Garlic, Roselle and Ginger on Quality Attributes of Chicken Patties. *African Journal of Biotechnology*, 4(8):688-694

Rahmani, A.A., F.M. Alshabrmi and Aly, M.S. (2014). Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. *International Journal Physiology, Pathophysiolology and Pharmacology*, 6(2): 125-136

Rosimini, M. R., Perlo, F, Perez- Alvarez, J.A., Pagan-Moreno, M. J., Gagogago, A, Lopez- Satin, M., (2002). Use of irradiation for microbial decontamination of meat: situation and perspectives. *Meat Sci*ence, 62: 277–83

Saeed, A., Muhammad, W., Nazir, A., Tariq, I., Zulifiqar, A., Muhammad, F. M. and Azhari, S. (2019). Polyphenol-rich extracts of traditional culinary spices and herbs and their antibacterial activity in minced beef. *Journal of Food Quality*, 2019:pp 1-9 https//doi.org/10.1155/2019/1702086

Satin, M., (2002). Use of irradiation for microbial decontamination of meat: situation and perspectives. *Meat Sci*ence, 62: 277–83

Seemat, R. (2015). Food spoilage: Microorganisms and their prevention. *Asian Journal of Plant Science and Research,* 5(4):47-56

Shamsudden, U. and Ameh, J. B. (2008). Survey on the possible critical control points in kilishi (a traditional dried and grilled meat snack) produced in Kano. *International Journal of Bioscience*,3(2):34-38

Shang, A. Cao, S.Y., Xu,Y.X., Gan,R.Y. Tand, G.Y. Corke, H. Mavumengwana, V. and Li, H.B. (2019). Bioactive compounds and biological functions of garlic (*Allium sativum* L.). *Journal of Foods*, 8:246-276 doi:10.33901foods8070246

Shah, M. A., Don Bosco J. S. and Ahmad, M. S. (2014). Plants extracts as natural antioxidants in meat and meat products. *Meat science*, 98(1): 22-33

Sirag, N. Elhadi, M. M, Algaili, M. A., Hassan, H. M and Ohaj, M. (2014). Determination of total phenolic and antioxidant activity of roselle (*Hibiscus sabdariffa* L.) calyx ethanolic extract. *Standard Research Journal of Pharmacy and Pharmacology*; 2: 034-039 https;//www.standardresjournals.org/journals/SRJPP

SPSS (2012). Statistical Package for Social Sciences, IBM Corporation Released 2012. IBM SPSS, Statistics for Windows, Version 21.0. Armonk, New York: IBM, Corporation SPSS

Sukisman, H. P., Djala, I. R. and Lilik, E. R. (2014). Quality properties, antioxidant capacity and total phenolic content of traditional deep fried shredded meat (abon) of Palu, central Sulawesi American. *Journal of Food Technology*, **9**(2): 80-88. DOI:10.3923/ajft.2 b014.80.88