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SHELF-LIFE AND NUTRITIONAL QUALITY OF BEEF HOTDOGS EXTENDED WITH DIFFERENT LEGUME FLOURS

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Abstract: Four legumes' flours as meat extenders in beef hotdogs allotted into 5-treatments [without extender (P1), extended using flour from African yam bean (P2), Lima beans (P3), Pigeon peas (P4) and Soybeans (P5)] for lipid oxidation-(mg MDA/kg), microbial load-(log_{10} cfu/g), cholesterol-(mg/100g) and proximate on 0, 3, 6 and 9-days at 4±1^{o}C. Lipid oxidation in P4 [0.47±0.01 (day-0) to 0.61±0.01 (day-9)] lower ($p<0.05$) than other treatments. Total bacterial count -TBC, Total fungi count - TFC, Total coliform count - TCC, *Escherichia coli* and *Staphylococcus aureus* were absent on day 0 in all treatments. Microbial-load in P1 [TBC (0.75±0.01), TFC (0.20±0.01), TCC (0.70±0.02), *Escherichia coli* (0.65±0.02) and *Staphylococcus aureus* (0.75±0.04)] were similar to P4 [TBC (0.40±0.03), TFC (0.02±0.01), TCC (0.55±0.01), *E. coli* (0.40±0.01) and *Staphylococcus aureus* (0.60±0.01)] but lower ($p<0.05$) than other treatments on day-9. Cholesterol in P4 [61.77±0.02 (day-0) to 61.60±0.01 (day-9)] lower ($p<0.05$) than other treatments and highest in P1 [76.09±0.01 (day-0) to 75.65±0.01 (day-9)]. Protein in P4 [13.55±0.03 (day-0) to 13.25±0.03 (day-9)] lower than P5 only. Crude-fat [17.83±0.03 (day-0) to 17.71±0.03 (day-9)] in P4 was lower ($p<0.05$) than other treatments. Beef hotdogs with pigeon peas flour was best in limiting lipid oxidation, microbial-load, cholesterol and fat but lower than hotdogs with soybeans flour in protein composition.

Keywords: Lipid oxidation, microbial load, cholesterol, legume flours, beef hotdogs, meat extenders

Introduction

The meat processing industry sustainability have much to do with reducing overall cost of production while improving nutritional and shelf life of meat products. It is on this premise that the inclusion of non-meat ingredients is considered an important factor especially those of plant origin in meat products (Asgar *et al*., 2010). Legumes in human nutrition are of prime importance due to their high protein content and are readily available as well as affordable (Boye *et al*., 2010). The use of plant protein in meat products as functional ingredients or extenders to improve the stability and lower cholesterol as well as the nutritional quality of the product or reduce cost of production leading to affordable price by consumers is much propagated (Messina, 1999). Flours from legumes are consumed worldwide as nutritious protein source, and its consumption has been proven to reduce cholesterol content and the risk associated with cardiovascular diseases as well as the acquisition of type-2 diabetes (Shand *et al*., 2011). However, animal protein sources such as whole milk and egg are considered as meat extenders. But their usage in sausage production increases the level of cholesterol (Omojola *et al*., 2013) and the cost of production of the meat products compared to plant protein sources such as legumes as meat extenders (Heinz and Hautzinger, 2007).

Notably, lipids are important components of all types of meat and are responsible for many desirable characteristics of meats; however, lipid oxidation is the main process responsible for the quality deterioration of meat and meat products (Amaral *et al*., 2018). Lipid oxidation among other things like microbial load negatively affect the nutritional value and shelf life of meat and meat products as well as health implications like cancer and tumor development in humans (Alvarez-Parrilla *et al*., 2010). Considering that ‘quality’ and ‘health’ are some of the most important factors that influence food choice, the control, or at least minimization, of the lipid oxidation process and microbial growth during storage are of great interest to the meat industry (Malav *et al*., 2013). Thus, non-meat ingredients use as meat extenders (especially of plant origin) should play a positive contributory role in improving nutritional value and shelf life of the meat products by limiting lipid oxidation and microbial load during storage (Belloque *et al*., 2002).

Hence, research attention has been directed toward increasing utilization of plant protein sources in meat products. Such research includes the use of pigeon pea (Gomezulu and Mongi, 2021), soya bean flour (Singh *et al*., 2008; Amadi, 2020), lima beans and Africa yam bean flours (Okere *et al*., 2022). Though, extensive works have been done on utilization of these plant proteins for food, there is insufficient information on the use of African yam bean, lima beans, pigeon peas and soybeans flour as meat extenders in sausage production with respect to shelf life

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and nutritional quality. Therefore, the objective of this study was to compare the shelf life by determining the oxidative stability and microbial load as well as the nutritive quality in terms of the cholesterol level and proximate composition of beef hotdogs prepared using flours from four different legumes species as meat extenders.

Materials and Methods

Location of study

The research was conducted at the Food Science Laboratory of the Agricultural Value Addition Programme, Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, Ibadan, Nigeria.

Preparation of Legume Flours

The seeds of the African yam bean (*Sphenostylis stenocarpa*), pigeon peas (*Cajanus cajan*) and soybeans (*Glycine max*) were obtained from a local market while Lima beans (*Phaseolus lunatus*) was obtained from the Institute of Agricultural Research and Training (I.A.R. &T.). The seed of the legumes were screened to remove spoilt seeds and impurities. The screened seeds were soaked in water for 24 hours and sundried for 36 hours at an ambient temperature of 29^{o}C and ground into flour using a conventional corn mill.

Sausage Preparation

Fresh boneless beef (12.5 kg) was obtained from the thigh muscle (*Semi membranous* muscle) immediately after slaughter, chilled at 2^{o}C for 4 hours and it was stored at a freezing temperature (-4^{o}C) for 12 hours. It was thawed for 8 hours at 4^{o}C and chopped into smaller pieces and minced using a 5 mm sieve in a tabletop mincer (Breville, Model UTP141, United Kingdom). The minced meat was apportioned into five treatment groups of 2.5 kg and cooked to doneness of an internal temperature of 72^{o}C. Group one contained no extender (P1) while groups 2, 3, 4 and 5 contained African yam bean (P2), lima beans (P3), pigeon peas (P4) and soybean (P5) flour each at 0.25 kg to 1 kg of the minced meat used in formulating the beef hotdogs sausage (Table 1) adopted after Okere *et al*., 2022. Each treatment group was replicated three times in a completely randomized design.

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Table 1. Beef hotdogs formulation using different legume flours as extenders

Ingredients (%)	Samples				
	P1	**P2**	**P3**	**P4**	**P5**
Lean beef meat	93.75	75.00	75.00	75.00	75.00
Extender	0.00	18.75	18.75	18.75	18.75
[1]Binder	3.00	3.00	3.00	3.00	3.00
[2]Fat	1.25	1.25	1.25	1.25	1.25
[3]Spices	1.25	1.25	1.25	1.25	1.25
Monosodium glutamate	0.25	0.25	0.25	0.25	0.25
Salt	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100

[1]**Binder**: Corn flour, [2]**Fat**: Margarine, [3]**Spices**: Red chilli paste (30%), Green pepper (20%), Powdered nutmeg (20%), Garlic (10%), Ginger (10%), Onion (10%). P1: Beef hotdog without extender, P2: Beef hotdog extended with African yam bean flour, P3: Beef hotdog with Lima beans flour, P4: Beef hotdog extended with pigeon peas flour, P5 Beef hotdog extended with soybean flour. Adopted after Okere et al., 2022.

Lipid oxidation of the beef hotdogs sausages

The oxidative stability was monitored on beef hotdogs sausage from each of the 5-treatments at day 0, 3, 6 and 9 of cold (4ºC) storage. Evaluation of lipid oxidations in the sausage was assayed in 2-thiobarbituric acid (TBA). Measurements were taken twice on 10g samples at each sampling day for values of thiobarbituric acid reactive substance (TBARS) using the methods of Adeyemi *et al.* (2013) with some modification. The 10 g of sample from each of the 5-treatments was mixed with 25% Tricholoroacetic acid (TCA) and 20 mL of deionized water. The mixture was homogenized for 2 minutes and thoroughly filtered using Whatman filter paper. The filtrate was mixed with an equal volume of 0.02M TBA at 100ºC for 35 minutes. A running tap was used to cool the filtrate for 10 minutes. At 532mm spectrophotometer, solution of the absorbance was measured and TBARS was derived from reading from spectrophotometer at 532mm x 7.8 expressed in mg MDA/kg.

Microbiological assessment of the beef hotdogs samples

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Total Bacterial Count (TBC) or the total bacterial load of the beef hotdogs sausage: The culture medium used was nutrient agar for the overall bacterial load. The growths were evaluated at day-0 on freshly beef hotdogs each from the 5-treatment. Subsequently, the evaluation was carried out on day 3, 6 and 9 in a cold (4 ºC) storage. Ten (10 g) of the beef hotdog samples from each of the 5-treatments were homogenized with 90 mL of 0.1% (W/V) peptone for 1 minute at room temperature using a blender. Appropriate serial dilution was prepared in 0.1% (W/V) peptone water solution. A spread on petri plate was done using a 1ml of the mixture of each sample and was incubated at 37ºC for 24 hours as used by Heinz and Hautzinger (2007). All colonies that appeared afterward were counted and as expressed as colonies forming unit per gram (cfu/g). Analysis of the beef hotdogs samples were carried out on days 0, 3, 6 and 9 of the storage periods. The method of microbiological analysis described by Abdullahi *et al.* (2004) for Total *Coliform* count, Shamsuddeen (2009) for *Staphylococcal* counts, Tagoe *et al.* (2011) for Total *fungal* count, Adesiji *et al.* (2011) for *Escherichia coli* were adopted for the analysis of the beef hotdogs samples.

Proximate analysis of the beef hotdogs samples

The methods described by Heinz and Hautzinger (2007) were employed in evaluating the moisture, crude protein, crude fat, and ash contents of the beef hotdogs samples on days 0, 3, 6 and 9 of storage. Moisture content was evaluated using the oven drying method by drying 10 g of the milled beef hotdogs at 125ºC for 1 hour in an air oven. The protein content was evaluated using the Kjeldahl method by assaying crude protein in 10 g of milled beef hotdogs samples where the distillate obtained was titrated with 0.01N HCl. The derived crude protein was deduced by the conversion of nitrogen (%N) content of beef hotdogs samples obtained when titrated with a constant (6.25). Thus, it was expressed as (6.25 x %N). Beef hotdogs samples for crude fat analysis were semi-dried before being subjected to ether-extraction using the Soxhlet apparatus. After complete extraction, the fat was obtained by evaporating and recovering the ether. Ash content was obtained using the muffle furnace method at 600ºC for 2 hours.

The crude fiber was according to AOAC (2000) method for beef hotdogs samples on days 0, 3, 6 and 9 of storage. In assessing the crude fiber, the weight of crucible with 10 g of the

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beef hotdog samples and weight of crucible with ash as well as the weight of the beef hotdog samples were determined. These determined weights were used in calculating the crude fiber content as expressed below:

$$Crude\ fibre\ content = 100\frac{(A - B)}{C}$$

Where: A= weight of crucible plus beef hotdog samples (g)

B = weight of crucible plus ash (g)

C = weight of sample (g)

Results and discussion

The rate of lipid oxidation in the beef hotdogs samples were assessed by the thiobartituric reactive substances (TBARS) assay which expresses the amount of malondialdehyde formation that imparted rancid odours and off-flavour to the beef hotdogs samples. The lipid oxidation in the beef hotdogs as shown in Table 2 showed that there were significant variations ($p<0.05$) among the beef hotdogs samples throughout the storage days. The lipid oxidation increased in all the beef hotdogs samples as the storage days increases. The initial (day-0) lipid oxidation (mg MDA/kg) in the beef hotdogs samples ranged from 0.50±0.02 mg MDA/kg in non-extended beef hotdogs to 0.48±0.02 mg MDA/kg in beef hotdogs extended with lima bean flour and 0.47±0.01 mg MDA/kg in beef hotdogs extended with pigeon peas flour. Notably, the differences in lipid oxidation among the beef hotdogs samples at the beginning (day-0) were non-significant ($p>0.05$). However, significant variations ($p<0.05$) in lipid oxidation were observed among the beef hotdogs samples on days 3, 6 and 9 at cold storage of 4^{o}C. This could be due to the fact that lipid oxidation increases in food with high fat content, particularly those with higher levels of unsaturated fatty acids (Tee *et al.*, 2012). The unsaturated fatty acid present in the hotdogs samples which arouse from the meat and the addition of margarine as source of fat in the products could have reacted with oxygen, leading to lipid oxidation which produces rancid flavor (Warriss, 2010). Inspite of this reaction, the retarding effect of the legume flours as extenders in the hotdog samples on lipid oxidation was noticeable on days 3, 6 and 9 of storage with significant lower values of lipid oxidation in hotdogs samples extended with legume flours than in the non-extended samples.

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The lipid oxidation of 0.49±0.01 mg MDA/kg in beef hotdogs extended with pigeon pea flour on storage day-3 was observed to be significantly ($p<0.05$) lower than the other extended beef hotdogs and non-extended beef hotdogs samples, except for the beef hotdogs extended with Lima bean flour (0.53±0.01 mg MDA/kg). Although, beyond day 3, it was observed that the lipid oxidation of 0.58±0.01 mg MDA/kg (day-6) and 0.61±0.01 mg MDA/kg (day-9) in beef hotdogs extended with pigeon pea flour were not significantly ($p>0.05$) lower than those of 0.63±0.01 mg MDA/kg (day-6) extended with lima beans flour but was significantly ($p<0.05$) lower than those of 0.66±0.01 mg MDA/kg (day-9) extended with lima beans flour, [0.56±0.03 mg MDA/kg (day-3), 0.64±0.02 mg MDA/kg (day-6) and 0.69±0.01 mg MDA/kg (day-9)] extended with African yam bean flour and [0.57±0.02 mg MDA/kg (day-3), 0.65±0.02 mg MDA/kg (day-6) and 0.70 ±0.01 mg MDA/kg (day-9)] extended with soybeans flour. Notably, as the storage days increases the highest lipid oxidation among the samples was reckoned with non-extended beef hotdogs, so much that at storage day-9 the lipid oxidation of 0.75±0.02 mg MDA/kg in non-extended beef hotdogs was significantly ($p<0.05$) higher than those extended which ranged from 0.61±0.01 mg MDA/kg with pigeon peas flour to 0.70±0.01 mg MDA/kg with soybeans flour. This result indicates that beef hotdogs extended with legume flours is an effective alternative not just in cutting down production cost but also in retarding lipid oxidation and this is in agreement with reports by Asgar et al. (2010). However, the results obtained for all the beef hotdog samples are within the acceptable limits of the maximum level of 2 mg MDA/kg lipid oxidation value (Possamai *et al*., 2018), indicating good quality of the beef hotdogs samples during the 9 days cold storage of 4°C.

Table 2. Lipid oxidation of beef hotdog extended using four different species of legume flours

Storage	Lipid oxidation of samples (mg MDA/kg)				
Days	P1	P2	P3	P4	P5
0	0.50 ± 0.02	0.49 ± 0.04	0.48 ± 0.02	0.47 ± 0.01	0.49 ± 0.01
3	0.59 ± 0.03^{c}	0.56 ± 0.03^{b}	0.53 ± 0.01^{ab}	0.49 ± 0.01^{a}	0.57 ± 0.02^{b}
6	0.69 ± 0.04^{c}	0.64 ± 0.02^{b}	0.63 ± 0.01^{ab}	0.58 ± 0.02^{a}	0.65 ± 0.02^{b}
9	0.75 ± 0.02^{c}	0.69 ± 0.01^{b}	0.66 ± 0.01^{b}	0.61 ± 0.01^{a}	0.70 ± 0.01^{b}

*P1= Beef hotdogs without extender, P2 = Beef hotdogs extended using African yam bean flour, P3 = Beef hotdogs extended using lima beans flour, P4 = Beef hotdogs extended using pigeon peas flour, P5 = Beef hotdogs extended using soybean flour.

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The rate at which meat deteriorate is partly influenced by the microbial load at the point of slaughtering, processing, packaging and handling of the finished product. The result of the microbial properties of the beef hotdogs sausages with and without extenders are presented in Table 3. The total bacterial count-TBC (log_{10} cfu/g) as shown in Table 3 revealed that there were significant variations among the beef hotdogs samples only on days 6 and 9. The TBC of 0.10±0.01 log_{10} cfu/g (day-6) and 0.75±0.01 log_{10} cfu/g (day-9) in beef hotdogs non-extended was significantly ($p<0.05$) higher than those extended using the legumes flour with the least TBC of 0.0±0.02 log_{10} cfu/g (day-6) and 0.40±0.03 log_{10} cfu/g (day-9) found in beef hotdogs extended with pigeon peas flour. However, the TBC of 0.05±0.01 log_{10} cfu/g (day 6) and 0.65±0.02 log_{10} cfu/g (day 9) in beef hotdogs extended with African yam bean had the highest proliferation of bacterial among the beef hotdogs extended with the legumes flour. For days 0 and 3 TBC was completely absence in all the beef hotdogs samples. This could be that the beef hotdogs samples were freshly prepared and have not been stored for enough time to allow bacterial proliferation to take place. This phenomenon is in harmony with the observations reported by (Onibi and Osho, 2007) where chicken meat from broiler's chicken fed roselle calyx extract had low bacterial count in freshly cooked and un-stored meat. In all the samples, as the storage days increases, the total bacterial count (log_{10} cfu/g) increases and this observation harmonizes with the report of Iheweagu *et al.* (2015). Also, it is noteworthy that following the recommendation by Heinz and Hautzinger (2007) for good microbiological standard the beef hotdogs sausages both extended and non-extended stored (at 4^{o}C) for 9 days did not exceed the maximum threshold point 4 log_{10} cfu/g for the total bacterial count.

The *Escherichia coli* loads as shown in Table 3 showed that there were significant variations among the beef hotdogs samples only on days 6 and 9 of storage. The *Escherichia coli* of 0.10±0.01 log_{10} cfu/g (day 3), 0.20±0.02 log_{10} cfu/g (day 6) and 0.40±0.01 log_{10} cfu/g (day 9) were least in beef hotdogs extended with pigeon peas flour but highest in the non-extended beef hotdogs. Noteworthy, is that in all the samples (extended and non-extended beef hotdogs) stored (at 4^{o}C) for 9 days was far below the maximum threshold point of 2 log_{10} cfu/g as recommended by Heinz and Hautzinger (2007) for good microbiological standard. This was achieved because in the preparation of the hotdogs basic food hygiene practices were observed such as washing hands and cooking tools thoroughly with soap and water as well cooking the hotdogs to

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doneness. The Total coliform count-TCC of 0.10±0.01 $\log_{10}$ cfu/g (day 3), 0.20±0.03 $\log_{10}$ cfu/g (day 6) and 0.50±0.01 $\log_{10}$ cfu/g (day 9) in the beef hotdogs samples extended with pigeon peas flour was the least among the beef hotdogs extended with the legume flours. However, the non-extended beef hotdogs had the highest TCC than those of the beef hotdogs extended with the legume flours. The total coliform count is considered particularly useful as indicators of contamination when it is present in small numbers. Their occurrence in large numbers could be due to mishandling such as temperature misappropriation. The total coliform count of the beef hotdogs extended with legumes flour and non-extended increases as the storage days increases and also decreases with the use of extender in the beef hotdogs samples. This is in agreement with report of Venia *et al.* (2006) for dehydrated kilishi stored for 2 days after production.

The *Staphylococcus aureus* count of the beef hotdogs samples as shown in Table 3 increased as the storage days increases. Similarly, non-extended beef hotdogs had the highest *Staphylococcus aureus* count of 0.20±0.01 $\log_{10}$ cfu/g (day 6) and 0.75±0.04 $\log_{10}$ cfu/g (day 9) while the beef hotdogs extended with pigeon peas flour had the least *Staphylococcus aureus* count of 0.00±0.01 $\log_{10}$ cfu/g (day 6) and 0.60±0.01 $\log_{10}$ cfu/g (day 9). The higher *Staphylococcus aureus* count of 0.75±0.04 $\log_{10}$ cfu/g observed in non-extended beef hotdogs on day-9 than those present in the beef hotdogs extended with legumes flour with a range of 0.60±0.01 – 0.70±0.01 $\log_{10}$ cfu/g raises concern since the growth of *Staphylococcus aureus* to a population of 10^6 cfu/g is considered crucial for the production of 1µg of enterotoxin sufficient to cause intoxication if such food is consumed (Shamsuddeen, 2009). The Total fungal count-TFC ($\log_{10}$ cfu/g) of the beef hotdogs samples during the 9 days reveals that there were no significant variations ($p>0.05$) among the beef hotdogs samples. The fungal count of the beef hotdogs non-extended and extended with the legume flour were low and indicates that aside the extenders other non-meat ingredient like the spices used in the formulation of the beef hotdogs could have conferred anti-fungal potential to both extended and non-extended beef hotdogs. This phenomenon agrees with the report by Tagoe *et al.* (2011).

Table 3: Microbial Load of beef hotdog extended using four different species of legume flours

Storage	Microbial load of samples

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Days	P1	P2	P3	P4	P5
	Total Bacterial Count (TBC) of Samples ($\log_{10}$ cfu/g)				
0	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.02	0.00 ± 0.01	0.00 ± 0.02
3	0.00 ± 0.03	0.00 ± 0.02	0.00 ± 0.02	0.00 ± 0.03	0.00 ± 0.02
6	0.10 ± 0.01^{c}	0.05 ± 0.01^{b}	0.00 ± 0.01^{a}	0.00 ± 0.02^{a}	0.00 ± 0.02^{a}
9	0.75 ± 0.01^{d}	0.65 ± 0.02^{c}	0.60 ± 0.01^{c}	0.40 ± 0.03^{a}	0.50 ± 0.03^{b}
	Escherichia coli of Samples ($\log_{10}$ cfu/g)				
0	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.02	0.00 ± 0.01
3	0.15 ± 0.02^{b}	0.15 ± 0.01^{b}	0.10 ± 0.02^{a}	0.10 ± 0.01^{a}	0.10 ± 0.03^{a}
6	0.35 ± 0.02^{c}	0.30 ± 0.01^{b}	0.30 ± 0.03^{b}	0.20 ± 0.02^{a}	0.30 ± 0.01^{b}
9	0.60 ± 0.02^{d}	0.50 ± 0.02^{c}	0.45 ± 0.01^{b}	0.40 ± 0.01^{a}	0.45 ± 0.02^{b}
	Total Coliform Count (TCC) of Samples ($\log_{10}$ cfu/g)				
0	0.00 ± 0.03	0.00 ± 0.01	0.00 ± 0.02	0.00 ± 0.01	0.00 ± 0.01
3	0.15 ± 0.01^{a}	0.10 ± 0.03^{b}	0.10 ± 0.01^{b}	0.10 ± 0.01^{b}	0.10 ± 0.02^{b}
6	0.45 ± 0.01^{d}	0.40 ± 0.01^{c}	0.30 ± 0.01^{b}	0.20 ± 0.03^{a}	0.40 ± 0.01^{c}
9	0.70 ± 0.02^{e}	0.65 ± 0.01^{d}	0.60 ± 0.01^{c}	0.50 ± 0.01^{a}	0.55 ± 0.02^{b}
	Staphyloccocus aureus (SA) of Samples ($\log_{10}$ cfu/g)				
0	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.02	0.00± 0.01
3	0.00 ± 0.01	0.00 ± 0.04	0.00 ± 0.01	0.00 ± 0.01	0.00± 0.01
6	0.20 ± 0.01^{b}	0.15 ± 0.01^{c}	0.00 ± 0.03^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}
9	0.75 ± 0.04^{d}	0.70 ± 0.01^{d}	0.70 ± 0.01^{c}	0.60 ± 0.01^{a}	0.65 ± 0.03^{b}
	Total fungi Count (TFC) of Samples ($\log_{10}$ cfu/g)				
0	0.00 ± 0.01	0.00 ± 0.03	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.03
3	0.00 ± 0.02	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.02	0.00 ± 0.01
6	0.00 ± 0.01	0.00 ± 0.02	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01
9	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.03

*P1= Beef hotdogs without extender, P2 = Beef hotdogs extended using African yam bean flour, P3 = Beef hotdogs extended using lima beans flour, P4 = Beef hotdogs extended using pigeon peas flour, P5 = Beef hotdogs extended using soybean flour.

Nutritional composition of beef hotdogs extended using 4 different species of legumes was presented in Table 4. The result showed that the least moisture content observed in beef hotdogs extended with Pigeon peas flour which was considered significantly ($p<0.05$) lower than those extended with the other 3 sources of legumes (African yam bean, lima beans and soybean flours) and the non-extended beef hotdogs from day 0 to day 9 of the storage periods. The observed trend for the moisture content of the beef hotdog samples was in the order of P1≥P2>P3>P5>P4 throughout the storage periods of 9 days. It was remarkable to note that there were no significant ($p>0.05$) differences between the beef hotdogs extended with African yam bean flour and that of the non-extended beef hotdogs for moisture content. The observed trend in the value of beef hotdogs been least in moisture content is collaborated in the report of

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Gomezulu and Mongi, (2021); where beef sausages extended with Pigeon peas flour had a lower moisture content compared to that extended using soybeans flour. Conversely, the beef hotdog extended with African yam bean flour had significantly ($p<0.05$) higher moisture content of the range [54.80±0.01 (day-0) to 55.84±0.01% (day-9)] than those of the other 3 sources of legumes (lima bean, pigeon pea and soybean) flours throughout the storage period of 9 days and this observation harmonized with the findings of Bunde *et al*. (2021) where with the increase in percentage of African yam bean flour added to food product the higher the moisture content.

The crude protein in beef hotdogs extended with soybeans flour being of the range of 14.66±0.01 (day-0) to 14.27±0.01 (day-9) was significantly ($p<0.05$) higher than those of beef hotdogs extended with African yam bean, lima beans, pigeon peas and the non-extended beef hotdogs throughout the 9-days of refrigerated (4^{o}C) storage. The trend observed was in the order of P5>P4>P3>P2>P1 throughout the storage period. The findings in crude protein of beef hotdogs extended with the 4 leguminous protein sources been higher that the non-extended beef hotdogs in this study provided further evidence that leguminous protein sources (like African yam bean, lima beans, pigeon peas and soybean flours) can extend meat products while providing an economical, high quality protein source (Serdaroglu *et al*., 2005, Egbert and Borders 2006, Singh *et al*., 2008).

The crude fat in beef hotdogs extended with pigeon peas flour was of 17.65±0.01 (day-0) to 17.47±0.02 (day-9) been significantly ($p<0.05$) lower than those of beef hotdogs extended with African yam bean flour, soybean flour and the non-extended beef hotdogs throughout the 9-days of refrigerated (4^{o}C) storage. Also, throughout the 9-days of refrigerated (4^{o}C) storage; on one hand there was no significant ($p>0.05$) difference between the beef hotdogs extended with Pigeon peas flour and Lima beans flour while on the other hand there were no significant ($p>0.05$) differences among the beef hotdogs extended using Lima beans flour, African yam bean flour, soybean flour and the non-extended beef hotdogs. Notably, the least crude fat content in beef hotdogs extended using Pigeon peas flour had a positive impact in the reduced level of lipid oxidation observed for beef hotdogs extended with Pigeon peas flour in this study. Remarkably, this positive impact observed could also be seen in the trending relationship that exist between the beef hotdog extended with pigeon peas and respectively lima beans flour; which were considered least among the beef hotdogs samples for crude fat [17.47 ± 0.02% ≤

17.71 ± 0.03%] and lipid oxidation [0.63 ± 0.03% ≤ 0.64 ± 0.02%] on day 9 of cold (4^{o}C) storage. Thus, this trend indicates that there could be a direct proportional relationship between crude fat content and lipid oxidation of the beef hotdogs samples.

The crude fiber content in beef hotdogs non-extended and those extended using soybeans flour were significantly (p<0.05) lower than those extended using African yam bean, lima beans and pigeon peas flour respectively during the 6-days of storage period. The exemptional case was observed on storage day-9 where the crude fiber content in beef hotdogs extended with African yam bean flour (0.45±0.01%) was significantly (p<0.05) lower than those extended with lima beans (0.62±0.04%) and pigeon peas (0.66±0.02%) flour respectively, but was significantly (p<0.05) higher than those of non-extended (0.21±0.02%) and extended using soybeans flour (0.30±0.01). This trend observed in this study harmonized with report of Offia-Olua *et al.* (2019) where the crude fiber content of African yam bean flour was considered higher than that of soybeans flour while in the report of Oke *et al.* (2013) the crude fiber content of lima beans flour was higher than that of African yam bean flour. Also, the crude fiber content of pigeon peas flour was considered higher than that of lima beans flour in the report made by Adamu *et al.* (2016). These reports gave evidence as to why the beef hotdogs extended with soybeans flour was least in crude fiber content among the beef hotdogs extended with the legume's flours used in this study.

The ash content of the beef hotdogs extended with Pigeon peas flour was of 2.72±0.02% (day-0) to 2.50±0.03 (day-9) been significantly (p<0.05) higher than those extended with African yam bean flour, lima beans flour, soybean flour and the non-extended beef hotdogs throughout the 9-days of storage. Conversely, the non-extended beef hotdog was of 2.40±0.01 (day-0) to 2.18±0.01 (day-9) been significantly (p<0.05) lower than those extended with African yam bean flour, lima beans flour, Pigeon peas and soybean flour throughout the 9-days of storage. The trend for ash content of beef hotdogs extended and non-extended was the same for the crude fiber content of beef hotdogs extended and non-extended used in this study. Implying that there is a direct proportional relationship between ash content and crude fiber content of the beef hotdogs extended and non-extended. This phenomenon is consistent with several research documentation on ash and crude fiber contents of some underutilized legumes (Aletor *et al.*, 1989, Ogunlade *et al.*, 2014; Amadi 2020).

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The cholesterol content of the beef hotdogs extended with Pigeon peas flour was of 61.77±0.02 mg/100g (day-0) to 61.60±0.01 mg/100g (day-9) been significantly ($p<0.05$) lower than those of the non-extended and extended beef hotdogs using African yam bean flour, lima bean flour and soybean flour respectively throughout the 9 days of storage. Conversely, the cholesterol content of the non-extended beef hotdogs was of 76.09±0.01 mg/100g to 75.65±0.01 mg/100g (day-9) been significantly ($p<0.05$) higher than those extended with African yam bean flour, lima beans flour, pigeon peas flour and soybean flour respectively throughout the 9 days of storage. Notably, there were no significant ($p<0.05$) differences among the beef hotdogs extended with African yam bean flour, lima beans flour and soybean flour respectively during the 9-day storage periods. The cholesterol content in the beef hotdogs samples were similar in trend to that of lipid oxidation with the non-extended beef hotdogs having a significantly ($p<0.05$) higher cholesterol and lipid oxidation than those of the beef hotdogs extended using African yam bean flour, lima beans flour, pigeon peas flour and soybean flour respectively during the 9-days of storage. The phenomenon observed in this study is consistent with that documented by Fellows, (2017) where leguminous protein sources can help to reduce the fat content in sausages. Thereby improving shelf-life of the product by reducing lipid oxidation and cholesterol content of the meat products.

Table 4: Nutritional Composition of beef hotdog extended using four different species of legume flours

Storage Days	Nutritional Composition of Samples				
	P1	P2	P3	P4	P5
	Moisture Content (%)				
0	57.23 ± 0.01^{d}	54.80 ± 0.01^{d}	51.94 ± 0.01^{c}	49.65 ± 0.01^{a}	49.77 ± 0.02^{b}
3	57.31 ± 0.01^{d}	54.90 ± 0.03^{d}	52.03 ± 0.01^{c}	49.71 ± 0.01^{a}	49.87 ± 0.01^{b}
6	58.27 ± 0.02^{d}	55.74 ± 0.01^{d}	53.15 ± 0.04^{c}	50.24 ± 0.01^{a}	50.80 ± 0.03^{b}
9	58.36 ± 0.01^{d}	55.84 ± 0.01^{d}	53.25 ± 0.04^{c}	50.36 ± 0.01^{a}	50.90 ± 0.01^{b}
	Crude Protein (%)				
0	13.55 ± 0.01^{a}	13.75 ± 0.04^{b}	14.06 ± 0.01^{c}	14.35± 0.01^{d}	14.66 ± 0.01^{e}
3	13.49 ± 0.01^{a}	13.69 ± 0.01^{b}	14.03 ± 0.01^{c}	14.30 ± 0.01^{d}	14.59 ± 0.01^{e}
6	13.38 ± 0.03^{a}	13.53 ± 0.01^{b}	13.88 ± 0.01^{c}	14.17± 0.02^{d}	14.46 ± 0.02^{e}
9	13.25 ± 0.01^{a}	13.37 ± 0.01^{b}	13.67 ± 0.01^{c}	14.01± 0.04^{d}	14.27 ± 0.01^{e}
	Crude fat (%)				
0	18.37± 0.01^{b}	18.19± 0.01^{b}	17.83± 0.01^{ab}	17.65± 0.01^{a}	18.25± 0.01^{b}
3	18.31 ± 0.02^{b}	18.16 ± 0.01^{b}	17.78 ± 0.01^{ab}	17.59 ± 0.01^{a}	18.23 ± 0.01^{b}

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6	18.25± 0.01^{b}	18.11± 0.01^{b}	17.74 ± 0.01^{ab}	17.55 ± 0.01^{a}	18.13 ± 0.03^{b}
9	18.20± 0.01^{b}	18.05 ± 0.01^{b}	17.71 ± 0.03^{ab}	17.47 ± 0.02^{a}	18.09 ± 0.01^{b}
			Crude fibre (%)		
0	0.37 ± 0.01^{a}	0.65 ± 0.03^{b}	0.76 ± 0.03^{b}	0.84 ± 0.01^{b}	0.43 ± 0.01^{a}
3	0.35 ± 0.01^{a}	0.60 ± 0.03^{b}	0.73 ± 0.01^{b}	0.81± 0.02^{b}	0.41 ± 0.02^{a}
6	0.27 ± 0.03^{a}	0.53 ± 0.03^{b}	0.66 ± 0.02^{b}	0.72 ± 0.01^{b}	0.36 ± 0.01^{a}
9	0.21 ± 0.02^{a}	0.45 ± 0.01^{b}	0.62 ± 0.04^{c}	0.66 ± 0.02^{c}	0.30 ± 0.01^{a}
			Ash Content (%)		
0	2.40 ± 0.01^{a}	2.53 ± 0.02^{b}	2.59 ± 0.01^{c}	2.72 ± 0.02^{d}	2.50 ± 0.01^{b}
3	2.35 ± 0.03^{a}	2.48 ± 0.01^{b}	2.56 ± 0.01^{c}	2.67 ± 0.01^{d}	2.45 ± 0.01^{b}
6	2.23 ± 0.02^{a}	2.41 ± 0.01^{b}	2.45 ± 0.03^{c}	2.56 ± 0.01^{d}	2.33 ± 0.01^{b}
9	2.18 ± 0.01^{a}	2.33 ± 0.02^{b}	2.39 ± 0.04^{c}	2.50 ± 0.03^{d}	2.25 ± 0.01^{b}
			Cholesterol (mg/100g)		
0	76.09 ± 0.01^{c}	66.13 ± 0.01^{b}	67.84 ± 0.01^{b}	61.77 ± 0.02^{a}	68.21 ± 0.01^{b}
3	76.07 ± 0.01^{c}	66.10 ± 0.04^{b}	67.83 ± 0.01^{b}	61.73 ± 0.01^{a}	67.18 ± 0.01^{b}
6	75.77 ± 0.01^{c}	65.76 ± 0.01^{b}	66.37 ± 0.03^{b}	61.60 ± 0.01^{a}	66.83 ± 0.01^{b}
9	75.65 ± 0.01^{c}	65.71 ± 0.01^{b}	66.29 ± 0.01^{b}	61.60 ± 0.01^{a}	66.77 ± 0.02^{b}

*P1= Beef hotdogs without extender, P2 = Beef hotdogs extended using African yam bean flour, P3 = Beef hotdogs extended using lima beans flour, P4 = Beef hotdogs extended using pigeon peas flour, P5 = Beef hotdogs extended using soybean flour.

Conclusions

The study has revealed that the use of legumes protein as meat extender is beneficial in improving the nutritional qualities of beef hotdogs which was ascertain by the lower level of cholesterol content in the four legume protein sources (African yam bean, lima beans and pigeon peas and soybean flour) than the non-extended beef hotdogs. The protein content also, was higher in the four legumes protein sources than the non-extended beef hotdogs. Besides, the beneficial effect of the four legumes protein sources as meat extender was in improving the shelf-life stability of the beef hotdogs. This was appraised by the lower lipid oxidation and microbial loads on beef hotdogs extended using the four legumes' proteins sources than the non-extended during the 9 days of cold storage. Noteworthy is the use of pigeon peas flour as meat extender in this study because of its greater positive impact on the nutritional and shelf-life quality of the beef hotdogs as was justified in that it was least in cholesterol content, microbial load and in lipid oxidation while maintaining a comparable appreciable level of protein content second to soybean flour among the three sources of legume proteins. Thus, it is recommended that the legumes protein sources as meat extenders, could be used to improve the nutritional qualities and shelf life of beef hotdogs and provide the consumer with food containing less cholesterol content, which might be more healthful in reducing cardiovascular diseases.

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