QUALITY CHARACTERISTICS OF FERMENTED BUFFALO BEEF SAUSAGE

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ABSTRACT

Suitability of using meat of swamp buffalo (carabeef) alone or in combination with pork in production of fermented sausage was assessed. Four combinations of sausage mix *viz.*, 80% carabeef lean +20% carabeef fat (Combination I), 80% carabeef lean +10% carabeef fat (Combination II), 60% carabeef lean +20% pork lean +20% carabeef fat (Combination III) and 60% carabeef lean +20% pork lean +10% carabeef fat +10% pork fat (Combination IV) were used. pH of the sausages of all the four combinations declined from the beginning to the end of the production process and the sausages of Combination I recorded the lowest pH of 4.62±0.02. There was a gradual fall in the moisture content of the sausages with the advancement of production schedule and on 25th day, the moisture content were 40.13±1.05, 38.12±0.90, 40.22±1.12 and 39.04±0.15% in sausages of Combinations I to IV, respectively. Expulsion of moisture from the sausage mix might have caused an increase in crude protein (CP), ether extract (EE) and total ash (TA) content of sausages and in the finished product with the mean CP, EE and TA content recorded at 28.41±1.01 to 32.14±1.02, 25.97±1.10 to 30.22±1.09 and 2.58±0.09 to 2.98±0.03%, respectively.

Ready-to-eat sausages contained residual nitrite between 21.45±3.67 to 23.87±5.09 mg/kg. The total viable counts, coliform and yeasts and moulds counts of the finished products were 5.38±0.12 to 5.50±0.04 $\log_{10}$cfu/g, 3±0 (MPN/g) and 3.28±0.12 to 3.39±0.09 $\log_{10}$cfu/g, respectively. Panel score for appearance and colour of sausages prepared with different combinations of the sausage mix were not different significantly. Sausages of Combinations I and II enjoyed significantly ($P<0.01$) higher panel ratings for flavour and juiciness, respectively. Good quality fermented sausage could be prepared with swamp buffalo beef alone or in combination with pork.

Keywords: buffalo beef, *Staphylococcus carnosus*, *Pediococcus pentosaceus,* residual nitrite, organoleptic properties

INTRODUCTION

India is endowed with a very rich livestock heritage and its buffalo population accounts for 57.77% of the world buffalo population (FAO, 2014) contributing immensely to the economy of the nation. With a growth rate of 27% per annum, the buffalo meat (carabeef) sector is showing a promising prospect and buffalo is regarded as 'Black Gold' of the Indian meat industry. During 2012 to 2013 India exported buffalo valued at US$ 2.9 billion (FICCI, 2013). Carabeef is exported

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mainly to the Gulf countries either in frozen or chilled form. The inherent difficulties in export of such meat such as cold shrinkage, oxidative rancidity, drip loss, microbial growth, surface discolouration and loss of bloom, and high cost of transportation etc. have restricted expansion of the Indian carabeef trade into the lucrative European and American markets.

Therefore, development of suitable state-of-the-art-technology for production of shelf stable processed meat products like fermented sausages which are highly nutrient dense and have good microbiological quality due to its reduced pH, a_W etc. and superior taste and flavour is considered highly pertinent. Ferreira *et al*. (2006) indicated that fermented sausages are considered safe foods due to the reduction in water activity and pH that occurs during processing and storage and inhibits the development of pathogenic bacteria. *Lactobacillus*, *Micrococcus* and *Staphylococcus* used as meat starter cultures play a vital role during fermentation and ripening of fermented sausage. Availability of cheap labour, natural casings and good quality spices and condiments are the added advantages. Keeping these in view, the investigation was undertaken to study the suitability of using buffalo beef in preparation of fermented buffalo beef sausage either alone or in combination with pork and pork fat.

MATERIALS AND METHODS

The lean of carabeef and fat, pork lean and pork fat were collected from healthy animals slaughtered in the local market and immediately brought to the laboratory in polyethylene bags. Carabeef and pork were trimmed off visible fascia and cartilages, deboned and cut into small pieces of approximately 100 g. The lean and fat were separately stored at -20^{o}C until use. Best quality spices were procured from local market and natural hog casings were used for stuffing and the recipe was formulated (Table 1).

The lean of carabeef and pork were thawed and then minced in a meat mixer grinder. The carabeef and pork fats 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 1min at slow speed followed by 2 minutes at high speed. Spices and curing ingredients were added as per recipe to the bowl chopper. The sausage mix thus prepared were kept for 48 h at 4^{o}C to 5^{o}C to cure. After the initial curing process, freeze-dried commercial meat starter cultures ('Bactoferm T- SP', M/s. Chr. Hansen, Denmark) comprising of *Pediococcus pentosaceus* and *Staphylococcus carnosus* were added to the sausage mix at the dose level prescribed by the manufacturer (25 g per 100 kg) and were mixed uniformly in the bowl chopper by operating the instrument at high speed for 1min. Ice flakes were added during bowl chopping. The sausage emulsions thus prepared were then stuffed into hog casings with the help of a vacuum sausage stuffer. The sausages thus prepared were then transferred to the fermentation cabinet maintained at temperature of 10^{o}C to12^{o}C, relative humidity of 90% to 85% and air circulation rate of 0.2 to 0.3 m/second for 24 h. The fermentation process was accomplished under conditions detailed in Table 2.

Fermentation process was continued till moisture content of the sausages was reduced to 35 to 45%. The sausages were pressed on 6th day by placing the sausages in between two wooden planks surfaced with mica and layered with butter paper to give a flattened shape. A total load of 60 kg distributed over the wooden plank

Table 1. Recipe of buffalo beef sausage.

Raw Material, Spices and Condiments	Quantity per 100 kg
Combination I:	
Carabeef lean	80
Carabeef fat	20
Combination II:	
Carabeef lean	80
Carabeef fat	10
Pork fat	10
Combination III:	
Carabeef lean	60
Pork lean	20
Carabeef fat	20
Combination IV:	
Carabeef lean	60
Pork lean	20
Carabeef fat	10
Pork fat	10
Curing ingredients, spices and condiments:	
Black pepper	0.3
Cane sugar	1.0
Corn flour	3.0
Cumin	0.3
Ice flakes	1.0
Skim milk powder	1.5
Sodium chloride	2.0
Sodium nitrite	0.025

Table 2. Conditions of ripening and drying of buffalo beef sausage.

Parameters	Stages of ripening and drying		
	Stage I (1st to 10-12d)	**Stage II (10-12 to 22d)**	**Stage III {22nd to end day of the process (28 to 30day)}**
Air temperature (°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

was applied. Sausages of identical diameter were placed in between the wooden planks to press the sausages uniformly. This pressing was done for 48 hours and thereafter the sausages were hung in the fermentation cabinet for further drying and ripening. This process was repeated on the 13th day.

pH

pH of the sausages was determined by using a digital pH meter (Cyberscan, Model 1000) by following the method as described by Pippen *et al*. (1965).

Proximate composition

The moisture, protein, ether extract and ash contents of the sausage mix and the sausages at different stages of ripening and drying were determined as per methods of AOAC (1986).

Residual nitrite

Residual nitrate content of the finished product was determined as per method described by AOAC (1986).

Microbiological examination

Total Viable Plate Count: Enumeration of the total viable count of the sausage samples was done in standard plate count agar medium, pH 7.0±0.1 by following the 'pour plate technique' as described by Harrigan and McCance (1976).

Colititre

The colititre of the sausage samples were recorded by following the 'multiple tube technique' as described by Harrigan and McCance (1976). The results were expressed as most probable number (MPN) per gram by using the conversion table designed by AOAC (1986).

Yeasts and Moulds Count: Yeasts and moulds of the sausage samples were enumerated on Rose Bengal Agar, pH 7.2±0.1 (Harrigan and McCance, 1976).

Organoleptic evaluation

Ready-to-eat fermented sausages prepared with the different sausage mix formulations were subjected to evaluation for organoleptic qualities with the help of a 9 membered semi-trained panelists. Samples were evaluated for appearance, colour, juiciness, flavour, taste and overall acceptability by using a 7-point hedonic scale as described by Hsiao, Clarke and Kohnke (1999).

Statistical analysis

The data of the experiment were analyzed statistically as per the methods described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

pH

The results of the pH values of the different combinations of sausage mix and at different intervals of the ripening and drying process are presented in Table 3. There was a gradual fall of pH values from the beginning to the end day of the production process indicating that *Staph. carnosus* and *P. pentosaceus* used as starter cultures could produce acid from the inherent and the carbohydrates added to the sausage mix. Of all the four combinations, sausages of Combination I showed faster fall in the pH value and recorded the lowest pH value of 4.62±0.02 which is significantly lower than the other combinations. Erkkilä *et al*. (2001) observed that when *P. pentosaceus* was used as starter culture together with *Lactobacillus rhamnosus* and *L. plantarum,* it could grow very fast and could brought down the pH of dry fermented sausages from 5.6 to 4.9-5.0. Antara *et al*. (2004) also reported that *P. pentosaceus* when used as a starter organism with other LAB could decrease the pH efficiently in *Urutan*, a Balanese fermented sausage. Gönülalan *et al*. (2004) reported that a final pH of 4.94 to 5.46 could be achieved in fermented sausages when *Staph. carnosus* and *P. pentosaceus* together with other LAB were used as starter cultures. Hugas and Monfort (1997) concluded that *Staph. carnosus* was essentially required to lower the pH of fermented sausages. Salem and Ibrahim (2010) noticed a sharp decrease in the pH values of fermented buffalo meat sausage on the 2nd day of the ripening from 6.9 to 4.52.

Proximate composition

Moisture

The proximate composition of the different sausage mix formulations and of sausages during different stages of the production process are presented in the Table 4. There was a gradual fall in the moisture level of the sausages during the entire production schedule ending on the 25th day of fermentation. On 25th day, sausages of combination II contained the lowest moisture level (38.12±0.09%) followed by those of combination IV, III and I, respectively. The mean moisture content of the sausage mix samples prepared with combination II differed significantly (P<0.01) from the other three combinations. From the results presented in Table 4, it is seen that the moisture content of sausage samples of all the combinations showed a speedier fall on the 8th and 15th day of fermentation process. This might be due to the pressure applied externally over the sausages which might have facilitated release of excess water from the sausage mass to achieve faster and uniform drying. Analysis of the data revealed that the moisture content of the fermented sausages at different stages of the production process differed significantly (P<0.01) amongst the four combinations. The mean moisture content of the ready-to-eat fermented sausages was in between 38.12% to 40.22%, 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 cause faster lowering of pH of the sausage and on attainment of ultimate pH of 5.5 to 5.4 which is also the isoelectric point of major muscle proteins, denaturation starts and the water holding capacity of the meat becomes the poorest (Weber and Meyer, 1933) resulting in faster drying of the sausage. Bacus (1984b) noted that lower pH denatured the meat protein whereby the native protein structure or conformation got altered with the unfolding of the polypeptide chains. As a result of denaturation of muscle proteins, the intracellular water was released which in turn reduced the moisture content 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

All the four sausage mix formulations showed a gradual rise in crude protein content from the beginning of the production process till the 25th day of drying. In the finished product, the crude protein ranged between 28.41 to 32.14% (Table 4). The mean percent crude protein content showed significant (P<0.01) differences amongst the sausage formulations. The rise in crude protein content might be due to the concomitant increase in the solid contents of the sausage resulting from gradual decrease in moisture content. The results

Table 3. Changes in pH value of fermented buffalo beef sausage during ripening and drying process.

Combination	Before curing	After curing	24 h	48 h	Periods of ripening and drying (days)							
					3	5	6	8	10	13	16	25
I	5.55^{j}_{A} ±0.02	5.50^{ij}_{A} ±0.02	5.48^{hi}_{A} ±0.03	5.46^{gh}_{A} ±0.04	5.31^{fg}_{B} ±0.03	5.26^{ef}_{A} ±0.02	5.10^{e}_{A} ±0.05	5.00^{d}_{A} ±0.04	4.91_{A} ±0.05	4.83^{b}_{A} ±0.06	4.72^{ab}_{A} ±0.02	4.62^{a}_{A} ±0.02
II	5.71^{i}_{B} ±0.05	5.66^{hi}_{B} ±0.06	5.60^{gh}_{B} ±0.05	5.49^{g}_{B} ±0.06	5.23^{de}_{A} ±0.02	5.18^{f}_{B} ±0.04	5.21^{ef}_{B} ±0.03	5.15^{de}_{BC} ±0.07	5.08^{cd}_{C} ±0.05	5.00^{b}_{C} ±0.04	4.91^{b}_{C} ±0.05	4.74^{a}_{B} ±0.02
III	5.80^{j}_{C} ±0.04	5.70^{i}_{C} ±0.03	5.67^{hi}_{C} ±0.02	5.52^{gh}_{C} ±0.03	5.48^{fg}_{C} ±0.04	5.40^{f}_{C} ±0.05	5.30^{e}_{BC} ±0.05	5.24^{de}_{C} ±0.04	5.14^{c}_{C} ±0.03	5.02^{b}_{C} ±0.01	4.90^{b}_{BC} ±0.02	4.78^{a}_{B} ±0.02
IV	5.76^{i}_{B} ±0.10	5.68^{h}_{BC} ±0.04	5.60^{h}_{BC} ±0.09	5.47^{g}_{BC} ±0.10	5.40^{fg}_{C} ±0.03	5.32^{e}_{B} ±0.03	5.31^{ef}_{C} ±0.15	5.16^{d}_{B} ±0.13	5.00^{c}_{B} ±0.10	4.91^{bc}_{B} ±0.05	4.85^{ab}_{B} ±0.04	4.73^{a}_{B} ±0.06

n=5

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. Proximate composition of fermented buffalo beef sausage during different stages of ripening and drying.

Parameter	Combination	Before curing	After curing (h)			Periods of ripening (days)							
			1	24	48	3	5	6	8	10	13	16	25
Moisture	I	77.45^{j}_{A} ±0.42	77.50^{j}_{A} ±0.31	75.56^{j}_{A} ±0.27	72.17^{j}_{A} ±0.47	68.79^{h}_{A} ±0.39	65.14^{g}_{B} ±0.44	60.23^{f}_{B} ±0.63	55.24^{e}_{C} ±1.01	51.36^{d}_{C} ±1.08	48.15^{c}_{C} ±0.11	44.23^{b}_{C} ±0.35	40.13^{a}_{C} ±1.05
	II	77.91^{k}_{AB} ±0.53	78.22^{k}_{AB} ±0.42	75.63^{j}_{A} ±0.53	70.45^{j}_{A} ±0.15	68.00^{h}_{A} ±0.11	63.16^{g}_{A} ±0.18	59.51^{f}_{A} ±0.14	52.58^{e}_{A} ±0.78	50.12^{d}_{A} ±1.09	45.13^{c}_{A} ±1.16	42.06^{b}_{A} ±1.05	38.12^{a}_{A} ±0.90
	III	77.67^{k}_{A} ±0.24	77.53^{k}_{A} ±0.55	75.78^{j}_{A} ±0.64	70.80^{j}_{A} ±0.81	68.09^{h}_{A} ±0.63	64.25^{g}_{AB} ±1.01	60.85^{f}_{B} ±1.04	53.32^{e}_{BC} ±1.12	50.07^{d}_{B} ±1.06	47.14^{c}_{C} ±0.09	43.15^{b}_{C} ±1.03	40.22^{a}_{C} ±1.12
	IV	78.75^{k}_{B} ±0.07	77.96^{jk}_{B} ±0.10	76.12^{J}_{B} ±0.45	71.45^{j}_{A} ±0.18	68.16^{h}_{A} ±0.47	64.35^{g}_{AB} ±1.10	60.23^{f}_{B} ±1.20	53.42^{e}_{B} ±0.72	50.08^{d}_{B} ±0.15	46.17^{c}_{B} ±0.17	42.96^{b}_{B} ±1.05	39.04^{a}_{B} ±0.15
Crude Protein	I	16.12^{a}_{B} ±0.28	16.17^{a}_{B} ±0.34	16.57^{b}_{B} ±0.43	18.01^{c}_{C} ±0.18	20.01^{c}_{C} ±0.21	21.75^{d}_{B} ±0.19	23.12^{de}_{C} ±0.24	24.03^{ef}_{B} ±1.01	25.12^{f}_{B} ±1.05	26.51^{g}_{B} ±1.09	28.37^{h}_{B} ±0.09	32.14^{i}_{C} ±1.02
	II	15.42^{a}_{A} ±0.36	15.51^{a}_{A} ±0.23	17.50^{b}_{B} ±0.05	18.33^{bc}_{BC} ±0.43	19.05^{c}_{C} ±0.56	21.23^{d}_{B} ±0.14	21.80^{d}_{B} ±0.18	25.43^{e}_{C} ±0.13	26.25^{f}_{C} ±0.19	27.75^{f}_{C} ±0.09	29.76^{g}_{C} ±0.19	$31.87^{h}c$ ±0.18
	III	15.55^{a}_{B} ±0.18	15.62^{a}_{A} ±0.04	16.78^{ab}_{A} ±0.15	17.20^{bc}_{A} ±0.16	18.56^{cd}_{B} ±0.17	19.05^{de}_{A} ±0.10	20.12^{e}_{A} ±0.19	21.77^{f}_{A} ±0.11	23.54^{g}_{A} ±0.08	25.34^{h}_{A} ±0.09	27.03^{i}_{A} ±0.18	28.41^{i}_{A} ±1.01
	IV	15.76^{a}_{AB} ±0.12	15.79^{a}_{A} ±0.37	16.44^{ab}_{A} ±0.08	17.55^{b}_{AB} ±0.13	16.27^{a}_{A} ±0.09	20.09^{c}_{A} ±1.06	20.55^{cd}_{A} ±1.03	21.66^{d}_{A} ±1.09	24.12^{e}_{A} ±1.07	25.21^{e}_{A} ±0.74	27.98^{f}_{A} ±1.11	32.09^{g}_{B} ±1.05

n=5

Parameter-wise, means in a row bearing a common superscript (lower case) and means in a column bearing a common subscript (upper case) do not differ significantly.

Table 4. Proximate composition of fermented buffalo beef sausage during different stages of ripening and drying. (Cont.)

Parameter	Combination	Before curing	After curing (h)			Periods of ripening (days)							
			1	24	48	3	5	6	8	10	13	16	25
Ether extract	I	5.66^{ab}_{A} ±0.10	5.60^{a}_{A} ±0.09	5.72^{a}_{A} ±0.18	6.83^{b}_{A} ±0.10	8.77^{c}_{A} ±0.12	11.04^{d}_{A} ±0.78	14.21^{c}_{A} ±0.15	17.86^{f}_{A} ±1.08	20.11^{g}_{A} ±1.09	21.88^{h}_{A} ±1.04	24.55^{i}_{A} ±1.23	25.97^{i}_{A} ±1.10
	II	5.70^{a}_{A} ±0.06	5.73^{a}_{AB} ±0.20	5.81^{a}_{A} ±0.10	7.85^{b}_{B} ±0.18	10.05^{c}_{B} ±0.18	12.93^{d}_{B} ±0.19	17.12^{c}_{C} ±1.07	20.12^{f}_{B} ±1.10	23.12^{g}_{B} ±0.80	25.56^{h}_{BC} ±0.09	27.67^{i}_{C} ±0.15	29.21^{j}_{C} ±0.16
	III	5.59^{a}_{A} ±0.10	5.64^{a}_{B} ±0.10	6.32^{a}_{B} ±0.04	9.26^{b}_{C} ±0.0.9	11.55^{c}_{C} ±1.07	14.19^{d}_{C} ±1.03	16.27^{c}_{B} ±1.01	22.45^{f}_{C} ±1.05	23.83^{g}_{BC} ±1.06	25.45^{h}_{B} ±1.09	26.52^{hi}_{B} ±1.00	27.87^{i}_{B} ±1.02
	IV	5.54^{a}_{A} ±0.11	5.59^{a}_{A} ±0.04	5.77^{a}_{A} ±0.15	8.97^{b}_{B} ±0.08	13.10^{c}_{D} ±1.10	13.45^{c}_{BC} ±0.18	16.23^{d}_{B} ±1.09	22.05^{e}_{C} ±1.06	24.19^{f}_{C} ±1.02	26.65^{g}_{C} ±0.18	27.66^{g}_{C} ±1.12	30.22^{h}_{D} ±1.09
Total Ash	I	0.87^{a}_{B} ±0.02	1.41^{bC} ±0.07	1.45^{cD} ±0.03	1.62^{dD} ±0.07	1.87^{e}_{D} ±0.02	2.01^{f}_{C} ±0.05	2.03^{fg}_{C} ±0.03	2.17^{g}_{C} ±0.04	2.30^{h}_{C} ±0.05	2.46^{i}_{B} ±0.02	2.59^{j}_{B} ±0.04	2.74^{k}_{B} ±0.09
	II	0.83^{a}_{AB} ±0.05	1.22^{b}_{B} ±0.05	1.57^{c}_{C} ±0.07	1.76^{d}_{C} ±0.05	1.62^{d}_{C} ±0.10	1.93^{e}_{C} ±0.06	2.07^{e}_{C} ±0.06	2.42^{f}_{D} ±0.10	2.47^{f}_{D} ±0.06	2.60^{g}_{C} ±0.10	2.83^{h}_{C} ±0.05	2.98^{i}_{C} ±0.03
	III	0.79^{a}_{A} ±0.03	1.13^{b}_{A} ±0.02	1.20^{bc}_{A} ±0.03	1.26^{c}_{A} ±0.5	1.42^{d}_{A} ±0.04	1.61^{e}_{A} ±0.02	1.78^{f}_{A} ±0.05	1.93^{g}_{A} ±0.04	2.16^{h}_{A} ±0.10	2.30^{i}_{A} ±0.10	2.45^{j}_{A} ±0.10	2.59^{j}_{A} ±0.09
	IV	0.84^{a}_{B} ±0.06	1.20^{b}_{B} ±0.04	1.33^{c}_{B} ±0.10	$1.42^{cd B}$ ±0.12	1.53^{d}_{B} ±0.10	1.67^{e}_{B} ±0.12	1.86^{f}_{B} ±0.10	2.07^{g}_{B} ±0.05	2.19^{h}_{B} ±0.08	2.37^{i}_{B} ±0.07	2.51^{j}_{AB} ±0.03	2.73^{k}_{B} ±0.05

n=5

Parameter-wise, means in a row bearing a common superscript (lower case) and means in a column bearing a common subscript (upper case) do not differ significantly.

Table 5. Changes in microbiological quality of fermented buffalo beef sausage during ripening and drying process.

Parameter	Combination	Before curing	After curing (h)			Periods of ripening (days)							
			1	24	48	3	5	6	8	10	13	16	25
TVC (log_{10}cfu/g)	I	5.76^{c} ±0.03	5.81^{d} ±0.03	6.19^{e} ±0.06	6.24^{f} ±0.032	6.71^{gh} ±0.05	6.88^{ij} ±0.032	6.91^{j} ±0.03	6.81^{i} ±0.24	6.67^{h} ±0.04	6.62^{g} ±0.03	5.53^{b} ±0.04	5.38^{a} ±0.12
	II	5.67^{a} ±0.04	5.69^{ab} ±0.05	5.92^{c} ±0.04	6.12^{d} ±0.04	6.56^{f} ±0.04	6.89^{i} ±0.02	6.92^{hi} ±0.03	6.80^{h} ±0.02	6.71^{g} ±0.03	6.56^{e} ±0.20	5.87^{ab} ±0.04	5.45^{b} ±0.03
	III	5.60^{a} ±0.26	5.80^{ab} ±0.02	5.98^{c} ±0.03	6.20^{d} ±0.05	6.68^{f} ±0.04	7.10^{h} ±0.03	6.85^{h} ±0.06	6.80^{g} ±0.03	6.77^{f} ±0.04	6.72^{e} ±0.04	5.69^{b} ±0.02	5.38^{b} ±0.03
	IV	5.71^{b} ±0.019	5.82^{b} ±0.03	6.02^{c} ±0.02	6.30^{d} ±0.04	6.76^{f} ±0.04	7.01^{h} ±0.05	7.11^{gh} ±0.05	6.82^{g} ±0.03	6.56^{f} ±0.03	6.45^{e} ±0.05	5.56^{a} ±0.02	5.50^{a} ±0.04
Colititre (MPN/g)	I	270^{e}_{A} ±10.54	155^{d}_{A} ±8.50	115^{c}_{A} ±11.60	55^{b}_{A} ±12.45	37.8^{b}_{A} ±5.60	15.46^{a}_{A} ±2.36	8.82^{a}_{A} ±2.09	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0
	II	249^{g}_{D} ±20.55	231^{f}_{C} ±14.40	133^{e}_{B} ±12.45	102.4^{d}_{E} ±16.07	53^{c}_{B} ±6.07	25.24^{b}_{AB} ±4.40	9.68^{ab}_{A} ±2.44	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0
	III	309^{h}_{C} ±26.33	231^{g}_{C} ±12.26	165^{f}_{C} ±12.04	133^{e}_{D} ±14.28	77^{d}_{C} ±6.59	43.8^{c}_{C} ±5.70	22.4^{b}_{B} ±2.24	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0
	IV	224^{g}_{B} ±11.60	174^{h}_{B} ±24.12	133^{e}_{B} ±26.63	80.4^{d}_{B} ±18.70	53^{c}_{B} ±3.97	26^{b}_{B} ±3.22	10.46^{a}_{A} ±1.47	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0	3^{a}_{A} ±0
Yeasts & Moulds Count (log_{10}cfu/g)	I	2.10^{a} ±0.03	2.23^{a} ±0.04	2.32^{b} ±0.05	2.45^{c} ±0.11	2.70^{d} ±0.06	2.80^{de} ±0.10	2.88^{ef} ±0.10	3.02^{f} ±0.10	3.10^{f} ±0.12	3.21^{g} ±0.11	3.33^{g} ±0.21	3.28^{g} ±0.12
	II	2.21^{a} ±0.05	2.25^{ab} ±0.04	2.40^{bc} ±0.07	2.45^{cd} ±0.06	2.58^{d} ±0.04	2.74^{e} ±0.03	2.77^{ef} ±0.03	2.85^{fg} ±0.05	2.94^{gh} ±0.05	3.15^{h} ±0.10	3.30^{i} ±0.06	3.28^{i} ±0.09
	III	2.08^{a} ±0.02	2.12^{ab} ±0.06	2.28^{bc} ±0.06	2.40^{cd} ±0.07	2.62^{d} ±0.05	2.81^{e} ±0.04	2.90^{ef} ±0.10	3.17^{fg} ±0.05	3.22^{g} ±0.11	3.30^{h} ±0.12	3.42^{hi} ±0.10	3.39^{i} ±0.09
	IV	2.15^{a} ±0.08	2.30^{ab} ±0.06	2.38^{bc} ±0.10	2.46^{cd} ±0.10	2.51^{de} ±0.06	2.72^{ef} ±0.10	2.80^{fg} ±0.05	3.10^{gh} ±0.10	3.16^{hi} ±0.05	3.25^{hi} ±0.06	3.34^{jk} ±0.10	3.31^{k} ±0.12

n=5

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.

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 crude protein content ranged in between 22.0 to 35.0%. Pereira *et al.* (2000) noted rise of protein content of fermented Brazilian poultry sausages as the days of fermentation increased. Ambrosiadis *et al*. (2004) reported that the crude protein content of Greek-style fermented sausages ranged between 19.67 to 32.08%. Ortiz-Somovilla *et al*. (2006) reported that as the moisture content decreased in pork sausages, crude protein content increased significantly.

Ether extract

The different combinations of sausage mix exhibited a gradual increase in the mean ether extract up to the 25th day of the production schedule (Table 4). At the initial stages of ripening, the **e**ther extract of the different sausage mix formulations did not differ significantly. However, at the end of the fermentation process, significant differences were noticed in ether extract content amongst the sausages prepared with the four different combinations. This increase in ether extract content could be attributed to the gradual fall in the moisture content of the product. The ether extract content in the finished product ranged between 25.97 to 30.22%. Terrell, Smith and Carpenter (1978) reported that typical European-style fermented sausages had an ether extract content ranging from 16.0 to 39.0%. Pereira *et al.* (2000) reported that the ether extract content of various Brazilian fermented poultry sausages were more than 17.0%. The traditional Greek sausages were also found to have a ether extract content around 29.74% (Ambrosiadis *et al*., 2004). Ortiz-Somovilla *et al*. (2006) reported that the ether extract content of final sausages with pork as a raw material should contain a fat content of 25.0 to 30.0% for better juiciness.

Total Ash

After curing there was a rise in the total ash content of the sausages (Table 4) which might be due to the addition of salt, salt-petre and other curing ingredients. The trend in increase of total ash content continued till the end day of the production process, which might reasonably be attributed to the fall in the moisture content of the product. The mean total ash content of the sausages at the end of the production process was recorded at 2.59 to 2.98%. The total ash content of the final products of combinations I and IV did not differ significantly while the other two combinations (II and III) differed significantly ($P<0.01$) between themselves and also with those of combinations I and IV. Terrell, Smith and Carpenter (1978) reported that various raw-dried fermented sausages usually had ash content in between 3.4 to 4.8%. Pereira *et al*. (2000) also reported that there was a significant rise in the total ash content of the Brazilian poultry sausages with the advancement of the fermentation process. Ambrosiadis *et al.* (2004) reported that the traditional Greek sausages prepared from pork and beef had a total ash content of 2.99%. They also reported that the total ash content went up as the sausages approached the final stage of ripening. Ortiz-Somovilla *et al*. (2006) reported 2.5 to 3.2% of total ash content of the fermented sausages prepared with pork and noted that there was a gradual rise in the amount of total ash content as the moisture content of the sausages reduced. The total ash 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).

Residual nitrite

The mean residual nitrite content of the ready-to-eat fermented carabeef sausages of the present study was much below the permissible limit of 200 mg/kg (Figure 1).

In the present study, the amount of nitrite added was perhaps optimum as it did not suppress the starter organisms as the finished products recorded pH and flavour scores that were desirable for a fermented sausage. Zaika *et al*. (1976) reported that in some products like Hungarian salami and Lebanon bologna, a high quantity of nitrate is used (200 to 600 mg/kg). During curing, nitrite is added to the sausage emulsion or is formed from the added nitrate by the microorganism that generally fulfills three functions in the raw sausages: the development of cured meat colour, the inhibition of autoxidative processes leading to rancidityand its role in the establishment of desired Gram-positive "ripening microflora" (lactobacilli and micrococci). In addition, some strains of micrococci and staphylococci can also reduce nitrite due to the action of nitrite reductase enzyme (Lücke, 1985).

Microbiological quality

The mean total viable count (TVC) increased up to the 6th day of ripening in combination I and up to 5th day in the other three combinations (Table 5). The TVC tended to decline up to the end day of the production process and the finished product of all the four combinations had good microbiological quality with a mean TVC of 5.38±0.12, 5.45±0.03, 5.38±0.03 and 5.50±0.04 $\log_{10}$cfu/g for sausage samples of combinations I, II, III and IV, respectively and the differences amongst the four sausage formulations were statistically non-significant. However, the TVC of the four sausage mix formulations enumerated at different periods of the production schedule differed significantly (P<0.01) amongst themselves. Decrease in the number of total viable organisms could be attributed mainly to drop in the pH of meat mass. Klettner and Rödel (1978) reported that lactic acid formation due to the addition of starter culture caused fall of pH up to 4.8 to 5.0, that ensured a decrease in the number of surviving organisms in the sausages and also increased the firmness of the product. Morioka *et al*. (1999) recorded higher bacterial count at the beginning of the process in soft salami prepared with the use of starter culture. Holley, Lammerding and Tittiger (1988) reported much higher. TVC in Italian dry sausage inoculated with starter culture than the traditional dry sausages without any starter organism. They also noted that at the end of the experiment, the total viable counts decreased both in starter inoculated and traditionally manufactured sausage products. Comi *et al.* (1992) also observed that the total microbial count increased in Italian dry sausage with the start of production process and that the change in number of organism did not follow a specific pattern. Further, they reported that with the advancement of fermentation process, the total number of organisms decreased and stabilized at the end of ripening period. Andersen (1995) stated that it would be a wrong interpretation, if the quality of the meat is evaluated on the basis of undifferentiated total cell enumeration including the added starter cultures, which have been inoculated with the contaminating microflora either for fermentation or for biopreservation. Curing ingredients, starter cultures and the conditions of ripening and drying of the fermented buffalo beef sausage seemed to exert inhibitory effect on the coliforms resulting in gradual fall in their numbers with the advancement of the production schedule. Beyond 6th day of the ripening process, the coliform organisms were found to be £3.0 MPN/g. The decrease in the mean coliforms could be attributed to the antagonism by the starter organisms which might be due to the acid production or due to the production of specific antibacterial substances. Holley *et al*. (1988) reported that *P. pentosaceus* and *Staph. carnosus* could effectively eliminate coliforms from dry sausages. Baran and Stevenson (1975) reported a decline in the number of enteropathgenic *E. coli* in fermented Turkish sausage inoculated with *P. acidilactici* and *P. cerevisiae*; however, the degree of inactivation of the coliforms was dependent on the amount of the inoculum and the starter strain used. Lücke (1985) reported that a significant decrease in coliform count due to the organisms competitive growth that aggravated the conditions of the meat mass including low pH, low oxygen tension and the presence of salt. Grau (1981) and Gill (1982) also reported that a decreased pH in the meat mass was the main factor for elimination of the Enterobacteriaceae from the fermented sausages. Morioka *et al*. (1999) in a study on utilization of starter culture comprising of *Staph. carnosus* and *P. pentosaceus* at the ratio of 2:1 for the production of soft salami sausage observed that the number of *Escherichia coli* decreased to levels of below detection on 15th day of fermentation of the product. They further observed that when pH and a_w values were decreased to levels of lower than 5.1 and 0.91, respectively, the *E. coli* counts decreased considerably. With the progress of the fermentation process, yeasts and moulds counts increased gradually till the end of the fermentation process. Capita *et al*. (2006) while evaluating the microbiological profiles of Chorizo and Salchichon (two Spanish dry fermented sausages) recorded average yeasts and moulds count between 2.27 to 5.11 log_{10}cfu/g in the finished products. There was also an increasing trend of yeasts and moulds counts as the production process proceeded. Ordonez *et al*. (1999) reported that the yeasts and moulds count of dry fermented sausages increased along with the progress of fermentation process. Gönülalan, Arslan, and Köse (2004) studied the effects of different starter culture combinations including *Staph. carnosus* and *P. pentosaceus* in production of fermented sausages and reported yeasts and moulds counts of 3.31 to 3.96 log_{10}cfu/g in the finished products. Samelis *et al*. (1998) evaluated the microbial ecology, stability and safety of traditional Greek salami and reported that the yeasts and moulds counts did not increase significantly during the ripening period and were below 10^5 log_{10}cfu/g.

Organoleptic properties

Panel scores in respect of appearance and colour of the sausages prepared with the different formulations did not differ significantly (Figure 2). As regards to juiciness, panel ratings for sausages of Combinations II and IV were almost identical but were significantly (P<0.01) higher than those of I and III. Sausages of Combination I enjoyed the highest score for flavour and the differences with the other combinations were significant (P<0.01). Likewise, the taste of the sausages of Combination I was found to be the best amongst all the combinations and the difference being statistically significant (P<0.01). Sausages of Combinations I and IV were rated equally and were found to be significantly better than those of Combinations II and III. Wirth (1973) and Puolanne (1977) opined that the colour development in the fermented sausages were mainly due to the addition of nitrite. The specific sensory properties of fermented sausages are built up from many components like the salt, spices, smoke etc. while others are formed without the direct participation of microorganisms,

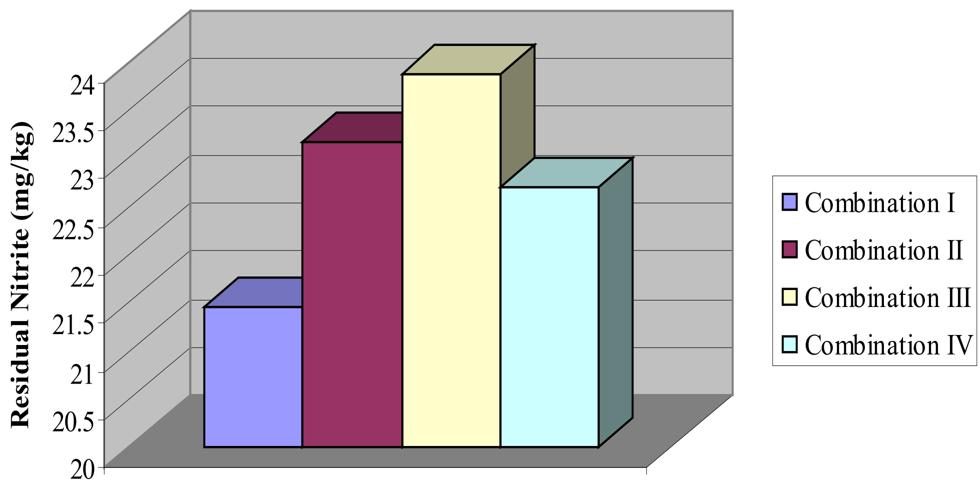

Figure 1. Residual nitrite content of fermented buffalo beef sausage.

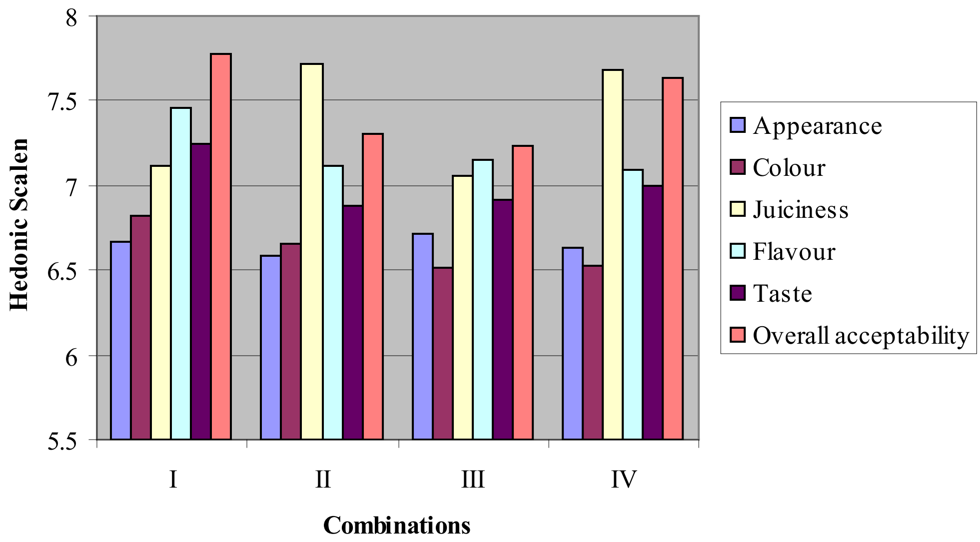

Figure 2. Organoleptic properties of fermented buffalo beef sausage.

and many originate from microbial breakdown of carbohydrates, lipids and proteins. Halvarson (1973) concluded that the lipids of the meat products were the precursors of many odoriferous compounds, which included aldehydes, ketones and short-chain fatty acids although some of these compound (e.g., branched-chain aldehydes) are originated from amino acids as well. Maillet and Henry (1960) and Alford, Smith and Lilly (1971) opined that the distinctive flavour of fermented sausages was related partly to the hydrolytic and oxidative changes occurring to the lipid fraction during ripening. Berdague *et al.* (1993) reported that *P. pentosaceus* and *Staph. carnosus* had major effect on the flavour quality as well as on the colour stability of dry sausages. The ripening process of fermented sausage gives the product its particular slicing ability, firmness, colour and flavour (Ordonez *et al*., 1999). Erkkilä *et al*. (2001) reported that the flavour could be increased notably by using *P. pentosaceus* as a starter culture. It may be concluded that fermented sausages with superior organoleptic properties could be prepared from the lean and fat of carabeef alone. Unpleasant mouth coating has not been reported. Addition of pork fat may be considered to improve the juiciness property of fermented carabeef sausage.

CONCLUSION

Sausage mix formulations did not affect the pH quality of the ready-to-eat fermented buffalo beef sausage. While there was a gradual fall in the moisture contents, the CP, EE and TA registered a gradual rise from the beginning to the end of the production process. Sausage mix formulations did not affect the microbiological quality of fermented buffalo beef sausage. Formulation I was rated to be the best for appearance colour and flavour attributes. However, combination II was better for juiciness. Good quality fermented swamp buffalo beef sausage could be prepared alone or in combination with pork.

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