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Research Article

Production and Quality Evaluation of Probiotic Beef Sausage

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Abstract

Background and objectives: The growing concern of consumers regarding food health and safety issues has led to the development of products that promote health and well-being beyond its nutritional effect. The objective of this study was to develop fermented sausages, incorporating probiotic bacteria as well as determine the viability, bio-chemical qualities and acceptability of the sausage. **Materials and Methods:** Fermented Sausages were produced from beef with the addition of *Lactobacillus acidophilus* (*L. acidophilus*) and *Streptococcus thermophilus* (*S. thermophilus*) as probiotics. The quality was compared with control fermented beef sausage, without the addition of probiotics. Both sausage samples were stored at 10-15°C for 10 weeks. The chemical properties, microbial quality, probiotic viability and sensory acceptance of the sausages were evaluated. **Results:** The results of the chemical composition showed that the probiotic sausages had higher protein (30.28%), ash (11.07%) and carbohydrate (19.02%) contents and lower moisture (31.80%) and fat (7.90%) values than the non probiotic sausage (CS). The pH ranges of the probiotic sausage (5.34-4.11) and control sausage (5.88-5.70) decreased during storage. The percentage lactic acid content of the probiotic sausage was higher (1.35%) than that of the control sausage (0.761%) after storage. The total bacterial counts increased for both probiotic sausage (5.40-7.80 log CFU g^{-1}) and control sausage (8.11-9.10 log CFU g^{-1}) during storage. The probiotics were viable in the fermented sausage during storage with higher population in probiotic sausage (5.98-9.32 log CFU g^{-1}) than that of the control sausage (2.48-2.65 log CFU g^{-1}). The sensory preference evaluation revealed that probiotic beef sausage was more preferred in terms of texture, colour, taste and overall acceptability. **Conclusion:** The use of probiotic bacteria (*L. acidophilus* and *S. thermophilus*) in the production of dry fermented sausage would be beneficial in the meat industry as probiotics delivery vehicles.

Key words: Beef sausage, fungi, lactic acid bacteria, meat industry, probiotic microbes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sausage is a prepared food product, usually made from ground meat, animal fat, salt and spices and generally packed in a casing[1]. Sausages can differ dramatically depending on their ingredients, shapes, curing techniques, level of dryness, weather fresh, cooked, or fermented[2].

The growing concern of consumers regarding healthy foods and safety issues has led to the development of product that promotes health and well-being beyond its nutritional effect. Functional foods are those which promote beneficial effects to human's health beyond nutrition[2,3]. They also play active role in reducing the risk of diseases[4]. Their beneficial effects are due to the addition of bioactive ingredients and the removal or the replacement of undesirable compounds in its composition[5,6].

Among the different types of functional foods, probiotics represents a large share of the functional food market[7,4]. Probiotics can be formulated into many different types of products; mainly in dairy, beverages, cereal products, infant feeding formula, fruit juices and ice-cream[8,1]. Strains of the genera *Lactobacillus* and *Bifidobacterium* are the most widely used probiotic bacteria, other microorganisms such as yeast and some *Escherichia coli* and *Bacillus* species are also used as probiotics[5,9,3].

In the meat industry the demand for new products has greatly influenced its development, especially in sausage type products. Most meat products are considered unhealthy by some population because of the high fat contents and the use of additives in their formulation[10,6]. Therefore the addition of probiotics into fermented sausage to promote the health benefits associated with the consumption of such products is an excellent idea for health conscious consumers. The objective of this study was to develop fermented sausages, in which probiotic bacteria were incorporated and to evaluate the viability of the probiotics, the bio-chemical qualities as well as the sensory acceptability of the sausages.

MATERIALS AND METHODS

Sourcing of raw materials: The meat (beef), full cream milk powder and other ingredients were purchased from Kaduna Central Market. The Probiotic starter culture and the cellophane casing were purchased from Abuba super-market in Kaduna. The chemicals used for the enumeration of the probiotics were obtained from Sigma Aldrich, Australia. The reagents and facilities used for the study were of analytical grade and standard qualities.

Preparation of probiotic stater cultures: Commercial probiotic starter cultures (Yo-Flex-L812), a highly concentrated yogurt starter culture of *Lactobacillus acidophilus and Streptococcus thermophilus* were added as liquid inoculums by dissolving 10 g granules of the freeze dried probiotic starter cultures in 10 mL of distilled water.

Production of probiotic beef sausage: The probiotic sausages were produced as shown in Fig. 1. Freshly cut lean meat of excellent hygienic quality was washed in clean water to remove dirt and reduce the bacterial load. The beef was chilled for 3 days for ripening and to help reduce the bacterial load. The meat was then ground through a 3 mm plate to achieve a uniform particle size and desired texture. One kilogram of the minced beef meat was weighed from the lot and mixed with non-meat ingredients (Table 1). The probiotic starter cultures were added and all the ingredients thoroughly mixed and dispersed for even distribution. The sausage-mix was stuffed into cellophane casings of 45 mm in diameter, was allowed to ferment for 48 h, at 25°C and oven dried at 35°C for 24 h. The dried probiotic sausages were then packed in glass jars to prevent reabsorption of moisture and oxygen into the product and labeled PS. The control sample (CS), was also produced by similar procedure but without the probiotic

Table 1: Recipe formulation of fermented sausage

Ingredients (%)	CS	CS
Meat (beef)	81.13	81.13
Sodium chloride	2.03	2.03
Full cream milk	16.23	15.43
Garlic	0.16	0.16
Onion	0.16	0.16
White pepper	0.20	0.20
Monosodium glutamate	0.08	0.08
Sodium nitrate	0.01	0.01
Probiotic culture	0.00	0.80

CS: Control sausage, PS: Probiotic beef sausage

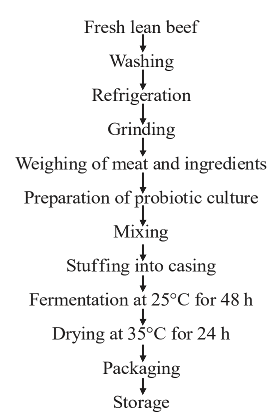

Fig. 1: Flow chart for the production of probiotic beef sausage

starter culture. All the sausage samples were stored in the refrigerator at a temperature between 10-15°C, to maintain the viability of the probiotic bacteria.

Physico-chemical analysis: The proximate composition of the sausage samples was determined as described by Onwuka[11]. The moisture content was determined by indirect distillation drying method, ash content was determined by the muffle furnace ignition method, fat content through the solvent extraction method in a continuous reflux system using the soxhlet apparatus. The protein content was determined by the formal titration method, while the carbohydrate content was determined by recommended mathematical procedures. The pH was measured by using the digital meter. The Lactic acid content of the samples was determined by titration method as described by AOAC[12].

Microbiological assay: The enumeration of the microbial content was determined using the method described by APHA[13] with some modifications. Briefly; 25 g of the sample was homogenized with 225 mL of sterile 0.1% (w/v) peptone water. Decimal dilutions were prepared in 9 mL of sterile 0.1% (w/v) peptone water and aliquots were plated in triplicates on specified media. The plate count agar was used for the total viable bacteria counts; McConkey agar for the coliform counts and Potato dextrose agar with 10% tartaric acid was used to suppress the growth of bacteria for the total fungi counts. The plates were allowed to solidify and then incubated at 37 for 24 h for bacteria and 72-120 h for the growth of fungi. The colonies of each plate were counted. The colony counts were expressed in colony forming units and converted to log CFU g^{-1}.

Enumeration of probiotic microorganisms: The viability of probiotics was determined immediately after production and at 2 weeks intervals during storage. The sausage samples (10 g) were homogenized and decimally diluted with sterile peptone water. One milliliter of the aliquot dilutions was pour plated in triplicate on MRS agar for *L. acidophilus* and *Streptococcus* agar (M17 agar) was used for S. *thermophilus*. The plates were incubated at 38±1°C for 72 h under aerobic condition for *L. acidophilus* and anaerobic condition for *S. thermophilus*. The bacterial viability was represented as survival rate. The number of colonies appearing in the incubated plates of the respective media were counted, averaged and expressed as $\log_{10}$ of the colony forming units per gram (log CFU g^{-1}).

Sensory analysis: The sausage samples were evaluated using 20-member semi-trained panelists drawn from staff and students of the university, as described by Iwe[14]. The sensory attributes of appearance, taste, aroma, texture and acceptability, were evaluated using a 9-point Hedonic Scale with one representing the least score (dislike extremely) and 9 the highest score (like extremely).

Statistical analysis: Data were analyzed using analysis of variance followed by Tukey's test for comparisons among means with a significance level of 5%. Statistical analysis was performed using SAS statistical package, version 9.2. Mean and standard deviation of the data was determined.

RESULTS AND DISCUSSION

Physico-chemical properties: Table 2 shows the proximate composition of the fermented beef sausages after 10 weeks of storage. The moisture (31.80%) and fat (7.90%) contents of probiotic sausage were lower than that of the control sausage (33.17 and 10.35%). The crude protein (30.28%), ash (10.03%) and carbohydrate (19.02%) contents of probiotic sausage were higher than that of the control sausage (29.75, 9.21 and 13.73% respectively). This showed that the nutrient value of probiotic sausage was superior to the nutrient value of control sausage which agrees with the findings of Abdolghafour and Ahmad[15] on the chemical properties of fermented sausage with probiotics.

The pH and lactic acid contents of the fermented beef sausages are presented in Fig. 2 and 3 respectively. The pH values of the fermented sausages followed the opposite trend of the percentage Lactic acid. The pH of probiotic sausage (3.64-4.84) was generally lower than that of control sausage (5.55-5.75). The pH helps to determine the acidity and alkalinity of foods. Microbes are classified according to their survival in different pH environment[1].

The percentage Lactic acid in probiotic sausage (1.12-1.73) was higher than that of the control sausage (0.81-0.98%). The Lactic acid content in probiotic sausage was

Table 2: Proximate composition of sausage samples (%)

Sample	Moisture	Protein	Fat	Ash	Carbohydrate
CS	33.17±0.24^a	29.75±0.13^a	10.35±0.15^a	9.21±0.29^a	13.73±0.05^a
PS	31.80±0.28^b	30.28±0.10^a	7.90±0.20^b	10.03±0.31^a	19.02±0.03^b

Values are Mean±standard deviation, Column with different superscript are significantly different (p<0.05), CS: Control sausage, PS: Probiotic beef sausage

Table 3: Total microbial counts of sausage samples (Log CFU g^{-1})

Weeks	Samples	TBC	TFC	TCC
0	CS	5.40	Nil	Nil
	PS	8.11	Nil	Nil
2	CS	5.67	1.17	Nil
	PS	8.66	Nil	Nil
4	CS	6.04	2.34	Nil
	PS	9.36	Nil	Nil
6	CS	6.54	2.72	Nil
	PS	8.80	Nil	Nil
8	CS	7.70	3.04	Nil
	PS	8.93	Nil	Nil
10	CS	7.80	3.50	Nil
	PS	9.10	Nil	Nil

TBC: Total bacteria counts, TFC: Total fungi counts, TCC: Total coliform counts

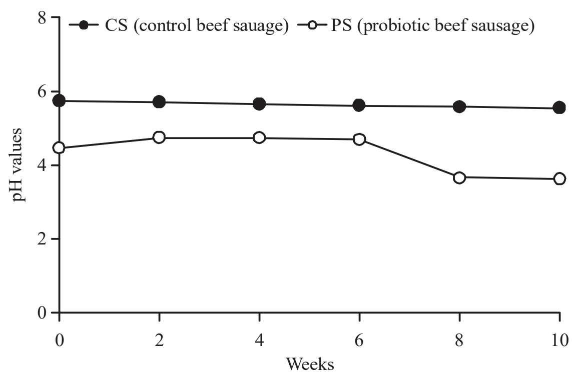

Fig. 2: pH values of sausage samples

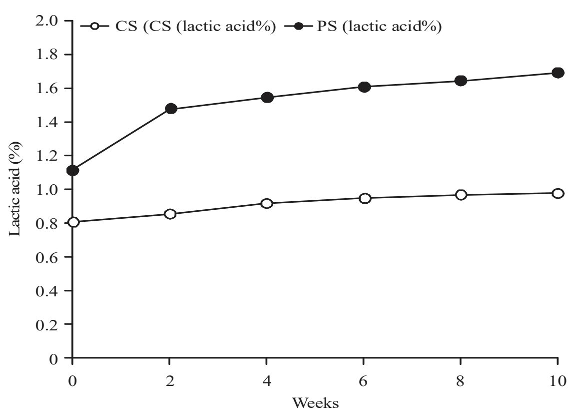

Fig. 3: Lactic acid content of sausage samples

increased after production and during storage compared to control sausage. Production of lactic acid was reduced in both sausage samples after week 6 but the decrease was less in control sausage.

It is plausible since the presence of *L. acidophilus and S. thermophilus* drives the fermentation process which enhances the production of organic acids[16,17]. Changes in both pH values and lactic acid contents have implications in sugar metabolism by Lactic acid bacteria (LAB). The sugar is converted into lactic acid thus lowering the pH values of the sausages[18,19,7]. This showed that LAB organisms were viable with resultant proliferation. This agrees with the findings of Blagoeva *et al.*[20] on the physicochemical and microbiological properties of fermented lamb sausage using probiotic *Lactobacillus* and *Enterobacteriaceae.*

Microbiological analysis of the sausage samples: Table 3 shows the microbial quality of both probiotic (PS) and non-probiotic fermented beef sausage samples. The total bacterial counts increased for both PS (5.40-7.80 log CFU g^{-1}) and CS (8.11-9.10 log CFU g^{-1}) samples during the 10 weeks of storage. There was no fungi growth in both sausages immediately after production. However, fungi growth was observed in the control sausage (CS) which ranged from 1.17-3.50 log CFU g^{-1} at 2-10 weeks of storage. There were no observable fungi and coliform growths in the probiotic sausages during the storage period. It appears that the fermentation conditions were optimal for LAB growth and acid productions, which could have led to the inhibition of fungi and coliforms present in probiotic sausages compared to control sausage (without probiotics). This agrees with the findings of Chow[18] and Ahmad and Nawab[21] on the viability of probiotics in fermented sausages.

Viability of probiotic microbes: During the storage of the fermented beef sausages, the probiotic microorganisms proliferated in both sausage samples (probiotic and Control sausage). The *Lactobacillus* counts and the *Streptococcus* counts (Fig. 4 and 5) reflect the viability of both probiotic strains in the sausage samples during storage. The *Lactobacilli* counts and *Streptococci* counts were found much more in the probiotic sausage (5.98 and 5.57 log CFU) than the control sausage (2.48 and 1.45 log CFU) after production. This was because sample of probiotic sausage was mixed with

Table 4: Sensory evaluation of sausages after storage

Sample	Appearance	Aroma	Taste	Texture	Acceptability
CS	5.90[a]	6.40[a]	6.00[a]	6.63[a]	6.50[a]
PS	8.35[b]	6.45[a]	7.50[b]	7.15[b]	8.15[b]

Values are Mean±standard deviation; Column with different superscript are significantly different (p<0.05), CS: Control sausage, PS: Probiotic beef sausage

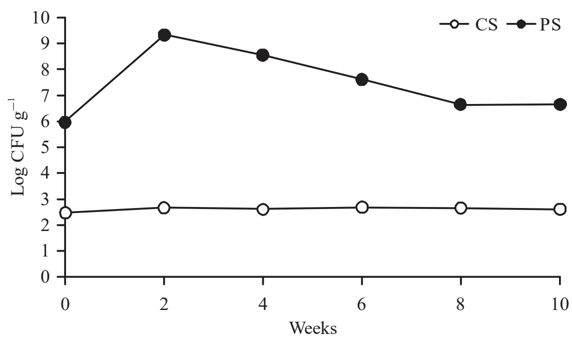

Fig. 4: *Lactobacillus* counts of sausage samples

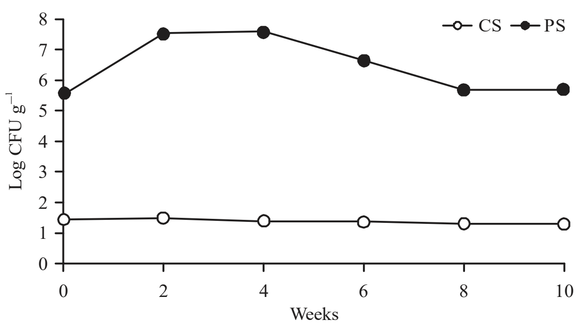

Fig. 5: *Streptococcus* counts of sausage samples

these starter cultures during production. Generally the *Lactobacilli* growth was found much more in probiotic sausage (5.98-9.32 log CFU g^{-1}) than in control sausage (2.48-2.65 log CFU g^{-1}). The increase in the *lactobacillus* was more pronounced in probiotic sausage during the initial period of storage (1-4 weeks). The same growth trend was observed for the *Streptococcus* in probiotic sausage sample. In this study, a drop in pH and increase in the lactic acid content of the sausage samples was observed which was reflected in the growth of the probiotics.

The probiotics were most viable before week 8, after which there was slight decrease in the *Lactobacillus* counts of both sausage samples from 2.65-2.60 log CFU g^{-1} in control sausage and 7.60-6.65 log CFU g^{-1} in the probiotic sausage (Fig. 4). The same trend was observed for the *Streptococcus* counts (Fig. 5). Aramide *et al.*[22] reported similar findings on the physicochemical and microbiological properties of fermented lamb sausage using probiotic *Lactobacillus plantarum*. The viable counts of the probiotics were within the minimum required limit (>7.0 log CFU g^{-1}) for probiotic foods[23-20].

Sensory evaluation of sausages after storage: The result of consumer sensory evaluation after 10 weeks of storage is presented in Table 4. With respect to the sensory attributes of appearance, aroma, taste, texture and overall acceptability; the probiotic sausage was more preferred than non-probiotic (control sausage) (p<0.05). However, the aroma of both sausages (probiotic and control) were not significantly different (p>0.05). The inoculation of sausage was batter with a starter culture composed of selected lactic acid bacteria, *lactobacilli* and *Pediococci* have been reported to improve sausage sensory attributes and safety properties[24,19].

CONCLUSION

The results of this study showed that the nutrient, microbial and organoleptic qualities of probiotic sausage were better than that of control sausage (without probiotics). The added probotics were found to be viable and proliferated with storage time. Also the reduced pH of the probiotic sausage suppressed the growth of yeast, coliform bacteria and other pathogenic organism, making it more stable and hygienic to consume than the control sausage.

SIGNIFICANCE STATEMENT

This study discovered that the use of probiotic bacteria of *Lactobacillus acidophilus and Streptococcus thermophilus* in the production of dry fermented sausage would be beneficial to the meat industry as innovative functional food which will help to deliver probiotics in meat products. However, further study is needed on how to enhance the flavour of the sausage as most consumers do not like the sour taste associated with the fermented sausage.

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