PHYSICOCHEMICAL AND ORGANOLEPTIC PROPERTIES OF FERMENTED BUFFALO MEAT (CARABEEF) SAUSAGE

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ABSTRACT

An experiment was carried out to develop fermented sausages from lean of carabeef mixed with different proportions of fat of carabeef and/or pork fat. Four different sausage mix formulations were used in the present study and a commercial starter culture 'Bactofam T-SP' comprising of *Staphylococcus carnosus*, *Pediococcus pentosaceus* was used to induce the fermentation process. The study revealed that the mean percent crude protein (CP), ether extract (EE) and total ash (TA) increased from the beginning of the fermentation process up to the end day of fermentation, whereas, there was a gradual fall in the percent moisture content and pH of the sausages of different combinations during the entire production schedule. The residual nitrite of the ready-to-eat fermented carabeef sausages was found to be much below the permissible limit. Organoleptic evaluation revealed that sausages prepared with Combination I (80% carabeef + 20% carabeef fat) and Combination II (80% carabeef + 10% carabeef fat+ 10% pork fat) enjoyed significantly ($p<0.01$) higher panel rating; however, the appearance and colour ratings were found to be non-significant between the sausages prepared with four different combinations of sausage mix.

Keywords: carabeef, fermented sausage, starter culture

INTRODUCTION

India has a very rich livestock heritage and Indian buffaloes account for 58% of the world buffalo population (APEDA, 2010). This enormous buffalo population has been contributing immensely to human nutrition as well as to the economy of the nation. With a growth rate of 27% per annum, the carabeef sector is showing a promising prospect, and buffalo is now regarded as the 'Black Gold' of the Indian meat industry. India exported 495,019.70 MT of buffalo meat during the year 2009-2010 valued at Rs. 5,840,600,000 (APEDA, 2010). Buffalo meat is exported to the Gulf countries either in frozen or chilled form. The inherent difficulties in export of carabeef such as cold shrinkage, oxidative rancidity, drip loss, loss of bloom, surface discoloration, microbial growth together with high cost of transportation etc. have restricted entry of Indian carabeef into the vast European and American markets. Moreover, animal health issues like foot-and-mouth disease, bovine spongiform encephalopathy (BSE) etc. have also been exerting downward pressure on meat imports from the developing countries like India. Therefore, development of suitable state-of-the-arttechnology for production of value added processed meat products like fermented sausages with superior eating and keeping qualities and high microbial standard is considered highly pertinent. Fermented

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sausages are highly nutrient dense and have good microbiological quality due to its peculiar ecology (reduced pH, a_W etc.). The product is prized for its high palatability characteristics with special reference to typical flavour and taste. Keeping these in view, the present investigation was undertaken to study the effect of sausage mix formulation with or without incorporation of pork and pork fat on the physico-chemical and organoleptic properties of fermented carabeef sausage to help select the best sausage mix formulation for production of fermented carabeef sausage. Carabeef fat is reported to give an unpleasant mouth coating (Padda *et al.*, 1996) and hence pork fat was included in the recipe of fermented carabeef sausage.

MATERIALS AND METHODS

The carabeef lean, fat, pork lean and pork fat were collected from healthy animals slaughtered in the local butcher's shop. The meat and fat were packed in polyethylene bags and brought to the laboratory immediately. The lean of carabeef and pork were trimmed off of visible fascia and cartilages, deboned and cut into small pieces weighing approximately 50 g. The fat and lean were then stored in a deep freeze maintained at -20°C. Best quality spices were used in the study. Natural casings obtained from small intestine of healthy pigs slaughtered in the local butcheries were used in the study.

A basic recipe (Table 1) was taken into consideration in the formulation of various combinations of sausage mix. Carabeef lean, carabeef fat, pork lean and pork fat were mixed as follows:

Combination I: 80% carabeef lean + 20% carabeef fat

Combination II: 80% carabeef lean + 10% carabeef fat + 10% pork fat

Combination III: 60% carabeef lean + 20% pork lean + 20% carabeef fat

Combination IV: 60% carabeef lean+20% pork lean+10% carabeef fat 10% pork fat

The lean of carabeef and pork stored at -20°C were thawed overnight and then minced in a meat mixer grinder. The carabeef and pork fat were introduced into the bowl chopper in frozen state. The minced lean and frozen fat of carabeef and pork were mixed according to different formulations and bowl chopped for 1 minute at slow speed and then for 2 minutes at high speed. Spices and curing ingredients were added as per recipe in the bowl chopper. The sausage mixtures thus prepared were kept overnight in the fermentation cabinet maintained at 10-12°C. Freeze-dried commercial meat starter cultures ('Bactoferm T- SP', M/s. Chr. Hansen, Denmark) comprising *Pediococcus pentosaceus* and *Staphylococcus carnosus* were added to the sausage mix at the dose level prescribed by the manufacturer on the next day and were mixed uniformly in the bowl chopper by operating the instrument at high speed for 1 minute. Finely crushed ice crystals were added during bowl chopping at the rate of about 1% of the sausage mix (w/w). The sausage emulsions were then stuffed into casings with the help of a sausage stuffer. The green sausages thus prepared were then transferred to the fermentation cabinet maintained at temperature of 10-12°C, relative humidity of 90 to 85% and air circulation rate of 0.2 to 0.3 m/sec for 24 h. The fermentation process was continued the till the moisture content of the sausages was reduced to 35 to 45%. The sausages were pressed overnight on the 6th and 13th d of drying and ripening to give a characteristic flattened shape.

Proximate Composition of the Sausage Mix and the Sausages:

The total moisture, CP, EE, TA and residual nitrite contents of the sausage mix and the sausages at different stages of ripening and drying were determined as per methods of AOAC (1986).

Physico-chemical Properties pH:

The pH of the sausage mix was determined immediately before and after adding the curing ingredients and at 24 and 48 h, 3rd, 5th, 6th, 8th, 10th, 12th, 14th and 20th d of ripening by using a spear type electrode of a digital pH meter (Cyberscan, Model 1000).

Organoleptic Evaluation of Sausage Samples:

Ready-to-eat (RTE) fermented sausages prepared with the different sausage mix formulations were subjected to evaluation for organoleptic qualities by a panelof nine semi-trained panelists. Samples were evaluated for appearance, colour, juiciness, flavour and overall acceptability by using a seven-point hedonic scale as described by Hsiao *et al.* (1999).

Statistical Analysis:

The data of the experiment were analyzed statistically as per the methods described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Proximate Composition:

Moisture

The mean percent moisture content of the sausage mix formulations and the sausages prepared from those formulations are presented in Table 3. There was a gradual fall in the percent moisture level of the sausages during the entire production schedule ending on the 20th d of fermentation. On 20th d of fermentation, the sausage of Combination II had the lowest percent of moisture (35.22±0.890) followed by those of Combinations IV, III and I, respectively. The mean percent moisture content of the sausage mix samples prepared with Combination II differed significantly ($p<0.01$) from the other three combinations. There was a sudden fall in mean percent moisture of sausage samples of all the combinations on the 8th and 15th d of fermentation process. This might be due to the pressure applied externally over the sausages to facilitate release of excess water in order to achieve faster drying and also to give a characteristicflattened shape to the fermented sausage in line with *Lukanka* – the famous Bulgarian raw-dried sausage.

Statistical analysis of the data of the four different combinations of sausage mix revealed that the sausage samples differed significantly ($p<0.01$) in respect to percent moisture content. The mean percent moisture levels of fermented sausage at different periods of production process also revealed significant differences ($p<0.01$) amongst the four combinations. The mean percent moisture content of the RTE fermented sausage were in between 35.22-41.35, which was well within the limits for fermented sausages (Bacus, 1984a).

Changes in the moisture profile of the products could partly be attributed to the starter organisms used. The starter cultures help in faster lowering of pH value of the sausages and when the meat attains the ultimate pH of 5.5-5.4, the denaturation of the meat proteins starts as it is also the isoelectric point of major muscle proteins. At ultimate pH, the water holding capacity of the meat is the poorest (Lawrie, 1985) resulting in faster drying of the sausage. Several other investigators (Bacus, 1984b) noted that lower pH denatured the meat protein whereby the native protein structure or conformation gets altered with the unfolding of the polypeptide chains. As a result of denaturation or coagulation of muscle proteins, the intracellular water is released, and this in turn reduces the moisture percent of the products.

Johansson *et al.* (1994) reported that *P. pentosaceus* and *Staph. xylosus* could effectively cause proteolysis when used as starter cultures in fermented sausage leading to lowering of the moisture content.

Crude protein

The mean percent CP content of the different combinations of sausage mix formulations is shown in Table 4. All the four sausage mix formulations showed a gradual rise in the CP content from the very first day of the production process up to the 20th d. In the RTE product, the percent CP ranged from 28.30 to 32.08. The mean percent CP content recorded at different periods of the production process showed significant differences ($p<0.01$) amongst the sausages.

The rise in the percent CP content of sausages might be due to the gradual decrease in moisture content and concomitant increase in the solid contents of the sausage. The results of the present study correlate with the reports of several other workers. Terrell *et al.* (1978) studied the proximate composition of various dry and semidry sausages and reported that the CP contents were in the range of 22 .0 to 35.0 percent. Ortiz-Somovilla *et al.* (2006) carried out a proximate analysis of pork sausages and reported that as the moisture percent of the sausages decreased, the level of crude protein content increased significantly.

Ether Extract

The mean percent EE content of the four different combinations of sausage mix exhibited a gradual increase in the mean percent EE contents up to the 20th d of the production process (Table 5).

Mean percent EE of the sausage mix of the four combinations did not differ significantly at the beginning of the production process. However, at the end of the fermentation process, significant differences were observed in EE contents. This increase in the EE content could also be attributed to the gradual fall in the percent moisture content of the product. It is an established fact that the moisture and fat content of a product are inversely related. The percent EE content in the final product ranged from 24.99 to 30.66.

Terrell *et al.* (1978) studied the fat content of various fermented sausages and reported that typical European-style fermented sausages had an EE content ranging from 16.0 to 39.0 percent. The traditional Greek sausages which are in high demand in European countries were also found to have an EE content of around 29.74 percent (Ambrosiadis *et al.* 2004).

Total Ash

The mean percent TA content of four different combinations of the sausage mix are presented in Table 6. After curing there was a rise in the percent TA content of the sausages which might be due to the addition of salt, salt-petre and other curing ingredients to the sausage formulations. The trend in increase of TA content continued till the final day of the production process, which might reasonably be attributed to the fall in the moisture content of the product. The mean percent total ash content of the sausage at the end of the production schedule was recorded at between 2.57 and 2.96.

Estimation of TA content in the final products revealed that Combinations I and IV did not differ significantly while the other two combinations (II and III) differed significantly between themselves and also with those of Combinations I and IV.

Ambrosiadis *et al.* (2004) reported that the traditional Greek sausages prepared from pork and beef had a TA content of 2.99 percent. They also reported that the TA content together with crude protein and ether extract went up as the sausages approached the final stage of ripening. The TA content, however, greatly depended on the amount of salt added, which was on the other hand dependent on the consumer preference for a particular taste (Bacus, 1984a).

From the data on the mean percent crude protein, ether extract and TA contents, it could be seen that, there was a rise in the percent levels of these nutrients on the 8th and 15th d of the fermentation process which might be accounted for by the pressure applied over the sausage to mobilize the water from the interior of the sausage to the surface facilitating faster and more effective drying.

Residual Nitrite Content

The mean residual nitrite content of the sausage prepared with the four different sausage mix formulations is presented in Table 7. The mean residual nitrite contents of all the RTE fermented carabeef sausages recorded in the study was much below the permissible limit of 200 mg/kg.

In some products like Hungarian salami and Lebanon bologna, a high quantity of nitrate is used (200 to 600mg/kg). However, the technological and microbiological necessity of using such a high amounts of nitrate is questionable, at least in the case of Lebanon bologna, which is a fermented product (Zaika *et al.* 1976).

Dry sausages cured with nitrate or low amounts of nitrite frequently taste better than those made with the usual quantities of nitrite. This clearly indicates that a high concentration of nitrite in the sausage mix at the beginning of the fermentation process may suppress the growth and activity of added starter organisms which synthesize flavour components or their precursors. In the present study, the amount of nitrate/ nitrite added during the curing process was perhaps reasonable as it did not suppress the starter organisms and the finished products recorded pH and the flavour score was acceptable and desirable for a fermented sausage product. Besides, the residual nitrite levels obtained in the different sausage formulations at the end of fermentation process was also indicative of the judicious use of these compounds in the manufacture of rawdried carabeef sausage.

Physico-chemical Properties of Fermented Carabeef Sausage pH

A gradual fall in the pH value of the sausage from the very first day up to the end day of the production process was recorded indicating that *Staph. carnosus* and *P. pentosaceus* used as starter cultures produced acid from the inherent and added carbohydrates of the meat mass. The pH of the final product ranged between 4.72-4.82 (Table 8). From the results presented in Table 8, it may be seen that the mean pH value of the finished product of Combination I was the lowest and differed significantly ($p<0.01$) from the pH values of the other three combinations. The mean pH values of sausage mix of Combinations II, III and IV, however, did not differ significantly amongst themselves.

P. pentosaceus, used as a component of starter culture together with *Lactobacillus rhamnosus* and *L. plantarum*, has been reported to be very fast growing organisms and could bring down the pH of dry fermented sausages from 5.6 to 4.9-5.0 (Erkkilä *et al.,* 2001).

Antara *et al.* (2004) also reported that *P. pentosaceus* when used as a starter organism with other lactic acid bacteria in *Urutan*, a Balanese fermented sausage, could decrease the pH efficiently.

Gönülalan *et al.* (2004) studied the combined effect of *Staph. carnosus* and *P. pentosaceus* together with other LAB on fermented sausages and recorded a final pH of 4.94 to 5.46 after completion of the fermentation process.

Hugas and Monfort (1997) studied bacterial starter cultures suitable for meat fermentation and concluded that *Staph. carnosus* was very much required for lowering the pH of sausages besides the other LAB.

Holley *et al.* (1988) observed that starter cultures containing staphylococcal and pediococcal cultures could effectively lower the pH of the sausages.

The type and amount of carbohydrates added to the sausage mix formulation play an important role in determining the rate and extent of lactic acid formation and the composition of the sausage microflora (Lücke, 1985). With glucose, sucrose or maltose, the rate and extent of lactic acid production is higher than with lactose, starch and dextrin (Klettner and List, 1980; Pyrcz and Pezacki, 1974).

The spices added to the sausage mix formulation also exert certain influence on the acid production capacity of the added starter cultures. Some spices like red pepper, mustard, and mace have been found to stimulate the rate of lactic acid formation, thereby lowering the pH value of the sausage (Vandendriessche *et al.* 1980; Nes and Skjelkvåle, 1982).

Puglia and Seperich (1983) reported that the manganese of spices stimulated the various enzymatic activities of LAB including the key enzyme of glycolysis, fructose-1, 6-diphosphate aldolase (Kandler, 1982).

'*Bactoferm'*, the commercial starter culture used in the present study, has been characterized as an efficient acid producer by the manufacturer.

Organoleptic Qualities of Fermented Carabeef Sausages

The results of sensory evaluation in respect of appearance, colour, flavour and juiciness of fermented carabeef sausages are presented in Table 9. It can be seen that in respect of appearance, Combination I sausages recorded the highest panel score of 5.71±0.11 while the sausages of Combination IV registered the lowest panel score of 5.55±0.16.

The differences in the mean panel score for appearance trait of sausage prepared with four different sausage mix formulations were, however, found to be statistically non significant.

In regards to colour, sausages of Combination I recorded the highest mean panel score of 6.73±0.08 followed by Combinations II and IV. Sausages of Combination III registered the lowest mean panel score of 6.42±0.11.

As regards juiciness, Combination II sausages with a mean score of 6.62±0.08 enjoyed the highest panel rating followed by Combination IV, III and I sausages, respectively. The mean panel score for juiciness of Combination I and III sausages did not differ significantly. Similarly, Combination II and IV sausages revealed no significant difference in respect of mean taste panel score for the juiciness attribute.

In respect to flavour quality, Combination I sausage with a mean score of 6.60±0.10 was rated to be the best followed by Combination III, IV and II sausages, respectively. It may be noted that the mean flavour score of Combinations II, III and IV sausages were similar. However, Combination I sausage differed markedly from the other three combinations in respect of the flavour trait. Organoleptic evaluation of the ready-to-eat fermented carabeef sausage prepared with the four different sausage mix formulations revealed that Combination I, composed of lean and fat of carabeef, registered the highest score in terms of appearance, colour and flavour. However, the juiciness score of the sausage prepared with this combination was less than the other three combinations, indicating the positive role of pork fat in improving the juiciness property of fermented sausage.

Wirth (1973) and Puolanne (1977) opined that the colour development in cured meat products including the fermented sausages were mainly due to the addition of nitrite. The specific flavour and taste of fermented sausages is built up from many components. Some are added to the sausage as such e.g., salt, spices, smoke etc., others are formed without the direct participation of microorganisms (e.g., autoxidation products), and many originate from microbial breakdown of carbohydrates, lipids and proteins.

Nurmi and Niinivaara (1964); Cantoni *et al.* (1967) and Smith and Alford (1968) reported that many Micrococcaceae present in fermented sausages actively attacked pork fat leading to the formation of long chain unsaturated fatty acids which were further degraded to carbonyls and short chain fatty acids that gave flavour to the products.

Halvarson (1973) observed that the lipid contents of meat products were the precursors of many odoriferous compounds, which include aldehydes, ketones and short-chain fatty acids, although some of these compounds (e.g., branched-chain aldehydes) originate from amino acids as well. Maillet and Henry (1960), Alford *et al.* (1971) and Nurmi and Niinivaara (1964) reported that the distinctive flavour of the fermented sausages was found to be related partly to the hydrolytic and oxidative changes occurring to the lipid fraction during ripening.

Berdague *et al.* (1993) evaluated the usefulness of *P. pentosaceus* and *Staph. Carnosus* on the formation of flavour and colour in dry sausages and reported that these two strains proved to have a major effect on the level of volatile compounds in dry sausages as well as on the colour stability of the product.

Molly *et al.* (1997) examined the importance of meat enzymes on the ripening and flavour development of meat products. They opined that meat enzymes also play a major role in the production of flavour compounds.

Erkkilä *et al.* (2001) studied the effect of *P. pentosaceus* on the flavour profile of dry sausages and reported that the flavour could be increased notably by using *P. pentosaceus* as a starter culture. Similar reports were also made earlier by Johansson *et al.* (1994).

Oleson *et al.* (2004) studied the effect of curing ingredients together with *Staph. xylosus* and *Staph. carnosus* on the development of flavour compounds in fermented sausage and reported that curing ingredients had a pronounced effect on formation of volatile compounds. Further they reported that nitrate, when used as a curing ingredient, could support the growth of *Staph. carnosus*, which increased the development of flavour compounds in fermented sausages.

Marco *et al.* (2006) studied the influence of nitrite and nitrate on the sensory properties of slow-dried fermented sausages and reported that the production of volatile compound were much higher

Table 1. Basic recipe of carabeef sausage.

Raw material, spices and condiments	**Quantity per 100 kg**
Lean of carabeef	60
Lean of pork	20
Carabeef fat	10
Pork fat	10
Sodium chloride	2.3
Sodium or Potassium nitrate	0.02
Cane sugar	0.3
Black pepper	0.3
Cumin	0.3
Natural casings	-

Table 2. Conditions of ripening and drying of carabeef sausage.

Parameters	**Stages of ripening and drying**		
	Stage I (1st to 10-12d)	**Stage II** (10-12 to 22d)	**Stage III** {22d to end day of the process (28 to 30d)}
Air temperature- (^{o}C)	10-15	10-15	10-15
Relative humidity-(%)	90-85	80-75	75-70
Air circulation rate- (m/sec)	0.05-0.1	0.05-0.1	0.1

Table 3. Moisture content of carabeef sausage at different stages of ripening and drying.

Combination	BC	AC	24 h	48 h	Periods of ripening (d)							
					3d	5d	6d*	8d	10d	13d*	15d	20d
I	77.56^{j}_{A} ±0.404	77.57^{j}_{A} ±0.380	75.68^{j}_{A} ±0.379	72.05^{j}_{A} ±0.730	69.13^{h}_{A} ±0.727	65.03^{g}_{B} ±1.282	61.03^{f}_{B} ±1.362	55.401^{e}_{C} ±2.016	51.88^{d}_{C} ±1.922	48.52^{c}_{C} ±1.870	44.16^{b}_{C} ±2.051	41.35^{a}_{C} ±1.50
II	78.69^{k}_{AB} ±0.385	78.71^{k}_{AB} ±0.409	75.93^{j}_{A} ±0.340	71.63^{j}_{A} ±0.555	68.20^{h}_{A} ±0.820	63.75^{g}_{A} ±1.138	58.75^{f}_{A} ±1.476	51.81^{e}_{A} ±1.783	47.43^{d}_{A} ±1.482	43.34^{c}_{A} ±1.490	39.05^{b}_{A} ±1.355	35.22^{a}_{A} ±0.890
III	77.74^{k}_{A} ±0.432	77.93^{k}_{A} ±0.451	75.67^{j}_{A} ±0.404	71.80^{j}_{A} ±0.538	68.49^{h}_{A} ±0.601	64.75^{g}_{AB} ±1.351	61.97^{f}_{B} ±1.843	54.12^{e}_{BC} ±2.434	50.17^{d}_{B} ±1.795	47.40^{c}_{C} ±1.409	43.58^{b}_{C} ±1.736	40.67^{a}_{C} ±1.297
IV	79.56^{k}_{B} ±0.272	79.20^{jk}_{B} ±0.393	77.17^{J}_{B} ±0.514	72.16^{j}_{A} ±0.862	68.07^{h}_{A} ±0.873	64.50^{g}_{AB} ±1.591	61.22^{f}_{B} ±2.846	53.76^{e}_{B} ±3.047	49.26^{d}_{B} ±2.695	45.70^{c}_{B} ±2.735	41.79^{b}_{B} ±2.569	37.05^{a}_{B} ±2.559

Mean in a row bearing a common superscript (lower case) do not differ significantly.
Mean in a column bearing a common subscript (upper case) do not differ significantly.

Table 4. Crude protein content of carabeef sausage at different stages of ripening and drying.

Combination	BC	AC	24 h	48 h	Periods of ripening (d)							
					3d	5d	6d*	8d	10d	13d*	15d	20d
I	16.00^{a}_{B} ±0.398	16.76^{a}_{B} ±0.463	17.65^{b}_{B} ±0.349	19.19^{c}_{C} ±0.861	20.12^{c}_{C} ±0.972	21.63^{d}_{B} ±0.967	22.91^{de}_{C} ±1.124	24.32^{ef}_{B} ±1.025	25.43^{f}_{B} ±1.250	26.96^{g}_{B} ±1.449	28.95^{h}_{B} ±1.255	31.76^{i}_{C} ±1.045
II	14.99^{a}_{A} ±0.378	15.56^{a}_{A} ±0.350	17.32^{b}_{B} ±0.353	18.41^{bc}_{BC} ±0.515	19.55^{c}_{C} ±0.684	21.35^{d}_{B} ±0.724	21.83^{d}_{B} ±0.602	25.26^{e}_{C} ±0.633	26.89^{f}_{C} ±0.845	27.8^{f}_{C} ±0.683	30.16^{g}_{C} ±0.941	32.0^{h}_{C} ±0.816
III	15.84^{a}_{B} ±0.380	15.65^{a}_{A} ±0.445	16.69^{ab}_{A} ±0.547	17.22^{bc}_{A} ±0.56	18.47^{cd}_{B} ±0.735	19.35^{de}_{A} ±0.637	20.06^{e}_{A} ±0.819	21.62^{f}_{A} ±0.551	23.93^{g}_{A} ±0.182	25.42^{h}_{A} ±0.489	27.43^{i}_{A} ±0.780	28.3^{i}_{A} ±1.489
IV	15.39^{a}_{AB} ±0.552	15.35_{A} ±0.287	16.41^{ab}_{A} ±0.405	17.76^{b}_{AB} ±0.653	15.68^{a}_{A} ±3.453	2014^{c}_{A} ±1.336	20.75^{cd}_{A} ±1.558	21.89^{d}_{A} ±1.759	24.06^{e}_{A} ±1.778	25.17^{e}_{A} ±2.124	27.91^{f}_{A} ±1.519	29.45^{g}_{B} ±1.591

Mean in a row bearing a common superscript (lower case) do not differ significantly.
Mean in a column bearing a common subscript (upper case) do not differ significantly.

Table 5. Ether extract content of carabeef sausage at different stages of ripening and drying.

Combination	BC	AC	24h	48h	Periods of ripening (d)							
					3d	5d	6d*	8d	10d	13d*	15d	20d
I	5.58^{ab}_{A} ±0.181	4.14^{a}_{A} ±0.491	5.06^{a}_{A} ±0.384	6.80^{b}_{A} ±0.780	8.90^{c}_{A} ±0.494	11.34^{d}_{A} ±0.702	14.18^{c}_{A} ±0.523	17.93^{f}_{A} ±1.218	20.16^{g}_{A} ±1.151	21.86^{h}_{A} ±1.214	24.26^{i}_{A} ±1.253	24.99^{i}_{A} ±1.178
II	5.57^{a}_{A} ±0.088	4.72^{a}_{AB} ±0.264	5.31^{a}_{A} ±0.210	7.89^{b}_{B} ±0.485	10.43^{c}_{B} ±0.608	12.90^{d}_{B} ±0.973	17.36^{c}_{C} ±1.160	20.33^{f}_{B} ±1.137	23.05^{g}_{B} ±0.810	25.89^{h}_{BC} ±0.829	27.81^{i}_{C} ±0.556	29.38^{j}_{C} ±0.775
III	5.49^{a}_{A} ±0.126	5.27^{a}_{B} ±0.275	6.41^{a}_{B} ±0.412	9.59^{b}_{C} ±0.788	11.62^{c}_{C} ±1.177	14.22^{d}_{C} ±1.638	16.39^{c}_{B} ±1.501	22.15^{f}_{C} ±2.370	23.86^{g}_{BC} ±1.767	25.37^{h}_{B} ±1.679	26.49^{hi}_{B} ±1.493	27.06^{i}_{B} ±1.627
IV	5.40^{a}_{A} ±0.130	4.25^{a}_{A} ±0.402	5.46^{a}_{A} ±0.549	8.67^{b}_{B} ±0.783	13.08^{c}_{D} ±1.724	13.51^{c}_{BC} ±0.282	16.15^{d}_{B} ±1.209	21.96^{e}_{C} ±1.364	24.28^{f}_{C} ±1.362	26.62^{g}_{C} ±1.313	27.75^{g}_{C} ±1.312	30.66^{h}_{D} ±1.279

Mean in a row bearing a common superscript (lower case) do not differ significantly.
Mean in a column bearing a common subscript (upper case) do not differ significantly.

Table 6. Total ash content of carabeef sausage at different stages of ripening and drying.

Combination	BC	AC	24 h	48 h	Periods of ripening (d)							
					3d	5d	6d*	8d	10d	13d*	15d	20d
I	0.76^{a}_{B} ±0.024	1.45^{bC} ±0.167	1.57^{cD} ±0.138	1.71^{dD} ±0.109	1.84^{e}_{D} ±0.067	1.99^{f}_{C} ±0.038	2.05^{fg}_{C} ±0.023	2.15^{g}_{C} 0.015	2.30^{h}_{C} ±0.035	2.44^{i}_{B} ±0.039	2.56^{j}_{B} ±0.43	2.71^{k}_{B} ±0.33
II	0.72^{a}_{AB} ±0.018	1.20^{b}_{B} ±0.60	1.41^{c}_{C} ±0.057	1.64^{d}_{C} ±0.095	1.69^{d}_{C} ±0.120	1.96^{e}_{C} ±0.097	2.04^{e}_{C} ±0.097	2.35^{f}_{D} ±0.099	2.44^{f}_{D} ±0.097	2.61^{g}_{C} ±0.124	2.81^{h}_{C} ±0.086	2.96^{i}_{C} ±0.072
III	0.66^{a}_{A} ±0.023	1.10^{b}_{A} ±0.026	1.19^{bc}_{A} ±0.032	1.28^{c}_{A} ±0.037	1.40^{d}_{A} ±0.054	1.60^{e}_{A} ±0.069	1.76^{f}_{A} ±0.080	1.94^{g}_{A} ±0.070	2.15^{h}_{A} ±0.106	2.29^{i}_{A} ±0.112	2.47^{j}_{A} ±0.127	2.57^{j}_{A} ±0.133
IV	0.73^{a}_{B} ±0.035	1.19^{b}_{B} ±0.090	1.30^{c}_{B} ±0.100	$1.40^{cd B}$ ±0.118	1.50^{d}_{B} ±0.127	1.69^{e}_{B} ±0.142	1.83^{f}_{B} ±0.127	2.04^{g}_{B} ±0.095	2.22^{h}_{B} ±0.097	2.38^{i}_{B} ±0.078	2.52^{j}_{AB} ±0.077	2.6^{k}_{B} ±0.082

Mean in a row bearing a common superscript (lower case) do not differ significantly.
Mean in a column bearing a common subscript (upper case) do not differ significantly.

Table 7. Residual nitrite content in fermented carabeef sausage (mg/kg).

Combinations	Residual Nitrite Content
I	$21.45^{a} \pm 3.67$
II	$23.16^{a} \pm 4.16$
III	$23.87^{a} \pm 5.09$
IV	$22.69^{a} \pm 4.08$

Mean in a column bearing a common subscript do not differ significantly.

Table 8. pH values carabeef sausage at different stages of ripening and drying.

Combination	BC	AC	24 h	48 h	Periods of ripening (d)							
					3d	5d	6d*	8d	10d	13d*	15d	20d
I	5.58^{j}_{A} ±0.023	5.52^{ij}_{A} ±0.025	5.44^{hi}_{A} ±0.030	5.40^{gh}_{A} ±0.037	5.32^{fg}_{B} ±0.030	5.24^{ef}_{A} 0.025	5.16^{e}_{A} ±0.049	5.06^{d}_{A} ±0.039	4.96^{C}_{A} ±0.046	4.86^{b}_{A} ±0.060	4.77^{ab}_{A} ±0.025	4.72^{a}_{A} ±0.022
II	5.71^{i}_{B} ±0.080	5.62^{hi}_{B} ±0.058	5.55^{gh}_{B} ±0.049	5.48^{g}_{B} ±0.057	5.19^{de}_{A} ±0.231	5.33^{f}_{B} ±0.067	5.27^{ef}_{B} ±0.078	5.18^{de}_{BC} ±0.079	5.10^{cd}_{C} ±0.071	5.04^{b}_{C} ±0.076	4.94^{b}_{C} 0.056	4.82^{a}_{B} ±0.023
III	5.82^{j}_{C} ±0.086	5.68^{i}_{C} ±0.044	5.61^{hi}_{C} ±0.043	5.54^{gh}_{C} ±0.046	5.47^{fg}_{C} ±0.043	5.41^{f}_{C} ±0.049	5.31^{e}_{BC} ±0.051	5.22^{de}_{C} ±0.042	5.10^{c}_{C} ±0.023	5.00^{b}_{C} ±0.011	4.92^{b}_{BC} ±0.011	4.82^{a}_{B} ±0.032
IV	5.76^{i}_{B} ±0.082	5.65^{h}_{BC} ±0.101	5.59^{h}_{BC} ±0.099	5.49^{g}_{BC} ±0.101	5.42^{fg}_{C} ±0.103	5.34^{e}_{B} ±0.130	5.36^{ef}_{C} ±0.195	5.14^{d}_{B} ±0.126	5.02^{c}_{B} ±0.106	4.94^{bc}_{B} ±0.076	4.88^{ab}_{B} ±0.069	4.81^{a}_{B} ±0.072

Mean in a row bearing a common superscript (lower case) do not differ significantly.
Mean in a column bearing a common subscript (upper case) do not differ significantly.

Table 9. Organoleptic properties of fermented carabeef sausage.

Combination	Appearance	Colour	Juiciness	Flavour
I	5.71±0.11	6.73±0.08	6.07^{a}±0.10	6.60^{b}±0.10
II	5.58±0.12	6.67±0.08	6.62^{b}±0.08	6.15^{a}±0.09
III	5.55±0.16	6.42±0.11	6.20^{a}±0.10	6.29^{a}±0.12
IV	5.69±0.14	6.56±0.10	6.47^{b}±0.10	6.16^{a}±0.10

Mean in a column bearing a common subscript do not differ significantly.

in sausages treated with nitrate, which favoured the growth of starter organisms and thereby increased the flavour components.

The results obtained in the present study on various sensory qualities viz., appearance, colour, flavour and juiciness of fermented carabeef sausages could be explained and interpreted in the light of the above findings in respect of the sensory qualities of fermented raw-dried sausages.

CONCLUSION

From the results it may be concluded that fermented sausages with superior physicochemical and sensory properties could be prepared from lean and fat of carabeef alone. However, the addition of pork fat may be considered to further improve the juiciness property of the sausage.

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REFERENCES

Alford, J.A., J.L. Smith and H.D. Lilly. 1971. Relationship of microbial activity to changes in lipids of foods. *J. Appl. Bacteriol*., **34**: 133.

Antara, N.S., I.N. Sujaya, A. Yokota, K. Asano and F. Tomoita. 2004. Effects of indigenous starter cultures on the microbial and physicochemical characteristics of *Urutan*, a Balinese fermented sausage. *J. Biosci. Bioeng*., **98**(2): 92-98.

AOAC. 1986. *Official Methods of Analysis*, 11th ed. Association of Official Analytical Chemists, Washington, D.C., USA.

APEDA. 2010. Agricultural and Processed Food Products Export Development Authority, www. apeda.com

Bacus J. 1984a. *Utilization of Microorganisms in Meat Processing: A Handbook for Meat Plant Operators*, Research Studies Press Ltd., New York, USA.

Bacus, J. 1984b. Update: Meat Fermentation. *Food. Technol*., (June), 59.

Berdague, J.L., P. Monteil, M.C. Montel and R. Talon. 1993. Effects of starter cultures on the formation of flavour compounds in dry sausage. *Meat. Sci*., **35**(3): 275-287.

Cantoni, C., M.R. Molnar, P. Renon and G. Giolitti. 1967. Lipolytic micrococci in pork fat. *J. Appl. Bacteriol*., **30**: 190.

Erkkilä, S., E. Petäjä, L. Lilleberg, T.M. Sandhoim and M.L. Suihko. 2001. Flavour profiles of dry sausages fermented by selected novel meat starter cultures. *Meat Sci.,* **58**(2): 111-116.

Gönülalan, Z., A. Arslan and A. Köse. 2004. Effect of different starter culture combinations on fermented sausage. *Turkish J. Vet. Anim. Sci*., **22**(10): 120-125.

Halvarson, H. 1973. Formation of lactic acid, volatile fatty acids and neutral, volatile monocarbonyl compounds in Swedish Fermented sausage. *J. Food Sci.,* **38**: 310-312.

Holley, R.A., P.A. Jui, M. Wittmann and P. Kwan. 1988. Survival of *S. aureus* and *S. typhimurium* in raw ripened dry sausages formulated with mechanically separated chicken meat. *Fleischwirt*, **68**(2): 194-201.

Hsiao, H.V., A.D. Clarke, C.S. Kohnke. 1999. Consumer acceptance of reduced-fat Ground Beef Products. *Food Service Res. Intl*., **11**(4): 265.

Hugas, M. and J.M. Monfort. 1997. Bacterial starter cultures for meat fermentation. *Food Chem*., **59**(4): 547-554.

Johansson, G., J.L. Berdague, M. Larsson, N. Tran and E. Borch. 1994. Lipolysis, proteolysis and formation of volatile compounds during ripening of a fermented sausage with *Pediococcus pentosaceus* and *Staphylococcus xylosus* as starter cultures. *Meat Sci.,* **38**(2): 208-213.

Kandler, O. 1982. Gärungsmechaismen bei Milchsäurebakterien. *Forum Mikrobiologie*, **5**: 16-22.

Klettner, P.G. and D. List. 1980. Beitrag zum Einfluss der Kohlenhydratart auf den Verlauf der Rohwurstreifung. *Fleischwirt.,* **60**: 1589-1593.

Lawrie, R. A. 1985. *Meat Science*, 4th ed., Pergamon Press. Oxford, England.

Lücke, F.K. 1985. Fermented sausages. *Appl. Sci.,* **2**: 49-83.

Maillet, J. and M. Henry. 1960. Etude de queiques produtis de la proteolyse et de la lipolyse dans le saucisson cru. *In Proceedings of 6th Symp. Subst. Etrang. dans les Aliments*.

Marco, A., J.L. Navarro and M. Flores. 2006. The influence of nitrite and nitrate on microbial, chemical and sensory parameters of slow dry fermented sausage. *Meat Sci*., **73**(4): 660-673.

Molly, K., D. Demeyer, G. Johansson, M. Raemaekers, M. Ghistelinck and I. Geenen. 1997. The importance of meat enzymes in ripening and flavour generation in dry fermented sausages. *Food Chem.,* **59**(4): 539-545.

Nes, I.F. and R. Skjelkvåle. 1982. Effect of natural spices and oleoresins on *Lactobacillus plantarum* in the fermentation of dry sausage. *J. Food Sci.,* **47**: 1618.

Nurmi, E. and F.P. Niinivaara. 1964. Lipolytic changes of fat in dry sausage. *In Proceedings of 10th Euro. Meet Meat Res Work,* Roskilde, Manuscript G 8.

Oleson, P.T., A.S. Meyer and L.H. Stahnke. 2004. Generation of flavour compounds in fermented sausages - the influence of curing ingredients, *Staphylococcal* starter culture and ripening time. *Meat Sci.,* **66**(3): 675-687.

Padda, G.S., R.C. Keshri, V.K. Rao, B.D. Sharma and N. Sharma. 1996. Organoleptic and physico-chemical properties of carabeef (buffalo meat) patties with different fat combinations. *Indian J. Anim. Sci.,* **56**(4): 478-481.

Puglia, M.L. and G.J. Seperich. 1983. Identification of a stimulatory agent in selected spices. *In Proceedings of 83rd Ann. Meet. American Soc. Microbiol*.

Puolanne, E. 1977. Der Einfluss von Verringerten Nitrite und Nitratzusätzen auf die Eigenschaften der Rohwurst. *J. Sci. Agric. Soc.,* **49**: 1-106.

Pyrcz, J. and W. Pezacki. 1974. Die Technologische Steuerung der Rohwurstreifung.III. Einfluss verschiedener Arten von Kohlenhydraten auf die Dynamik der Prozesse einer Ansammlung von sauren Fermentationserzeugnissen. *Fleischwirt*, **54**: 203-205, 208-211.

Smith, J.L. and J.A. Alford. 1968. Action of microorganisms on the peroxides and carbonyls of rancid fat. *J. Food Sci.,* **33**: 93-97.

Snedecor, G.W. and W.G. Cochran. 1994. *Statistical Methods*, East West Press Pvt. Ltd., New Delhi, India.

Vandendriessche, F., P. Vandekerckhove and D. Demeyer. 1980. The influence of some spices on the fermentation of a Belgian dry sausage, p. 128-133. *In Proceedings of 26th Euro Meet Meat Res. Work*, Colorado Springs, vol. 2.

Weber, H.H. and K. Meyer. 1933. *Biochem. Zentrabiol*., **266**: 137.

Wirth, F.F. 1973. Welche Konsequenzen hätte ein Verbot oder eine Reduzierung des Zusatzes von Nitrate und Nitritpökelsalz zu Fleischerzeugnissen? Aus technologischer Sicht. *Fleischwirt*, **53**: 363-368.

Zaika, L.L., T.E. Zell, J.L. Smith, S.A. Palumbo and J.C. Kissinger. 1976. The role of nitrite and nitrate in Lebanon bologna, a fermented sausage. *J. Food Sci*., **41**: 1457.