



Effect of adding different types of flour on the quality of low fat beef sausage

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Abstract

The experiment was conducted to find out the effect of different types of flour on the sensory, physicochemical, biochemical and microbiological properties of beef sausages. For this purpose, sausages were prepared into four different groups. They were treated control; beef sausage without flour, beef with 10% rice flour, beef with 10% wheat flour and beef with 10% corn flour. All parameters were analyzed at 0, 15th and 30th days of storage time. The proximate compositions of different sausage batters are analyzed and highly significant differences were found in dry matter and crude protein (%) content and significant differences were found in cooking loss (%), pH (%) and EE (%) content. While analyzing the different types of sausages, highly significant differences were found in pH, DM (%), CP (%) and EE (%) content and significant differences in Ash (%) content. Crude protein (%) and DM (%) increased, while Ash (%) and EE (%) decreased with increase of storage time. Significantly higher DM (%) and CP (%) were found in beef sausage without any flour, no significant differences were found among the flour groups in DM (%) and CP (%) content. The storage period had significant effect on different biochemical (FFA, POV and TBARS value) and microbial (TVC, TCC and TYMC) test. In all cases the values were increased with increase of storage time. The surface color (CIE L^* , a^* , b^*) of sausages of different treatments at different storage period were measured. No significant differences in L^* value were found among beef sausages and storage time. On the other hand, different types of sausages had a significant effect on a^* and b^* values. Significantly higher a^* value was found in beef sausage with 10% corn flour and b^* value in beef sausage without any flour (Control). Storage period had no effect on b^* values, but effect on a^* values. Significantly lower a^* value was found at 0 day of storage time. In sensory analysis, highly significant differences were found in flavor, off-flavor, juiciness, tenderness and overall acceptability among four types of sausages but no significant found in color. All the parameters were significantly lower in control sausage except off-flavour, which was significantly higher in control sausage. No significant differences were found in sensory parameters among the sausages manufactured from different types of flour. From this experiment, it might be concluded that addition of different types of flour increases the overall acceptability of beef sausage.

Keywords: sausage, beef, flour, acceptability

Introduction

Current health concerns have investigated numerous research projects on different types of foods and their constituents to determine whether certain food intakes should be increased, limited, or avoided to prevent heart and other diseases. Particular attention has focused on health problems associated with fat content in food and consumers are looking for no-fat or low-fat meat products (Miller and Groziak, 1996). Yet, fat is an important constituent of human nutrition and contribute to the flavor, tenderness, juiciness, appearance, texture and shelf life of meat products. On the other hand with excessive fat reduction, however, the desired flavor and

texture of the products can be affected, resulting in decreased demand by consumers. In recent years, cereals and their ingredients have been accepted as functional foods, primarily due to constant promotion of dietary fiber, proteins, energy, minerals, vitamins, and antioxidants required for human health. Cereals possess dietary fiber such as β -glucan and arabinoxylan and carbohydrates such as resistant starch and oligosaccharides (Ötles and Cagindi, 2006). It has been reported that carbohydrate-based fat substitutes from plant polysaccharides such as fibers and starches can retain moisture and provide textural qualities that are usually provided by fat (WylieRosett, 2002). However, different types of carbohydrate-based substitutes

will have varying influence on final flavor profile of a product (Lucca and Tepper, 1994).

Besides carbohydrate, rice flour is treated as a good source of vitamins and minerals such as thiamine, niacin, iron, riboflavin, vitamin D and calcium. This flour is extremely low in fiber, hence soothing to the digestive system of the body. Cereal ingredients wheat flour is a vehicle for vitamins and minerals and an important source of carbohydrates, fiber, magnesium, B vitamins, folic acid, antioxidants and phytochemicals. Corn is one of the world's most popular cereal grains. As a good source of antioxidant carotenoids, yellow corn may promote eye health. It's also a rich source of many vitamins and minerals. All segments of the meat industry are attempting to market low-fat products and offer a wider variety of products. Value-added meat products are positively becoming popular. Sausage is one of them. Therefore, the present study was to investigate how the addition of different cereal flours affects the quality of low-fat beef sausages and to find out good quality cereals for making beef sausages with higher consumer's preference.

Materials and Methods

Experimental Design

Four beef sausage formulations were developed (Table 1), as follows: i) beef sausage without flour (Control), ii) beef sausage with 10% rice flour, iii) beef sausage with 10% wheat flour and iv) beef sausage 10% corn flour.

Beef sausage preparation

Sausage batters were prepared by mixing all the ingredients in a plastic bowl. With different groups, flours were added at a level of 10% (wt/wt). For each batch of sausage batters, ingredients were mixed using a mixer machine. After mixing, the mixtures were stuffed into synthetic cellulose casings (approximate diameter of 30 mm) using a sausage stuffer. Then the sausages were cooked in a food steamer for 15 min. The prepared sausages were then packed in food grade poly ethylene bags and stored refrigerated for up to 30 days and assessed immediately after processing (0 day) and at an interval of 15- and 30-days post storage.

Product analysis

Proximate analysis

Moisture, protein, fat and ash of sausages and batters was determined as per the standard procedures of Association of Official Analytical Chemists (AOAC, 1995).

pH

The pH of emulsion and cooked products was determined by blending 10 g of sample with 50 ml of distilled water using an Ultra Turrax T25 tissue homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Germany) at 8,000 rpm for 1 min. The pH of the suspension was recorded by dipping combined glass electrode of Elico digital pH meter, Model LI 127 (Elico Limited, Hyderabad, India).

Cooking loss

To determine cooking loss of sausage batter, weighed 5 g sample and wrapped in a heat stable foil paper and kept in water bath at 80°C for 30 minutes. Samples surface are dried and weighed. Cooking loss was calculated as the percentage of the loss weight of the cooked sample (Symeon et al., 2010). Cooking loss (%) =

$$\frac{\text{Uncooked weight} - \text{cooked weight}}{\text{Uncooked weight}} \times 100$$

Color analysis

The surface color (CIE L*, a*, b*) of sausages samples were measured using a Minolta Chromameter (Minolta CR 410, Tokyo, Japan) standardized with a white plate (Y = 93.5, X = 0.3132, y = 0.3198). Five random reading were taken from each type of sausages. The measurements were averaged for each surface and the results were expressed as positive L*(lightness), a*(redness), b*(yellowness).

TBARS assay, peroxide value (POV) and free fatty acids (FFA)

The amount of malonaldehyde (MDA) was established using a procedure described by Buege and Aust (1978).

TBARS = Abs 530 nm × 7.8 (conversion factor) mg malonaldehyde/kg sausage

FFA value was determined according to Rukunudin et al. (1998). FFA was calculated as shown below: FFA (%) = (ml titration × Normality of KOH × 28.2) / g of sample

Peroxide value (POV) was determined according to (Sallam et al., 2004). POV was calculated as shown below:

$$\text{POV (meq / kg)} = \{(S \times N) / W\} \times 1000$$

Where, S, weight of oil sample; N, normality of sodium thiosulphate and W, weight of the sample.

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Table 1: Ingredient composition of beef sausage batter with different types of flour

Ingredients (g)	Different treatments			
	Beef	Beef+10% rice flour	Beef+10% wheat flour	Beef+10% corn flour
Breast meat (g)	1000	900	900	900
Flour (g)	00	100	100	100
Salt (g)	15	15	15	15
Sodium tripolyphosphate (g)	3.3	3.3	3.3	3.3
Sodium erthorbate (g)	0.37	0.37	0.37	0.37
Maltodextrin(g)	14.8	14.8	14.8	14.8

Microbiological analyses

Microbiological analyses

For microbial assessment total viable count, total coliform count and total yeast-mould count were undertaken. A quantity of 10 g of beef meat sample was aseptically excised from stored stock sample. Each of the stored beef meat samples was thoroughly and uniformly macerated in a mechanical blender using a sterile diluent (0.1% peptone water) as per the recommendation of International Organization for Standardization (ISO, 1995). A quantity of ten (10) grams of the minced meat sample was taken aseptically transferred into a sterile container containing 90 ml of 0.1% peptone water. A homogenized suspension was made in a sterile blender. Thus 1:10 dilution of the samples was obtained. Later on using whirly mixture machine different serial dilutions ranging from 10^2 to 10^6 were prepared according to the instruction of the standard method (ISO, 1995).

Sensory evaluation

Different sensory attributes were examined at day 1. Each sausage sample was evaluated by a trained panel of 6-honorable judges at Bangladesh Agricultural University. Recruitment, selection and training of panelist were performed according to sensory evaluation procedure (AMSA, 1995). The sensory questionnaires measured intensity on a 5-point balanced semantic scale (weak to strong) for the following attributes color, smell, tenderness, juiciness and overall acceptability. Sensory evaluation was carried out in individual booths under controlled conditions of light, temperature and humidity. Sensory qualities of the samples were evaluated after thawing of before cook and after cook using a 5-point scoring method. Sensory evaluation was accomplished at 0, 15th and 30th days.

Statistical analysis

The sausage batter data and the sensory evaluation of different sausages analyzed using analysis of variance technique with the principles of Completely Randomized Design, while sausage data during different storage period were analyzed by 4×3 factorial design (where, 4=different sausages and 3=different storage period) (SAS, 2009). DMRT was done to compare variations among means where ANOVA showed significant differences.

Results and Discussion

Proximate, pH and cooking loss of Sausage batter

Sausage batter is a mixture of ingredients by which sausage is prepared. The proximate, pH and cooking loss of sausage batter was shown in table 2. The data obtained from different sausage batter indicated that there were significant differences among the treatments in Cooking loss, pH, DM, CP and EE content ($p < 0.05$). The higher cooking loss was observed in beef sausage batter without any flour, no significant differences were found in cooking loss among the different flour group of sausage batters. Yang *et al.* (2009) found that duck sausage batter with 10% supplemented wheat flour showed the lowest cooking loss among the other sausage batters. The higher pH value was observed in beef sausage batter with 10%wheat flour (T_3) than others types of sausage batter. Significantly lower DM (%) and higher CP (%) and EE (%) were found in control sausage (beef sausage without any flour). Protein and fat content differed among the sausage batters and was significantly lower in batters with added cereal flours which were found by Yang *et al.* (2009). No significant differences were found in DM (%), CP (%) and EE (%) among the sausage batters manufactured with different types of flour.

Table 2: Proximate composition, cooking loss and pH of low fat beef sausage batter incorporate with different types of flour

Parameters	Treatments				Level of Significance
	Beef	Beef+10% rice flour	Beef+10% wheat flour	Beef+10% corn flour	
Cooking Loss (%)	6.69±0.71 ^a	5.19±0.15 ^b	4.63±0.35 ^b	5.05±0.10 ^b	*
pH	5.93±0.02 ^b	5.95±0.02 ^b	6.03±0.04 ^a	5.92±0.02 ^b	*
Dry matter (%)	25.53±0.34 ^b	26.59±0.08 ^a	26.94±0.28 ^a	27.49±0.36 ^a	**
Ash (%)	2.24±0.08	2.02±0.05	2.11±0.08	2.11±0.08	NS
Crude protein (%)	18.39±0.06 ^a	16.69±0.06 ^b	16.32±0.28 ^b	16.08±0.41 ^b	**
Ether extract (%)	2.40±0.15 ^a	2.05±0.08 ^b	1.92±0.06 ^b	1.82±0.06 ^b	*

* $p < 0.05$; ** $p < 0.01$; NS, Non-significant; Means with different superscripts within a row differ significantly ($p < 0.05$)

Table 3: pH of low fat beef sausages incorporate with different types of flour during different days of intervals

Parameter	days of interval (D)	Treatments (T)				Mean	Level of Significance		
		Beef	Beef+10% rice flour	Beef+10% wheat flour	Beef+10% corn flour		T	D	T*D
pH	0	6.11±0.06	6.13±0.05	6.29±0.07	6.20±0.06	6.18	**	NS	NS
	15	6.11±0.02	6.13±0.06	6.28±0.06	6.10±0.07	6.15			
	30	6.12±0.07	6.17±0.05	6.31±0.08	6.18±0.03	6.19			
	Mean	6.11 ^b	6.14 ^b	6.29 ^a	6.16 ^b				

Note: *, ($p < 0.05$); **, ($p < 0.01$); NS, ($p > 0.05$); a, b, c Different letters indicate significant difference among the treatments

pH of sausages during different days of interval

The pH of different treatments with days of intervals was shown in Table 3. The mean values observed from four treatment groups indicated that there were highly significant ($p < 0.01$) differences among the treatments. However, the higher value was observed in beef sausage with addition of 10% wheat flour, while the other 3 sausages did not differ significantly. McCarthy *et al.*, (2001) and Carpenter *et al.*, (2007) reported no difference in the pH of control and test antioxidants like grape seed, bearberry and rosemary extracts incorporated raw and cooked pork meat product.

The range of overall observed of different days of intervals of pH was 6.15 to 6.19. The mean values observed in 0, 15th and 30th days of observation indicated that there were no significant ($p > 0.05$) differences found among these three days of observation. The interaction between treatment and number of days it was stored does not have a significant difference ($p > 0.05$) on the level of pH.

Proximate analysis of sausages

The proximate analysis of different treatments with days of intervals is shown in Table 3. Result showed that treatment and storage periods have highly significant effect ($p < 0.01$) on DM content. DM content of beef sausage without any flour showed significantly higher DM content than the sausages made from beef incorporated with different types of flour. DM content was increased with the increase of storage period. The results agreed with results of Naveena *et al.* (2008), who have reported an increase in storage period with an increase in the dry matter content of chicken patties incorporated with pomegranate peel extract and pomegranate rind powder extract. The interaction between treatment and storage period did not show significant difference ($p < 0.05$) on DM content.

The range of overall observed CP content at different treatments was 19.56 to 23.22%. The mean value observed from different treatment groups indicated that there were significant ($p < 0.01$) difference found for CP content. Among four treatment groups, the highest CP content

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was observed from beef sausages without addition of flour, while no significant differences were observed among different sausage made from beef incorporated with cereal flours. The range of overall observed of different days of intervals of CP content was 28.13 to 29.16%. The mean value observed from 0, 15th and 30th days of observation indicated that there were significant ($p < 0.01$) differences among these three days of observation. The CP content was increased with the increase of storage period. The highest CP content was observed at 30th day and lowest CP content at 0 days. The interaction between treatment and number of days it was stored have no significant difference ($p > 0.05$) on CP content. The range of overall observed EE content at different treatments was 1.31 to 2.23%. Observation from four treatments, the mean values indicated that there were highly significant ($p < 0.01$) differences of EE content. Among four treatment groups, the lowest EE content was observed at sausage made from beef incorporated with corn flour group and the highest EE content was observed at beef sausage without any cereal flour group. The range of overall observed of different days of intervals of EE content was 1.23 to 2.16%. The mean values observed from 0, 15th and 30th days of observation indicated that there were significant differences ($p < 0.01$) among these three days of observation. The EE content was changed with the increase of storage period. Similar results

were found by Kumar *et al.* (2013) that protein and fat contents were decreased ($p > 0.05$) when investigated the effect of green banana (GBF) and soybean hulls flours (SHF) on chicken meat nuggets. Ali *et al.* (2011) found that crude protein and fat content were significantly lower in the group with added rice flour compared with the no flour group. The range of overall observed ash content at different treatments was 2.12 to 2.25%. The mean values observed from four treatment groups indicated that there were significant ($p < 0.05$) differences of ash content. Among these four treatments, the lowest ash content was observed beef sausage without flour group and the highest was observed from beef sausages with 10% wheat flour group. The range of overall observed of different days of intervals of ash content was 2.04 to 2.26%. The mean values observed from 0, 15th and 30th days of observation indicated that there were highly significant ($p < 0.01$) differences among these three days of observation. The ash content was significantly decreased with the increased storage period. The lowest ash content was observed at 30th day and highest ash content at 0 days. Kaur *et al.* (2015) prepared chicken nuggets incorporated with carrot and reported that with an increase in the storage period ash content is gradually decreased. The interaction between treatment and number of days it was stored did not have a significant difference ($p > 0.05$) on ash content.

Table 4: Proximate composition of low fat beef sausages incorporate with different types of flour during different days of intervals

Parameter (%)	Days of intervals	Treatments (T)				Level of Significance			
		Beef	Beef+10% rice flour	Beef+10% wheat flour	Beef+10% corn flour	Mean	T	D	T*D
DM	0	29.28±0.10	27.47±0.39	27.93±0.13	27.82±0.18	28.13 ^C	**	**	NS
	15	29.84±0.50	28.01±0.11	28.27±0.27	28.09±0.15	28.55 ^B			
	30	31.01±0.31	28.48±0.18	28.40±0.10	28.75±0.15	29.16 ^A			
	Mean	30.04 ^a	27.99 ^b	28.20 ^b	28.22 ^b				
CP	0	22.13±0.33	18.93±0.18	18.98±0.23	18.75±0.47	19.69 ^c	**	**	NS
	15	22.72±0.09	19.90±0.30	19.78±0.15	19.68±0.18	20.52 ^b			
	30	24.83±0.83	20.84±0.09	19.93±0.18	20.27±0.42	21.46 ^a			
	Mean	23.22 ^a	19.89 ^b	19.56 ^b	19.56 ^b				
Ash	0	2.25±0.05	2.23±0.05	2.32±0.02	2.22±0.02	2.26 ^a	*	**	NS
	15	2.16±0.08	2.23±0.05	2.20±0.02	2.18±0.02	2.19 ^a			
	30	1.95±0.07	1.99±0.05	2.23±0.05	2.00±0.06	2.04 ^b			
	Mean	2.12 ^b	2.15 ^b	2.25 ^a	2.13 ^b				
EE	0	2.78±0.08	2.20±0.10	2.05±0.05	1.63±0.18	2.16 ^a	**	**	NS
	15	2.05±0.10	1.28±0.08	1.23±0.08	1.33±0.13	1.47 ^b			
	30	1.85±0.00	1.03±0.07	1.05±0.05	0.99±0.04	1.23 ^b			
	Mean	2.23 ^a	1.50 ^b	1.44 ^{bc}	1.31 ^c				

DM, dry matter; CP, crude protein; EE, ether extract. * $p < 0.05$; ** $p < 0.01$; NS, Non-significant; Means with different superscripts within a row or column differ significantly.

Table 5: International commission on illumination color measurements (CIE*) of low fat broiler meat sausages incorporate with different types of flour during different storage time

Parameter	Days of intervals (D)	Treatments (T)				Level of Significance			
		Beef	Beef+10% rice flour	Beef+10% wheat flour	Beef+10% corn flour	Mean	T	D	T*D
L*	0	49.55±0.38	48.17±0.06	48.21±0.03	47.96±0.26	48.47			
	15	46.09±1.19	48.10±2.01	45.59±1.73	48.63±1.40	47.10	NS	NS	NS
	30	46.32±0.26	47.56±0.75	47.28±0.55	45.17±0.96	46.58			
	Mean	47.32	47.94	47.02	47.25				
a*	0	5.67±0.09	5.62±0.18	5.65±0.09	4.98±0.15	5.48 ^b			
	15	5.84±0.12	5.70±0.08	5.56±0.04	5.93±0.07	5.76 ^a	**	*	**
	30	5.84±0.05	5.98±0.15	5.66±0.25	5.01±0.06	5.62 ^{ab}			
	Mean	5.78 ^a	5.76 ^a	5.62 ^a	5.30 ^b				
b*	0	11.11±0.10	9.88±0.53	10.40±0.08	8.71±0.34	10.02			
	15	10.80±0.28	9.39±0.58	9.68±0.59	9.69±0.53	9.89	**	NS	NS
	30	10.30±0.44	9.42±0.45	10.70±0.30	8.76±0.49	9.79			
	Mean	10.73 ^a	9.56 ^{bc}	10.26 ^{ab}	9.05 ^c				

*p<0.05; **p<0.01; NS, Non-significant; Means with different superscripts within a row or column differ significantly.

Table 6: Biochemical properties of low fat broiler meat sausages incorporate with different types of flour during different storage time

Parameter	Days of interval	Treatments (T)				Level of Significance			
		Beef	Beef+10% rice flour	Beef+10% wheat flour	Beef+10% corn flour	Mean	T	D	T*D
FFA (%)	0	0.19±0.01	0.13±0.03	0.13±0.03	0.14±0.01	0.15 ^c			
	15	0.55±0.03	0.43±0.06	0.41±0.03	0.44±0.06	0.45 ^b	*	**	NS
	30	1.15±0.05	1.05±0.05	1.10±0.00	1.00±0.10	1.08 ^a			
	Mean	0.63 ^a	0.54 ^b	0.54 ^b	0.53 ^b				
POV (meq / kg)	0	1.73±0.01	1.56±0.07	1.65±0.02	1.65±0.02	1.65 ^b			
	15	1.77±0.03	1.72±0.02	1.68±0.05	1.72±0.02	1.72 ^a	**	**	NS
	30	1.92±0.02	1.69±0.02	1.72±0.02	1.71±0.02	1.76 ^a			
	Mean	1.81 ^a	1.66 ^b	1.68 ^b	1.69 ^b				
TBRS (mg malonaldehyde/ kg sample)	0	0.11±0.00	0.11±0.01	0.12±0.01	0.09±0.00	0.11 ^c			
	15	0.18±0.02	0.16±0.02	0.14±0.00	0.13±0.02	0.15 ^b	**	**	NS
	30	0.23±0.01	0.19±0.01	0.19±0.01	0.19±0.01	0.20 ^a			
	Mean	0.17 ^a	0.15 ^b	0.15 ^b	0.14 ^b				

FFA, free fatty acids; POV, per oxide value; TBRS, thiobarbituric acid reactive substance. *p<0.05; **p<0.01; NS, Non-significant; Means with different superscripts within a row or column differ significantly.

The instrumental sausage surface color (CIE L*, a*, b*)

The surface color (CIE L*, a*, b*) of sausages samples were measured using a Minolta Chroma meter (Minolta CR 410, Tokyo, Japan) standardized with a white plate (Y =93.5, X = 0.3132, y = 0.3198) shown in Table 5. The range

of overall observed color score at different treatment for lightness (L*) was 47.02 to 47.94. The mean values observed from four treatment indicate that there were no significant difference (P>0.05) exist among four treatments. Of the four treatments group highest reading was observed from beef sausage with 10% rice flour and lowest was observed from beef sausage with

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10% wheat flour group. Whereas, the range of different days of interval of overall observation of color score for lightness was 46.58 to 48.47. The mean values observed from 0, 15th and 30th days of observation indicated that there were no significant differences ($p > 0.05$) found among these three days of observation. The higher reading was observed from 0 day and lower reading was observed from 30th day. Again, there was no significant difference ($p > 0.05$) exist between the interaction of treatments and number of days it was stored under refrigerated condition. Singh *et al.* (2014) conducted an experiment on the shelf life evaluation of raw chicken meat by using different natural preservatives noticed that L^* value did not vary significantly among different treatment and storage period. Ali *et al.* (2011) found lightness decrease by adding rice flour to duck sausage.

The range of overall observed color score at different treatment for redness (a^*) was 5.30 to 5.78. The mean values observed from four treatment indicate that there were a highly significant difference ($p < 0.01$) found among four treatments. Of the four treatment group highest reading was observed from beef sausage without any flour and lowest color score was observed from beef sausage with addition of 10% corn flour group. Whereas, the range of different days of interval of overall observation of color score for redness was 5.48 to 5.76. The mean values observed from 0, 15th and 30th days of observation indicated that there were a significant differences ($p < 0.01$) found among these days of observation. The highest reading was observed from 15th day and lowest from 0 days of storage. The data showed that redness score not increased gradually with the increase in storage period. But there was significant difference ($p < 0.05$) exist between the interaction of treatments and number of days it stored under refrigerated condition. Results agreed with the results of Singh *et al.* (2014), who conducted an experiment on the shelf life evaluation of raw chicken meat by using different natural preservatives reported that redness (a^*) value increase significantly with the increase in storage period. On the other hand, Ali *et al.* (2011) found that redness decreased with adding rice flour in all meat type sausages.

Again, the range of overall observed color score at different treatment for yellowness (b^*) was 9.05 to 10.73. The mean values observed from four treatment indicated that there were highly significant difference ($p > 0.01$) found among four treatments. Of the four treatments highest score was observed from beef sausage without addition

of flour and lowest color score was observed from beef sausages with addition of 10% corn flour group. The range of different days of interval of overall observation of color score for yellowness was 9.79 to 10.02. The mean values observed from 0, 15th and 30th days of observation indicated that there were no significant differences ($p > 0.05$) exist among these days of observation. The highest color score was observed from 0 days and lowest from 30th day. There was no significant difference ($p > 0.01$) found between the interaction of treatments and number of days it stored under refrigerated condition. Anna *et al.* (2011) observed a decreased color test scores during storage resulted from the denaturation of proteins, particularly the myofibrillar protein that affects gel formation. In our experiment, we did not find any significant effect of b^* value during 30 days storage period.

Biochemical properties

The value of Biochemical components were shown in Table 6. The range of overall observed FFA value at different treatments was 0.53 to 0.63. Significant differences ($p < 0.05$) were found in Free Fatty Acid value among four treatment groups. The range of overall observed of different days of intervals of FFA was 0.15 to 1.08. The mean values observed in 0, 15th and 30th days of observation indicated that there were highly significant ($p < 0.01$) differences found among these three days of observation. The FFA value was increased with the increased storage period. Modi *et al.* (2004) reported that the FFA value gradually increase in fresh and smoked meat nuggets as 3.9 and 3.7 respectively during 6 months of frozen storage. Baker *et al.* (2013) reported that increasing storage period significantly rise in free fatty acids content which is similar to my findings.

The range of overall observed peroxide value at different treatment levels was 1.66 to 1.81. Highly significant differences ($p < 0.01$) were found in peroxide value among four treatments. The range of overall observed of different days of intervals of peroxide value was 1.65 to 1.76. The mean values observed at 0, 15th and 30th days of observation indicated that there were significant differences ($p < 0.01$) among these observations. During storage, the peroxide value increased in all treatments. The interaction between treatments and their storage time has also a significant difference on POV value. Das *et al.* (2011) reported a significant increase in peroxide value of the meat samples during refrigerated storage.

The range of overall observed TBARS value at different treatment levels was 0.14 to 0.17. The mean values observed from the treatment groups indicated that there were significant differences ($p < 0.01$) among four treatment groups. Among the treatments, the lowest TBARS value was observed from beef sausage with 10% corn flour. The highest TBARS value was found in beef sausage without any flour. The range of overall observed of different days of intervals of TBARS value was 0.11 to 0.20. The mean values observed from 0, 15th and 30th days of observation indicated that there were significant differences ($p < 0.01$) found among these three days observation. The interaction between treatments and number of days it is stored has no significant differences on TBARS value. The TBARS values increased significantly ($p < 0.01$) during storage in all treatments. Devatkal *et al.* (2008) observed that the TBARS value increased during the refrigerated storage in cooked goat meat patties added with different plant extract. Yadav *et al.* (2018) found a significant increase in TBARS value of control and fiber enriched sausage with an increase in storage period.

Microbiological assessment

The present study observed the presence of micro-flora (TVC) and food borne pathogens (Coliform and Yeast-Mold) on control and different treatment groups at different days of intervals and at different treatment levels. After 0 days of observation, four types sample was preserved at -20°C for the observation at 15th and 30th days.

The microbial properties of different sausages at different days of interval are shown in Table 7. The range of overall observed total coliform count from the beef sausage was 4.66 to 4.98log₁₀ CFU/g in different treatment groups. The mean values of different treatment indicated that there were a significant ($p < 0.05$) differences in TCC values found among four treatment groups. Of the four treatment groups the total coliform count was highest in sausage without cereal flour (4.98log₁₀ CFU/g) and lowest coliform count was found in beef sausage with rice flour (4.66log₁₀ CFU/g). On the other hand, the range of overall observed of different days of intervals of TCC value was 4.00 to 5.50log₁₀ CFU/g. The mean values observed from 0, 15th and 30th days of observation indicated that there were significant differences ($p < 0.01$) found among these three days of observation. The highest coliform count was found at 30th days of storage while lowest was noticed at 0 days of observation. There was no significant difference ($p > 0.01$) exist between the interaction of treatments and number of days it was stored. Reddy *et al.* (2017) observed a significantly ($P < 0.05$) lower coliform count in chicken meat sausages incorporated with natural antioxidant extracts i.e., rosemary and green tea. Sallam *et al.* (2004) reported that the initial aerobic plate count in sausage was 4.4 log₁₀ CFU/g and during the first 10 days of storage the count was observed below 7log₁₀ CFU/g which is the maximum permissible limit for aerobic plate count.

Table 7: Microbial properties of low fat beef sausages incorporate with different types of flour during different storage time

Parameter	Days of interval	Treatments (T)				Level of Significance			
		Beef	Beef+10% rice flour	Beef+10% wheat flour	Beef+10% corn flour	Mean	T	D	T*D
TCC (log ₁₀ CFU/g)	0	4.32±0.17	3.85±0.05	4.02±0.06	3.79±0.11	4.00 ^c			
	15	4.97±0.07	4.72±0.07	4.78±0.04	4.92±0.04	4.85 ^b			
	30	5.64±0.06	5.41±0.06	5.39±0.06	5.56±0.06	5.50 ^a	**	**	NS
	Mean	4.98 ^a	4.66 ^b	4.73 ^b	4.75 ^b				
TYMC (log ₁₀ CFU/g)	0	4.96±0.05	4.60±0.09	4.71±0.08	4.58±0.08	4.71 ^c			
	15	5.87±0.04	5.53±0.09	5.62±0.04	5.58±0.08	5.65 ^b			
	30	6.46±0.06	6.19±0.06	6.13±0.05	6.27±0.06	6.26 ^a	**	**	NS
	Mean	5.76 ^a	5.44 ^b	5.48 ^b	5.47 ^b				
TVC (log ₁₀ CFU/g)	0	6.64±0.01	6.31±0.01	6.50±0.00	6.44±0.01	6.47 ^c			
	15	7.13±0.03	6.77±0.07	6.81±0.21	7.01±0.10	6.93 ^b			
	30	7.71±0.01	7.49±0.00	7.44±0.01	7.57±0.01	7.55 ^a	**	**	NS
	Mean	7.16 ^a	6.86 ^c	6.91 ^{bc}	7.01 ^b				

TCC, total coliform count; TYMC, total yeast mold count; TVC, total viable count. * $p < 0.05$; ** $p < 0.01$; NS, Non-significant; Means with different superscripts within a row or column differ significantly.

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The range of overall observed total yeast-mold count from different chicken sausage was 5.44 to 5.76(log₁₀ CFU/g). The mean values of different treatment indicated that there were highly significant differences (p<0.05) of TYMC values found among four treatment groups. On the other hand, the range of overall observed of different days of intervals of TYMC value was 4.71 to 6.26log₁₀ CFU/g. The mean values observed from 0, 15th and 30th days of observation indicated that there were a significant differences (p<0.01) found among these three days of observation. The highest yeast mold count was found at 30th days while lowest was noticed at 0 days of observation. There was no significant difference (p>0.05) exist between the interaction of treatments and number of days it was stored. Reddy *et al.* (2017) observed a significantly (P<0.05) lower yeast molds count in chicken meat patties incorporated with natural antioxidant extracts i.e., rosemary and green tea.

The range of overall observed total viable count from the different chicken sausages was 6.86 to 7.16 (log₁₀ CFU/g). The mean values of different treatment indicated that there were a significant (p<0.01) differences of TVC values among four treatment groups. Of the four treatment groups the total viable count was highest in beef sausage without any flour (7.16log₁₀ CFU/g) and lowest viable count was found in beef sausage with rice flour (6.86 log₁₀ CFU/g). The range of overall observed TVC of different days of interval was 6.47 to 7.55log₁₀ CFU/g. The mean values observed from 0, 15th and 30th days intervals indicated that there were a significant differences found among these three days of observation. The highest TVC was found at 30th days while lowest was noticed at 0 days of observation. There was no significant difference (p>0.01) exist

between the interaction of treatments and number of days it was stored. Kumar *et al.* (2007) observed that chicken patties prepared by replacing spent hen meat with 5% sorghum flour, 10% barley flour and 5% pressed rice flour recorded higher total plate count and psychrophilic count, which increased significantly during storage up to 35 days of storage.

Sensory evaluation

Sensory evaluation is a scientific discipline that applies principles of experimental design and statistical analysis to the use of human senses (sight, smell, taste, touch and gearing) for the purpose of evaluating consumer products. The sensory analysis was done at 1 day old sausages. The effects of different types of flours on the sensory properties of beef sausages were shown in Table 8.

The data obtained from different treatment indicated that there was no significant difference among the treatments (p>0.05). However, the higher color score was observed in sausage made from beef with addition of 10% corn flour and lower value was observed in sausages made from beef with 10% rice flour. Highly significant differences were found in flavor among different sausages from sensory evaluation (p<0.01). The lowest value was observed in beef sausage without addition of flour and highest value was found in beef sausage incorporated with 10% corn flour. The data obtained from different treatment indicated that a highly significant difference (p<0.01) exist among the sausages. The off-flavor score in beef sausages without incorporation of flour has remarkably higher value than other three treatments. The off-flavor score of flour groups are statistically similar to each other.

Table 8: Sensory properties of cooked low fat broiler sausages manufactured from broiler meat incorporate with different types of flour during different storage time

Parameters	Treatments				Level of Significance
	Beef	Beef+10% rice flour	Beef+10% wheat flour	Beef+10% corn flour	
Color	4.78±0.16	4.68±0.07	4.70±0.12	4.90±0.16	NS
Flavor	3.95±0.11 ^b	4.78±0.17 ^a	4.60±0.07 ^a	4.95±0.14 ^a	**
Off-flavor	1.95±0.06 ^a	1.63±0.09 ^b	1.68±0.05 ^b	1.60±0.06 ^b	**
Juiciness	3.87±0.10 ^b	4.68±0.14 ^a	4.62±0.12 ^a	4.93±0.10 ^a	**
Tenderness	4.33±0.09 ^b	4.80±0.07 ^a	4.78±0.05 ^a	4.95±0.04 ^a	**
Overall acceptability	4.50±0.04 ^b	4.78±0.04 ^a	4.73±0.02 ^a	4.80±0.08 ^a	**

*p<0.05; **p<0.01; NS, Non-significant; Means with different superscripts within a row or column differ significantly.

Highly significant differences were found in juiciness value among different sausages ($p < 0.01$). The lowest juiciness was observed in beef sausage without addition of flour. Although there is no significant differences found among other three treatments, beef sausage treated with 10% rice flour, 10% wheat flour, 10% corn flour has higher juiciness value. Ravindranath *et al.* (1988) studied quantitative and qualitative characteristics of products prepared from buffalo meat and pork, and reported that addition of phosphates improved the sensory scores for juiciness of patties. Similarly, Santhi and Kalaikannan (2014) found that the juiciness score increased with the inclusion of Oat Flour to low-fat chicken nuggets.

A highly significant differences were found in tenderness property among different sausages ($p < 0.01$). Tenderness is interrelated with DM content of the sausages. Beef sausage without addition of different types of flours showed minimum tenderness, no significant differences were found among 3 flour sausages. Ali *et al.* (2011) found that tenderness value was increased by addition of rice flour while studying low-fat sausages with or without 10% hydrated rice flour made from duck, chicken and pork.

The data obtained from different treatment indicated that there is a highly significant difference exist among the treatments ($p < 0.01$). Beef sausage without any cereal flour is less overall acceptable than beef sausages incorporated with 10% rice flour, 10% wheat flour and 10% corn flour. Moreover, the flour sausages are equally acceptable. Ali *et al.* (2011) found that addition of rice flour increased the overall acceptability of duck sausage to that of pork and chicken sausages.

Conclusion

From sensory evaluation, it was found that flavor, juiciness, tenderness were lower, while off-flavor was higher in control sausage. Therefore, it might be concluded that low fat beef sausage incorporated with different types of flour increased the overall acceptability.

Conflict of interest

There is no conflict of interest among the authors.

References

Ali MS, GD Kim, HW Seo, EY Jung, BW Kim, HS Yang and ST Joo (2011). Possibility of making low-fat sausages from duck meat with addition of rice flour. *Asian-Australian Journal of Animal Science* 24: 421 – 428.

AMSA (1995). Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat. *American Meat Science Association* Chicago, IL. USA.

Anna A (2011). Shelf life of boiled restructured buffalo meat rolls in refrigerated storage under vacuum packaging condition. *Journal of Applied Animal Research* 43: 318-323.

AOAC (1995). Official method of analysis of the Association of Official Analytical Chemists 17th edition. *Association of Official Analytical Chemists*. Washington, DC, 7: 65-68.

Baker IA, JE Alkass and HH Saleh (2013). Reduction of oxidative rancidity and microbial activities of the Karadi lamb patties in freezing storage using natural antioxidant extracts of Rosemary and Ginger. *International Journal of Agricultural and Food Research* 2:31-42.

Buege J and SD Aust (1978). Microsomal lipid peroxidation. *Method Enzymology* 52:302-310.

Carpenter R, MN O'Grady, YC O'Callaghan, NM O'Brien and JP Kerry (2007). Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Journal of meat Science* 76:604-610.

Das AK, V Rajkumar and BK Dwivedi (2011). Antioxidant effects of curry leaf (*Murrayakoenigii*) powder on quality of ground and cooked goat meat. *International Journal of Food Science* 18: 559-565.

Devatkal S, SK Mendiratta and ASR Anjaneyulu (2008). Effect of calcium lactate on the quality and shelf life of restructured pork rolls. *Journal of Meat Science* 1:1-6.

ISO (1995). Recommendation of the meeting of the subcommittee, International Organization for Standardization, on meat and meat products. ISO/TC-36/Sc-6. The Netherlands 10-18.

Kaur M, A Kumar, S Kumar, HR Hakeem and S Gupta (2015). Effect of carrot on quality characteristics of chicken sausages. *Journal of Indian veterinary science* 92: 44-47.

Kumar RR, BD Sharma, M Kumar, S Chidanandaiah and AK Biswas (2007). Storage quality and shelf life of vacuum packaged extended chicken patties. *Journal of Muscle Foods* 15:253-263.

Kumar V, AK Biswas, J Sahoo, MK Chatli and S Sivakumar (2013). Quality and storability of chicken sausages formulated with green banana and soybean hulls flours. *Journal of Food Science and Technology* 50:1058-68.

Lucca, A and BJ Tepper (1994). Fat replacers and the functionality of fat in foods. *Trends in Food Science and Technology* 5:12-19.

Mccarthy TL, JP Kerry, JF Kerry, PB Lynch and DJ Buckley (2001). Assessment of the antioxidant potential of natural food and plant extracts in fresh and previously frozen pork patties. *Journal of Meat Science* 57:177-184.

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- Modi VK, NS Mahendrakar, NM Sachindra and DN Rao (2004). Quality of sausages prepared from fresh and smoked layer chicken meat. *Journal of Muscle Foods* 15:195-204.
- Miller, GD and SM Groziak (1996). Impact of fat substitutes on fat intake. *Lipids* 31(S): 293-296.
- Naveena BM, AR Sen, S Vaithyanathan, Y Babji, N Kondaiah (2008). Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. *Meat Science* 80:1304-1308.
- Ötles S and Ö Cagindi (2006). Cereal based functional foods and nutraceuticals. *Acta Scientiarum Polonorum* 5:107-112.
- Reddy DM, AJ Babu, BE Rao, S Moorthy and S Vani (2017). Comparative effect of rosemary extract, green tea extract and BHA on microbiological quality of chicken meat sausages during refrigeration storage. *International Journal of Livestock Research* 7:250-255.
- Rukunudin IH, PJ White, CJ Bern and TB Bailey (1998). A modified method for determining free fatty acids from small soybean sample sizes. *Journal of Ambion Oil Chemical Society* 75:563-568.
- Sallam KI, M Ishioroshi and K Samejima (2004). Antioxidants and antimicrobial effects of garlic in chicken sausage. *Lebensmittel-Wissenschaft Und-Technologie _ Food Science and Technology* 37:849-855.
- SAS Institute Inc (2009). User's Guide: Statistics, Version 9.1. SAS Institute Inc., Cary, NC.
- Singh P, J Sahoo, MK Chatli and AK Biswas (2014). Shelf life evaluation of raw chicken meat emulsion incorporated with clove powder, ginger and garlic paste as natural preservatives at refrigerated storage (4±1°C). *International Food Research Journal* 21:1363-1373.
- Symeon, GK, F Mantis, I Bizelis, A Kominakis and E Rogdakis (2010). Effects of caponization on growth performance, carcass composition, and meat quality of medium growth broilers. *Poultry Science* 89:1481-1489.
- Wylie-Rosett, J (2002). Fat Substitute and Health: An advisory from the nutrition committee of the American Heart Association. *Circulation* 105: 2800-2804.
- Yadav S, AK Pathera, RUI Islam, AK Malik and DP Sharma (2018). Effect of wheat bran and dried carrot pomace addition on quality characteristics of chicken sausage. *Asian-Australasian Journal of Animal Sciences* 10:1-9.
- Yang HS, MS Ali, JY Jeong, SH Moon, YH Hwang, GB Park, and ST Joo (2009). Properties of duck meat sausages supplemented with cereal flours. *Poultry Science* 88:1452-1458.