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Effect of ripening period and starter culture level on the quality characteristics of fermented beef sausages

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Abstract

This study was conducted to determine the impact of ripening period and starter culture level on physicochemical parameters, colour parameters, and total bacteria count (TBC) of fermented beef sausage under Sudanese conditions. Combination of equal portions of two strains of lactic acid bacteria (*Streptococcus ssp*. and *Lactobacillus ssp*.) were added as starter culture at two levels (level 1= 1.34 ml/kg and level 2 = 2.67 ml/kg) to formulate two batches of fermented beef sausage. Both the fermented sausages were stored for 0, 7, and 14 days under 85% relative humidity at 21 ºC. Three replicates from each starter level were subjected to analysis for physicochemical parameters, colour measurements, and TBC at the three ripening periods (0, 7, and 14 days). The results revealed significant increase ($P<0.01$) moisture, crude protein, and fat content of fermented sausages with increasing ripening period. The pH was higher at 7 day ripening followed by 0 day ripening, whereas lower pH was observed at 7 day ripening period. Lightness (L^*) increased ($P<0.01$) with ripening period, whereas the redness (a^*) decreased sharply ($P<0.01$). However, yellowness (b^*) value was lower ($P<0.01$) at 7 day ripening period followed by an increase at 14 day ripening and highest values was observed at 0 day ripening period. Furthermore, the higher values of all colour measurements were observed at lower starter level ($P<0.01$). Higher TBC was observed at 7 day ripening period compared to 0 day and 14 day ripening period which did not differ significantly from each other. In conclusion the 14 day ripening period and higher starter culture level results in higher values of physicochemical characteristics and colour measurements; 7 day ripening period results in higher total bacterial count of fermented beef sausages.

1. Introduction

Preservation of food items is achieved through two methods – fermentation and sun-drying (Dirar 1994). There are various indigenous fermented foods in the Sudan, depending on the raw materials which are used such as cereals, dates, honey, wild plants, milk, fish, and meat. Several studies reported that meat quality attributes are a group of physico-chemical, microbiological, organoleptic, and technological attributes (Naveena et al. 2013). Meanwhile, the desire of consumers and/or meat processing industry work on evaluation of meat quality including these attributes. The quality attributes of red meat are influenced by a set of integrated conditions such as pre-slaughter stress, stunning methods, post-mortem handling, and processing methods (Xiong et al. 2014).

Fermented sausages are the products obtained during ripening of meat mixture under well-defined temperature and relative humidity (RH) conditions by a series of biochemical, microbiological, and sensorial changes (Casaburi et al. 2007). Starter cultures are microbial preparations of one or more selected microorganism strains with certain enzymatic activity to be added in determined concentrations to raw materials to form a fermented food by boosting its fermentation process. Starter cultures play important role in processing of conventional cured meat products by increasing their preservation, enhancing their nutritional values, and improving the consumer satisfactoriness (Hammes and Hertel 1998; Leroy and Vuyst 2004). The most important meat-borne strains of starter cultures used in meat industry are lactic acid bacteria (LAB) and Gram positive/catalase-positive cocci (GCC+), particularly *staphylococcus* and *Kocuria* spp such as non-pathogenic coagulase negative staphylococci (CNS). LAB is

tolerant to low pH (acidity) and it produces lactic acid as end product of fermentation. CNS contributes in improvement of colour, constancy, flavour development, and decrease spoilage (Cocconcelli and Fontana 2015).

Fermented sausages have been widely produced in several parts of the world for a long time. However, in Sudan few researches have been done on fermented sausages. Therefore, the objective of this study was to investigate the effect of ripening period and levels of starter cultures on some quality properties of fermented beef sausage under Sudanese conditions.

2. Materials and methods

2.1 Preparation of the starter culture

Streptococcus spp. and *Lactobacillus spp.* were secluded from ordinary yoghurt and two types of culture media (M17 for *Streptococcus spp.* and de Man Rorosa Sharpe (MRS) for *Lactobacillus spp*.) were used. Serial dilutions of yoghurt in 90% normal saline were prepared and each dilution was cultured by streaking on M17 and MRS and incubated at 37° C for 72 hours under atmospheric conditions. One ml was taken from the fourth dilution (10^{-4}) from each bacterial type, the developing colonies were selected and incubated at 37°C for 24 hours on new petri dishes for both bacterial types. The colonies from countable plates were identified through morphological features (light color and smooth surface). The cultures of both *Streptococcus* spp. and *Lactobacillus* spp. used in combined form were cultivated on nutrient broth containing 10% reconstituted Skim Milk Powder at 4° C for one day until used as starter cultures.

2.2 Processing of fermented sausage mixture

Fresh lean beef with fat purchased from local market was stored at 4 ˚C up to processing. Meat and fat were cut up into small pieces, mixed with all non-meat ingredients on per kg basis and minced at low speed through 3.5 mm plate. The minced meat mixture was divided into two batches to formulate the experimental treatments (fermented sausages). Two levels of starter culture were added to each treatment as shown in Table 1, mixed well, then stuffed in 40 mm diameter cellulose casing, and transferred into ripening room at 85% relative humidity (RH) and 21˚ C temperature and stored for 14 days.

Table 1. Ingredients based on the total mixed base

Ingredients (%)	Level 1	Level 2
Lean beef meat	72.8	71.43
Fat	20	20
Salt	2.5	2.5
Black pepper	0.2	0.2
Paprika	0.2	0.2
Skim milk	3	3
Starter culture (ml/kg)	1.34	2.67

2.3 Quality attributes analysis

During fourteen days of ripening period the samples of fermented sausage from both starter culture levels were taken on day 0, 7, and 14 to determine quality attributes as follows:

2.3.1 Chemical analysis and pH determination

Sausage samples of each ripening period and starter culture level were prepared for proximate analysis using (AOAC 2016) official method. Ten gram of sample was blended with 100 ml distilled water at high speed for one minute. The pH measurement of the homogenate was done in the laboratory by Hanna pH meter, Japan (Okerman 1981).

2.3.2 Colour measurements

Color measurements including Lightness (L^*), Redness (a^*), and Yellowness (b^*) were determined using a CR-300 colorimeter (Bench top Spectrophotometer/ Hunter Lab, Japan).

2.3.3 Total bacterial count

Total viable bacterial count was determined according to International Commission on Microbiological Specification for Foods (ICMSF 1978).

2.4 Statistical analysis

Complete randomized design was used in this study. General Linear Model (GLM) was used to analyze the obtained data in factorial arrangement (3×2) followed by least significant difference test (LSD). Obtained data were analyzed using the program IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

3. Results

3.1 Effect on physicochemical parameters

Ripening period and level of starter culture significantly ($P<0.01$) affected physicochemical parameters and pH of fermented sausage (Table 2). Moisture content showed the highest levels (51.67%) at day 14 ripening period; also, crude protein level was high in day 14 and showed the lowest value in day 0 of ripening. Day 7 of ripening showed highest level of fat content followed by day 14. pH values tend to increase up to day 7 and then it declined during day 14 of ripening. However, ash content was not affected by ripening period.

As predictable, the higher level of starter culture resulted in higher ($P<0.01$) moisture, protein, fat, and ash content with lower pH of fermented sausages. Interaction between ripening period and level of starter culture showed significantly higher values of the studied physicochemical parameters at higher starter level and 14 days ripening period.

3.2 Effect on colour measurements

The effect of ripening period and starter culture levels on the colour parameters revealed significant differences ($P<0.01$)

Table 2. Effect of ripening period and starter culture levels on physiochemical parameters

Factor	Proximate composition (%)				pH
	Moisture	CP	Fat	Ash	
Main effect					
Overall mean	51.01	23.17	17.21	4.86	4.05
SEM	0.048	0.284	0.024	0.028	0.006
Ripening period (days)					
0	49.83^{c}	23.34^{a}	17.02^{b}	4.79	4.06^{b}
7	51.42^{b}	21.63^{b}	17.36^{a}	4.93	4.31^{a}
14	51.76^{a}	24.54^{a}	17.26^{a}	4.85	3.79^{c}
SEM	0.082	0.492	0.041	0.048	0.011
Significance	**	**	**	NS	**
Starter culture level (ml/kg)					
1.34	50.76	21.85	16.88	4.57	4.12
2.67	51.25	24.49	17.54	5.14	3.99
SEM	0.067	0.402	0.33	0.39	0.009
Significance	**	**	**	**	**
Reipening period x Starter culture level					
SEM	0.116	0.696	0.058	0.068	0.016
Significance	**	**	*	**	**

N = 3/treatments; ** P<0.01; * P<0.05; CP = Crude protein; NS= Nonsignificant
Different superscript within the same column means significant differences at P<0.01

(Table 3). Lightness (L*) tended to increase (P<0.01) with ripening period, whereas the redness (a*) decreased sharply (P<0.01). However, yellowness (b*) value was lower (P<0.01) at 7 day ripening period followed by an increase at 14 day ripening and highest values was observed at 0 day ripening period. Furthermore, the higher values of all colour measurements were observed at lower starter level (P<0.01).

3.3 Effect on total bacterial count

The TBC was significantly (P<0.01) influenced by ripening period (Table 4). Higher count was observed at 7 day ripening period compared to 0 day and 14 day ripening period which did not differ significantly from each other. However, no effect of starter culture level or the interaction effect was observed on the TBC.

4. Discussion

Several researchers reported significant effect of ripening period on various physicochemical parameters. In this study moisture content increased with ripening period, it could be due to the formation of water as secondary product during lactic acid generation (Casaburi et al. 2007; Drosinos et al. 2007; Kozacinski et al. 2008; Kim et al. 2014; El Adabi et al. 2014; Bingol et al. 2014). The increased protein content of fermented sausages might be attributed to the increase in free amino acid content (Casaburi et al. 2008; AroAro et al. 2010; Candogan et al. 2009; Lorenzo and Franco 2012). High content of fat during ripening period seems to be due to the biochemical reactions and fat retention during refrigerating. These observations were corroborated by earlier researchers too (Galgano et al. 2003; Zuber and Hovart 2007; Casaburi et al. 2007; Mahmoud and Badr 2011). Lowering of pH value during ripening period might be because of the breakdown of carbohydrates by bacteria especially after day 7 and similar observations have been made earlier (Radulovic et al. 2011; Nazli et al. 2017; Mitrovic et al. 2019). As observed in present study, high level of starter culture could accelerate the biochemical changes in the fermented sausages resulting in altered physicochemical measurements (Radulovic et al. 2011; Lorenzo and Franco 2012; Kozacinski et al. 2008; Bingol et al. 2014).

The absorption of moisture as resulted of ripening conditions (RH = 85%) in this study could have possibly resulted in the increment of lightness in beef sausages. Similar increase in lightness values were observed by Baka et al. (2011), however, in another study no significant effect of ripening was observed on the lightness values (Bozkurt and Bayram 2006). Reduction in redness is probably attributed to

Table 3. Effect of ripening period and starter culture levels on colour measurements

Factor	Colour parameters		
	Lightness (L*)	Redness (a*)	Yellowness (b*)
Main effect			
Overall mean	55.01	6.02	13.66
SEM	0.12	0.04	0.07
Ripening period (days)			
0	53.10^{b}	9.57^{a}	15.20^{a}
7	55.79^{b}	4.48^{b}	12.16^{c}
14	56.15^{a}	4.01^{c}	13.61^{b}
SEM	0.201	0.065	0.114
Significance	**	**	**
Starter culture level (ml/kg)			
1.34	56.68	6.35	14.61
2.67	53.35	5.69	12.70
SEM	0.164	0.053	0.093
Significance	**	**	**
Reipening period x Starter culture level			
SEM	0.285	0.091	0.162
Significance	**	**	**

N = 3/treatments; ** P<0.01
Different superscript within the same column means significant differences at P<0.01

Table 4. Effect of ripening period and starter culture levels on total bacterial count (TBC)

Factor	TBC (log CFU/g)
Main effect	
Overall mean	7.39
SEM	0.01
Ripening period (days)	
0	7.34[b]
7	7.49[a]
14	7.35[b]
SEM	0.018
Significance	**
Starter culture level (ml/kg)	
1.34	7.38
2.67	7.41
SEM	0.014
Significance	NS
Reipening period x Starter culture level	
SEM	0.025
Significance	**

N = 3/treatment; ** P<0.01; NS = Nonsignificant
Different superscript within the same column means significant differences at P<0.01

activity of catalyze enzyme produced by lactic acid bacteria which destroys lactate and generates hydrogen peroxide (H_2O_2) that attacks the heme pigments causing oxidative discoloration of sausages (Olivars et al. 2010; Baka et al. 2011; Kargozari et al. 2014). Decrement in yellowness (b^*) throughout ripening period could be attributed to similar factors (El Adabi et al., 2014). Because of low pH at 14 day ripening period low TBC was observed in this study and it was corroborated by earlier researchers too (Drosinos et al. 2007; Baka et al. 2011; El Adabi et al. 2014).

5. Conclusions

The study concludes that ripening period and starter culture level significantly influences the physicochemical characteristics, color measurements, and total bacteria count of fermented sausages. Improved quality parameters of fermented sausages occur at 14 days ripening period along with starter culture level of 2.67 ml/kg.

Declarations

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Conflict of interest: The author declares no conflict of interest.

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