Evaluation of quality and shelf life of buffalo meat keema at refrigerated storage

G. Kandeepan · A. S. R. Anjaneyulu · N. Kondaiah ·
S. K. Mendiratta · R. S. Rajkumar

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Abstract Scientific basis on the quality changes of traditional keema will boost and sustain meat production and utilization in buffalo abundant countries. A programme was undertaken to determine the influence of age and gender on the quality of buffalo meat keema at refrigerator storage (4 ± 1 °C). Buffalo meat keema was evaluated by analyzing the changes in physicochemical, microbiological and sensory attributes. The product characteristics and acceptability of keema were better in spent buffalo group than young male group. The values of pH, TBARS, total aerobic mesophils, coliforms, *Lactobacillus*, *Staphylococcus aureus*, anaerobic and psychrophilic counts of buffalo meat keema increased but appearance, flavour, juiciness, tenderness, connective tissue residue and overall acceptability scores decreased with progressing refrigerated

storage. The shelf life of refrigerated buffalo meat keema was 18 days with an overall acceptability score ranging from extremely acceptable to moderately acceptable.

Keywords Buffalo meat · Keema · Young · Spent · Quality changes · Refrigerated storage · Shelf life

Introduction

Meat processing improves preservation by inhibiting microbial decomposition and results in nutritious products. It provides convenience and variety to the meat to promote marketing. In India, meat is consumed mostly in curry or keema form. Only 2% of the meat is processed in India (APEDA 2008). Despite vast resource of population and contribution of buffaloes to total meat production in the country, their potential for utility in processed meat sector is not completely exploited.

Traditional buffalo meat products are infamous since time immemorial in several countries of Asia, Africa, Australia, Europe and South America where a significant buffalo population is found. But little effort has been undertaken to commercialize these traditional meat products. Although increasing urbanization and change in lifestyle have changed in the past few years, the liking for traditional meat products still exists among the people. Traditional Indian meat based foods require many preparatory steps and longer preparation time. In order to minimize such burden of processing in the kitchen and to fulfill needs of increasing working population, the concept of ready-to-eat meat products is growing in Indian and overseas markets.

Keema is an indigenous, delicious meat product prepared by cooking comminuted meat with spices and seasonings. It is consumed fresh because of its rapid

G. Kandeepan (✉) · S. K. Mendiratta
Division of Livestock Products Technology,
Indian Veterinary Research Institute,
Izatnagar,
Bareilly, Uttar Pradesh 243 122, India
e-mail: drkandee@yahoo.co.in

A. S. R. Anjaneyulu · N. Kondaiah
National Research Centre on Meat,
Chengicherla, Uppal,
Hyderabad, Andhra Pradesh 500039, India

R. S. Rajkumar
Division of Post Harvest Technology,
Central Avian Research Institute,
Izatnagar,
Bareilly, Uttar Pradesh 243 122, India

Present Address:

G. Kandeepan
National Research Centre on Yak,
Dirang,
West Kameng, Arunachal Pradesh 790101, India

perishability. Buffalo meat keema has great demand in consumer market due to its low price and high nutritional value. This meat product would receive huge acclaim among the consumers, if supplied ready to eat substantiated with adequate nutritional information and shelf life.

Refrigerated preservation is essential to maintain the inherent quality of the meat product. But gradual changes in physicochemical, microbiological and sensory quality are inevitable. According with Papadima and Bloukas (1999) storage conditions affected the microflora, pH, weight loss and water activity but had no effect on composition, colour and sensory attributes of traditionally processed Greek sausages. Karthikeyan et al. (2000) analysed the physicochemical, microbiological and sensory attributes of hurdle treated chevon keema and indicated it was fairly acceptable up to 5 days at ambient temperature. As per Boles and Swan (2002) age/gender significantly influenced processing characteristics but storage regime affected the sensory attributes of beef roasts. Badr (2004) found that refrigeration storage significantly increased thio-barbituric acid reactive substances value. Investigation by Morcuende et al. (2003) indicated oxidative and lipolytic deterioration lower shelf life of the product. According with Ruiz-Capillas et al. (2007) the storage temperature affected microbial development and production of biogenic amines. Rubio et al. (2007) established that increased storage days gradually decrease sensory quality of the meat product.

Thomas et al. (2010) developed pork sausage using hurdle technology which was acceptable up to 30 days. They found that hurdle treatment significantly ($P<0.05$) reduced the rate of deterioration of quality characteristics of pork sausages during storage, as indicated by TBARS and tyrosine values. Sureshkumar et al. (2010) developed buffalo meat sausages using Nisin and Butylated Hydroxy Anisole. The treatment exhibited a significant inhibitory effect on total viable count, staphylococcal, streptococcal and anaerobic counts. Also the products showed better appearance, flavour, texture and overall acceptability scores up to 5 days at 35 °C and 70–80% RH. Chicken meat balls stored at refrigeration temperature showed a significantly ($P<0.05$) high TBARS value, total plate count and psychrophilic count but a significantly ($P<0.05$) low sensory attributes (Bhat and PavanKumar 2011). Coliforms were not detected throughout the period of storage. Meat balls prepared using chicken skin and stored in the refrigerator was acceptable up to day 14. Bhat and Pathak (2011) found that mutton *harissa* stored under refrigerated condition was acceptable for a period of one week. They indicated that thiobarbituric acid reacting substances (TBARS) and free fatty acid (% oleic acid) values increased significantly ($P<0.05$) with storage period.

The scientific information on buffalo meat keema would add a new product to the list of ready to eat meat products. Besides, it will increase demand among consumers. However, buffalo meat keema has not been methodically investigated for quality changes during refrigeration temperature. Moreover, the influence of food animal source, packaging and storage condition on quality and shelf life of buffalo meat keema has not been explored. Hence, objective of this research was to evaluate quality and shelf life of buffalo meat keema (minced meat with thick gravy) at refrigeration temperature (4 ± 1 °C).

Materials and methods

Buffalo meat keema

The meat from young male (about 18 months old), aged/spent male (culled buffalo bullock) and female (culled buffalo female) buffaloes (>10 years) were procured from meat market, Bareilly, Uttar Pradesh, India. Meat samples were collected from *longissimus dorsi* muscle of carcasses of almost similar conformation. Each group of buffaloes were slaughtered according to traditional halal method. The meat was obtained within 6 h of slaughter, packed in low density polyethylene (LDPE) bags and conditioned at 4 ± 1 °C in a refrigerator (Godrej Cold Gold, India) for 24 h. Later, the separable fat and connective tissue were removed. The meat was portioned, packed in LDPE bags and transferred to freezer (Vest Frost, Denmark) maintained at -18 ± 1 °C, until it was processed. The meat was thawed at 4 ± 1 °C for 12 h before it was processed for meat keema. Refined salt (Tata salt, Tata chemicals Ltd. Mumbai, India), refined mustard oil (Dhara, Dhara Vegetable Oil and Foods Co. Ltd., Anand, India), spice mix ingredients and vegetables were procured from local market. Fine pastes of tomato, onion, garlic, ginger and green chilly were used as condiments. Spice ingredients, free from extraneous matter, were dried in hot air oven at 50 °C for 4 h. The ingredients were ground in a home mixer (Remi Equipments, India) and sieved through a fine mesh. The powders were mixed in suitable proportion to make a spice mix for buffalo meat keema. The composition of spice mix for buffalo meat keema comprised of coriander powder (*dhania*) 25%, cumin seeds (*zeera*) 12%, dried ginger (*sont*) 10%, aniseed (*soanf*) 10%, black pepper (*kali mirch*) 10%, capsicum (*mirch powder*) 5%, degi mirch 5%, turmeric (*haldi*) 5%, caraway seed (*ajowain*) 2.5%, cardamom (*bada elaichi*) 2.5%, cinnamon (*dal chini*) 2.5%, cloves (*laung*) 2.5%, nutmeg (*jaiphal*) 2.5%, split bengal gram (*channa dal*) 1.5%, mace (*javithri*) 1%, curry leaves 1%, bay leaf (*tej patha*) 1% and poppy seeds (*kaskas*) 1%.

About 1500 g of meat each from different groups of buffaloes was ground in a meat mincer with 4 mm plate. The standardized formulation for processing buffalo meat keema consisted of minced meat 100%, water 50%, onion paste 12%, oil 10%, tomato puree 6%, garlic paste 4%, spice mix 2%, salt 2%, green chilly paste 1.2%, ginger paste 1% and degi mirch powder 0.2%. The procedure used for processing buffalo meat keema is given in Fig. 1. The minced meat along with all the required quantity of water was precooked in a pressure cooker for 10 min. The precooked minced meat was kept ready. Onion paste, ginger paste, garlic paste, green chilly paste and spice mix were fried in oil as in order in a kettle. The precooked minced meat was added and fried for 5 min. Tomato puree was added followed by meat extract and salt. All the ingredients were mixed well. Then cooking was done on a medium heat for 25 min till the minced meat became tender. The buffalo meat keema was cooled to the room temperature and the cooking yield was recorded. The experiment was repeated three times.

About 250 g of ready to eat buffalo meat keema were vacuum packaged in laminated pouches (Nylon / LDPE)

Flow diagram for processing of buffalo meat keema

Fig. 1 Flow diagram for processing of buffalo meat keema

using a Rochermatic packaging machine (Model VM19S, Osnabruck, Germany) and reheated by steam cooking without pressure in a water boiler (Snow King, India). The time of reheating was done in such a way to attain a desired internal temperature of 90 °C in the finished product (Thomas et al. 2007). The temperature was recorded with a digital probe thermometer (Model CT-809, Century Instruments (P) Ltd, Chandigarh). It took approximately 10 min to reach 90 °C in the packaged cooked keema. The product was held at this temperature for about 10 min. The final product was cooled to room temperature and subjected to storage study at refrigeration temperature. The buffalo meat keema from different groups were assessed at 3 days interval for changes in quality parameters.

Physicochemical characteristics

pH of the homogenate prepared from buffalo meat keema was recorded by immersing combined glass electrode of digital pH meter (Model CP 901, Century Instruments Ltd, Chandigarh, India). The moisture content was determined by oven drying, protein by Kjeldahl nitrogen estimation and fat by Soxhlet extraction with petroleum ether (AOAC 2002). Cooking yield was calculated as the percentage of weight of meat product before and after cooking. Gross energy of buffalo meat products was determined by Gallenkamp Ballistic Bomb Calorimeter (Haque and Murari 1999). The samples were ignited and burnt in excess oxygen in the bomb to measure the rise in temperature by the thermocouple and galvanometer system. This was compared by burning a standard sample (benzoic acid) of known calorific value and the energy value was determined. The calorific value of the sample was calculated and expressed as Kcal/100 g.

Water activity of processed keema was measured by Pawkit water activity meter (Decagon Devices, Pullman, Washington, USA). The sample in duplicates was taken in a sample container provided with water activity meter and placed inside the apparatus. Proper care was taken not to touch the sensor and the upper lid was closed. Then the left button was pressed once to start measuring a_w . The lid was opened and the sample was taken out after getting a beep sound indicating the apparatus had finished estimating a_w . It took 5 min to take one reading. The water activity meter was calibrated at regular intervals.

The distillation method of Tarladgis et al. (1960) was followed to estimate Thio-Barbituric Acid Reactive Substances (TBARS) value. 2-Thiobarbituric acid mixed in glacial acetic acid develops pink colour in the distillate of buffalo meat keema. Absorbance of the colour developed was recorded at 538 nm using a spectrophotometer (Scanning mini SPEC, model SL 177, Elico Ltd,

Hyderabad). The optical density was multiplied by factor 7.8 to express TBARS value in mg malonaldehyde/kg (Koniecko 1979).

Microbiological quality

All microbiological parameters of buffalo meat keema were determined as per methods described by APHA (2001). Ready made media from Hi-Media Laboratories (P) Ltd, Mumbai, India were used for enumeration of different microbes. Preparation of samples and serial dilution of buffalo meat keema were done near flame in a horizontal laminar flow unit [Model YSI-188, Yarco Sales (P) Ltd., New Delhi, India]. It was pre-sterilized by ultraviolet radiation, observing all possible aseptic precautions. Sterile peptone water (0.1%) was used as diluent for making serial dilutions. The number of colonies were multiplied with reciprocal of the dilution and expressed as log₁₀ cfu/g.

Plate count agar (M091) was used to enumerate total plate count. The plates were incubated at 37±1 °C for 48 h. Plates were prepared in a similar fashion for psychrotrophic count and incubated at 4±1 °C for 14 days. The plates showing 30–300 colonies were counted. Violet Red Bile Agar (VRBA, M049A) was used for coliform and anaerobic agar (M 228) for total anaerobic count. The plates were incubated at 37±1 °C for 48 h. The number of red purple/pink colony in VRBA was counted as coliforms. Whereas, the white colour colonies in anaerobic agar was counted as total anaerobes.

MRS agar (M 6411), melted and maintained at 44–46 °C (added with 1 mL glycerol/100 mL media) was poured gently to prepare plates for the enumeration of *Lactobacilli*. The plates were incubated at 37±1 °C for 48 h and white colour colonies were counted. Baird Parker agar with sulphur (M 1140) cooled to 50 °C, aseptically mixed with 50 mL concentrated egg yolk emulsion and 3 mL sterile 3.5% potassium tellurite solution (or 5 mL of ready made egg yolk tellurite emulsion per 100 mL of media) was used to prepare plates for *Staphylococcus* count. The plates were incubated at 37±1 °C for 48 h. The number of intensely black, shiny, regular shaped colonies surrounded by clear halos was counted. The sterile cooled Potato Dextrose agar (M 096) medium acidified with 10% sterilized tartaric acid solution (1 mL/100 mL of media) was used for yeast and mold count. The plates were incubated at 25 °C for 7 days. Black, white, yellow, red or greenish black coloured colonies were counted.

Sensory evaluation

Standard sensory evaluation method using 8-point descriptive scale was followed, where 8=excellent; 1=extremely poor. A seven member trained sensory panel consisted of

scientists and post graduate students of Division of Livestock Products Technology, Indian Veterinary Research Institute, Izatnagar, India, judged the samples. The panelists were trained and well acquainted with different sensory attributes during their post graduate/doctoral programme. They were briefly explained about the nature of experiment without disclosing identity of the samples. The final product of ready to eat buffalo meat keema prepared fresh was evaluated for sensory attributes on same day. Whereas, buffalo meat keema held at refrigeration temperature (4±1 °C) was evaluated at 3 days interval. The stored samples were warmed (40–45 °C) using microwave oven (LG electronics India (P) Ltd., Mumbai) for 1 min and served to panelists. The panelists evaluated samples for appearance, flavour, juiciness, tenderness, connective tissue residue and overall acceptability using scores ranging from 1 to 8.

Statistical analysis

The data generated by repeating experiments for different quality characteristics were compiled and analyzed using SPSS (version 10.0 for Windows; SPSS, Chicago, Ill., U.S. A.) with randomized block design. Six replicates were used for each experiment to analyze the data statistically. The data were subjected to analysis of variance (2 way ANOVA), least significant difference and Duncan's multiple range test (Steel and Torris 1981) for comparing means to find difference between groups, storage periods and their interaction for various parameters. The smallest difference ($D_{5\%}$) between two means was reported as significantly ($P < 0.05$) different.

Results and discussion

Quality evaluation of buffalo meat keema

The meat pH of young buffaloes was significantly ($P < 0.01$) higher than other groups (Table 1). The lower and higher ultimate pH values might be attributed to the degree of stress exposed in each group of animals (Gregory 1998). There was no significant difference between the meat pH of spent male and female buffaloes. The product pH followed the trend of meat pH. The results resembled the pH of chevon keema (Karthikeyan et al. 2000). The yield of keema did not differ significantly between the groups.

The moisture content of meat keema from young male buffaloes was significantly ($P < 0.01$) higher than the other groups. The keema prepared from spent buffalo groups did not differ significantly for moisture content. The difference in moisture was related to the pH of the product in the present study.

Table 1 Physicochemical characteristics of keema prepared from different groups of buffaloes

Parameters	Groups		
	Young Male	Spent Male	Spent Female
Physicochemical characteristics			
Meat pH	5.7±0.03 ^a	5.5±0.01 ^b	5.5±0.01 ^b
Keema pH	6.0±0.02 ^a	5.9±0.01 ^b	5.9±0.01 ^b
Yield (%) [#]	58.6±2.31	59.7±1.71	57.9±1.65
Moisture (%)	63.1±0.63 ^a	59.9±0.75 ^b	61.2±0.31 ^b
Protein (%)	19.4±0.18 ^b	20.3±0.27 ^a	19.1±0.34 ^b
Fat (%)	9.8±0.48 ^b	13.7±1.01 ^a	12.6±0.71 ^a
Energy (Kcal/100 g DM)	520.0±6.40 ^b	549.5±2.11 ^a	541.9±6.87 ^a
Water activity	0.9±0.01	0.9±0.01	0.9±0.01

n=6, [#] *n*=3

Means with different superscripts in the same row indicate significant difference ($P<0.05$)

The protein content of keema from spent male buffalo meat was significantly ($P<0.05$) greater than other groups. The young male and spent female buffalo meat keema did not differ significantly ($P<0.05$) for their protein content. The difference in protein content of meat keema was due to their difference in protein content of the meat. The higher level of protein in the final product was contributed by the added ingredients in the meat keema and also due to moisture loss during cooking.

The fat content of meat keema from young male buffaloes was significantly ($P<0.05$) lower than the spent buffalo groups. Higher fat level in the spent buffalo group was due to markedly higher deposition of fat with advancing age of the animal. There was no significant difference in fat content of meat keema between the spent buffalo groups. The fat percentage was closely and inversely related to the moisture level of the product in each group. The higher fat content of meat keema in all the groups was closely related to the level of oil used for its processing.

The energy level followed the trend of protein and fat content of each group. The lower energy level of young male buffalo meat keema was related to its lower protein and fat content of the meat. The energy level of the meat increased with age and fat content of the animal (Charles 1982; Mohan et al. 1987). A significantly ($P<0.01$) higher calorie content of the product was observed in the spent buffalo groups.

The water activity (a_w) of buffalo meat keema did not differ significantly between the groups. This was attributed to the same ingredients and processing conditions applied to different groups of buffalo meat. As observed in the present study, the products in the vicinity of 0.94 a_w were preferred by the sensory panel.

Physicochemical changes at refrigerated storage

pH of buffalo meat keema increased significantly ($P<0.05$) with advancing refrigerated storage period in all groups (Table 2). A significantly ($P<0.05$) higher pH was

noticed in young male buffalo meat keema compared to the spent buffalo groups throughout the storage. pH reached a significantly ($P<0.05$) higher value on day 21 in all groups. The increase in pH was due to basic metabolites produced by bacteria, predominantly gram negative psychrophiles, from metabolism of protein (Bell and Shelef 1978).

The values of TBARS were found to be significantly ($P<0.01$) higher in young male group than others on day 18 and 21. TBARS value increased significantly ($P<0.01$) with progressing storage period and reached maximum on day 21 in all groups. The higher TBARS value was due to increased lipid oxidation in lower a_w values (Labuza et al. 1972). Cooking and reheating liberates heme iron from myoglobin thereby accelerating lipid oxidation, which subsequently increases lipid hydroperoxides, leading to warmed over flavour and decreased shelf life (Kerler and Grosch 1996). The acceptable limit of TBARS number of cooked meat products during storage was 0.50–1.0 mg/kg (Tarladgis et al. 1960). Rancid flavour was reported to be detected at a TBARS value above 1 mg malonaldehyde/kg sample (Watts 1962). Whereas the present research finding indicated development of off-flavor at a TBARS value of 0.35–0.46 mg/kg on day 21. The rancid level during spoilage was significantly ($P<0.05$) lower in keema from spent male buffalo group.

Microbiological changes at refrigerated storage

The total plate count of the buffalo meat keema increased significantly ($P<0.05$) with increasing refrigerated storage period in all the groups (Table 3). A significantly ($P<0.05$) reduced microbial load on initial day could be attributed partly to direct destruction of microorganisms at higher temperature (Lawrie 1998) and to metabolic injury to the microorganisms (Leistner 2000). The total plate count was significantly ($P<0.01$) lower in young male buffalo meat keema compared to spent buffalo group on day 21. The

Table 2 Physicochemical characteristics of buffalo meat keema at refrigerated storage (4±1 °C)

Parameters/ Groups	Storage period (days)							
	0	3	6	9	12	15	18	21
pH								
YM	6.0±0.02 ^{e1}	6.1±0.04 ^{d1}	6.2±0.05 ^{c1}	6.3±0.05 ^b	6.3±0.04 ^{b1}	6.3±0.04 ^{b1}	6.4±0.03 ^{a1}	6.4±0.01 ^{a1}
SM	5.9±0.01 ^{c2}	5.9±0.01 ^{c2}	6.0±0.01 ^{b2}	6.0±0.01 ^b	6.0±0.02 ^{b2}	6.0±0.02 ^{b2}	6.0±0.02 ^{b2}	6.1±0.01 ^{a2}
SF	5.9±0.01 ^{b2}	5.9±0.01 ^{b2}	5.9±0.02 ^{b2}	5.9±0.02 ^b	6.0±0.03 ^{a2}	6.0±0.04 ^{a2}	6.0±0.03 ^{a2}	6.0±0.03 ^{a3}
TBARS value (mg malonaldehyde/kg)								
YM	0.1±0.02	0.1±0.02	0.1±0.02	0.1±0.04	0.2±0.02	0.2±0.03	0.3±0.02	0.4±0.03 ¹
SM	0.1±0.01 ^c	0.1±0.01 ^c	0.1±0.02 ^c	0.2±0.02 ^b	0.2±0.01 ^b	0.2±0.01 ^b	0.3±0.01 ^a	0.3±0.01 ^{a2}
SF	0.1±0.01 ^d	0.1±0.01 ^d	0.1±0.01 ^d	0.1±0.02 ^d	0.2±0.01 ^c	0.2±0.02 ^c	0.3±0.02 ^b	0.4±0.02 ^{a1}

n=6

YM Young Male, SM Spent Male, SF Spent Female, ND Not Detected

Means with different superscripts (letters in the same row and numbers in the same column) indicate significant difference ($P<0.05$)**Table 3** Microbiological characteristics of buffalo meat keema at refrigerated storage (4±1 °C)

Parameters/ Groups	Storage period (days)							
	0	3	6	9	12	15	18	21
Total plate count (log cfu/g)								
YM	1.1±0.11 ^{cd}	1.2±0.10 ^{cd}	1.3±0.13 ^{cd}	1.4±0.13 ^c	1.6±0.14 ^{bc}	1.7±0.16 ^b	1.7±0.16 ^b	1.9±0.19 ^{a2}
SM	1.2±0.11 ^{cd}	1.3±0.09 ^{cd}	1.5±0.06 ^c	1.7±0.13 ^{bc}	1.7±0.13 ^{bc}	1.8±0.12 ^b	1.8±0.12 ^b	2.1±0.24 ^{a1}
SF	1.2±0.11 ^c	1.3±0.12 ^{dc}	1.4±0.12 ^{dc}	1.5±0.14 ^d	1.7±0.06 ^{cd}	1.8±0.05 ^c	2.1±0.08 ^b	2.6±0.22 ^{a1}
<i>Staphylococcus aureus</i> count (log cfu/g)								
YM	1.7±0.10 ¹	1.7±0.10 ¹	1.8±0.10 ¹²	1.8±0.10 ¹²	1.8±0.10 ¹²	1.9±0.09 ¹²	1.9±0.10 ¹²	2.0±0.11
SM	2.0±0.14 ¹	2.0±0.14 ¹	2.0±0.13 ¹	2.1±0.13 ¹	2.1±0.13 ¹	2.1±0.13 ¹	2.1±0.13 ¹	2.1±0.11
SF	1.2±0.15 ^{b2}	1.3±0.14 ^{ab2}	1.5±0.12 ^{ab2}	1.5±0.12 ^{ab2}	1.5±0.13 ^{ab2}	1.6±0.14 ^{ab2}	1.6±0.15 ^{ab2}	1.7±0.18 ^a
Total anaerobic count (log cfu/g)								
YM	ND	1.2±0.16 ^b	1.3±0.15 ^{ab}	1.4±0.15 ^{ab}	1.5±0.13 ^{ab}	1.6±0.14 ^{ab}	1.6±0.14 ^{ab}	1.7±0.10 ^a
SM	ND	1.2±0.13 ^c	1.2±0.13 ^c	1.4±0.11 ^{bc}	1.4±0.12 ^{bc}	1.5±0.13 ^{abc}	1.6±0.09 ^{ab}	1.8±0.07 ^a
SF	1.1±0.06 ^c	1.2±0.08 ^{bc}	1.3±0.11 ^{abc}	1.3±0.13 ^{abc}	1.4±0.13 ^{abc}	1.5±0.14 ^{abc}	1.5±0.16 ^{ab}	1.6±0.16 ^a
Lactobacillus count (log cfu/g)								
YM	2.2±0.09	2.2±0.08	2.2±0.08	2.2±0.08	2.2±0.08	2.2±0.08	2.2±0.08	2.2±0.08
SM	2.0±0.35	2.0±0.35	2.1±0.32	2.1±0.31	2.2±0.30	2.2±0.29	2.2±0.29	2.2±0.28
SF	2.7±0.10	2.7±0.10	2.7±0.10	2.7±0.10	2.7±0.11	2.7±0.11	2.7±0.11	2.8±0.11
Yeast and mold count (log cfu/g)								
YM	ND	ND	1.1±0.10	1.1±0.11	1.2±0.13	1.2±0.14	1.2±0.15	1.2±0.16
SM	ND	ND	1.0±0.01	1.0±0.05	1.0±0.08	1.1±0.10	1.1±0.12	1.1±0.13
SF	ND	ND	1.0±0.01 ^b	1.0±0.05 ^b	1.0±0.08 ^b	1.1±0.10 ^b	1.1±0.12 ^b	1.3±0.16 ^a
Psychrophilic count (log cfu/g)								
YM	ND	1.0±0.01	1.0±0.05	1.0±0.08	1.1±0.10	1.1±0.12	1.2±0.13	1.2±0.14
SM	ND	1.0±0.01 ^b	1.1±0.06 ^{ab}	1.1±0.09 ^{ab}	1.1±0.10 ^{ab}	1.2±0.15 ^{ab}	1.3±0.14 ^a	1.3±0.14 ^a
SF	ND	1.0±0.01	1.1±0.06	1.1±0.09	1.1±0.10	1.1±0.12	1.1±0.13	1.1±0.14

n=6

YM Young Male, SM Spent Male, SF Spent Female, ND Not Detected

Means with different superscripts (letters in the same row and numbers in the same column) indicate significant difference ($P<0.05$)

counts were higher in older buffaloes compared to young buffaloes (Uriyapongson et al. 1996). Increase in microbial count throughout storage period was related to increase in pH, TBARS value and conductive a_w in refrigerated keema. Similar increase in microbial load was observed by Kemp et al. (1988). A logarithmic increase in aerobic plate count in keema was mainly due to conductive a_w and pH (Smolka et al. 1974; Leistner et al. 1981). Spoilage was reported to occur when bacterial count reached more than 8 log cfu/g in fresh buffalo meat (Narasimha Rao et al. 1998). However, in processed and stored buffalo meat keema, spoilage was detected at a bacterial count of around 2–3 log₁₀ cfu/g.

It was reported that coliforms occur significantly greater in numbers in vacuum packaged meat and meat products and cause spoilage (Goktan et al. 1988). Coliforms were not found in any of the group throughout storage period. The absence of coliforms was because of thermal processing, hygienic practices followed during processing and antibacterial effects of spices (Grohs and Kunz 1999; Grohs et al. 2000).

Staphylococcus aureus count did not differ significantly between the storage periods in all buffalo groups. Keema from young male and spent male buffalo meat showed a significantly ($P<0.05$) higher *Staphylococcus aureus* count than spent female buffalo groups. This increase in count was due to slow adaptation for growth by *Staph. aureus* at lower temperature. The gradual increase in *Staphylococcus aureus* count of refrigerated keema may be related to conductive pH and a_w apart from good manufacturing practices.

Anaerobic count was not found except spent female buffalo group on day 1. The count increased significantly ($P<0.05$) towards day 21. There was no significant difference in anaerobic count among groups. The low initial count of anaerobes in keema may be attributed to temperature stress, slow adaptation to colder temperature and destruction of vegetative cells (Leistner and Gorris 1995). Subsequent increase in count was attributed to increase in pH, difference in a_w and vacuum packaging of product. The spoilage due to total anaerobes was reported to be 2.7×10^3 /g in vacuum packaged beef tripe (Goktan et al. 1988). The result of present study showed the product was slightly acceptable at an anaerobic count of 1.64–1.81 log cfu/g.

Lactobacillus count of meat keema did not differ significantly either between storage days or buffalo groups. It was reported that species of lactic acid bacteria responsible for spoilage depends on product composition (Korkeala and Makela 1989). Vacuum packaging caused a microbial shift in dark, firm, dry beef striploins, resulting in development of lactobacillus dominated population rather than high spoilage potential pseudomonas population (Newton and Gill 1978).

Yeast and mold was not noticed up to day 3 in any group. The absence and low count of yeast and mold was attributed to thermal processing, hygienic practices and antimicrobial effects of spices. The count did not differ significantly between storage periods except spent female buffalo group. Between groups, there was no significant difference in yeast and mold count.

The psychrophilic bacteria were not found on day 1. The count did not differ significantly between groups. There was no significant difference in count between days in young male and spent female buffalo groups. As storage period progressed, the count increased significantly ($P<0.05$) from initial level in spent male buffalo group. This showed preference of bacteria to grow at low temperature. Initially, low pH and reheating might have injured the bacteria to such an extent that their generation time was increased. An increase in psychrotrophic count in refrigerated wuerstel sausage was observed by Agosti and Ottaviani (1993). Cooked, sliced and vacuum packed chicken breasts stored in refrigerator was sensorily rejected at psychrotrophic bacterial counts of 7 to 8 log cfu/g (Rodriguez et al. 2003). But results in the present study showed the product was slightly acceptable at a count of 1.19–1.36 log₁₀ cfu/g.

Sensory quality changes at refrigerated storage

Appearance scores decreased significantly ($P<0.01$) with increase in storage period in all groups (Table 4). The scores did not differ significantly between groups except on day 3. The decrease in scores was attributed to discoloration of product turning pale. Higher appearance score was attributed to better colour of cooked product due to greater concentration of metmyoglobin. The decline in colour scores during storage might be due to lipid oxidation and subsequent oxidized compounds reacting with amino acids causing non-enzymatic browning in product (Che Man et al. 1995).

Flavour scores differed significantly ($P<0.05$) between groups up to day 9. During this period, flavour scores of spent buffalo groups were found to be better than young male group. This was attributed to higher fat content in keema from spent buffalo groups as reported by Kandeepan et al. (2009). As storage period increased, flavour scores decreased significantly ($P<0.05$). The flavour of keema reached to a slightly border level on day 21. Cooked beef flavours decreased uniformly after 21 days of chilled storage (Vaudagna et al. 2002). An increase in off odour was recorded as refrigerated storage period progressed (Sison et al. 1980). The reduction in flavour scores might be due to increased oxidation of free fatty acids liberated. This was in harmony with flavour changes reported in vacuum packaged ground buffalo meat (Sahoo and Anjaneyulu 1997). Oxi-

Table 4 Sensory attributes of buffalo meat keema at refrigerated storage (4 ± 1 °C)

Parameters/ Groups	Storage period (days)							
	0	3	6	9	12	15	18	21
Appearance								
YM	7.1 $\pm$ 0.05 ^a	7.1 $\pm$ 0.05 ^{a1}	7.0 $\pm$ 0.06 ^b	7.0 $\pm$ 0.01 ^b	6.9 $\pm$ 0.02 ^{bc}	6.8 $\pm$ 0.05 ^c	6.8 $\pm$ 0.06 ^c	6.1 $\pm$ 0.06 ^d
SM	7.2 $\pm$ 0.05 ^a	7.0 $\pm$ 0.02 ^{ab2}	7.0 $\pm$ 0.02 ^{ab}	6.9 $\pm$ 0.05 ^b	6.7 $\pm$ 0.07 ^c	6.7 $\pm$ 0.06 ^c	6.6 $\pm$ 0.11 ^d	6.2 $\pm$ 0.06 ^e
SF	7.1 $\pm$ 0.05 ^a	7.0 $\pm$ 0.02 ^{ab2}	7.0 $\pm$ 0.02 ^{ab}	6.9 $\pm$ 0.04 ^{bc}	6.8 $\pm$ 0.06 ^{bc}	6.7 $\pm$ 0.07 ^{cd}	6.6 $\pm$ 0.11 ^d	6.1 $\pm$ 0.05 ^e
Flavour								
YM	7.0 $\pm$ 0.08 ^{a2}	7.0 $\pm$ 0.03 ^{a2}	6.9 $\pm$ 0.03 ^{ab2}	6.9 $\pm$ 0.03 ^{ab12}	6.8 $\pm$ 0.05 ^{bc}	6.6 $\pm$ 0.15 ^c	6.3 $\pm$ 0.04 ^d	5.9 $\pm$ 0.07 ^e
SM	7.1 $\pm$ 0.05 ^{a12}	7.1 $\pm$ 0.05 ^{a1}	7.1 $\pm$ 0.05 ^{a1}	6.9 $\pm$ 0.09 ^{a1}	6.6 $\pm$ 0.12 ^b	6.4 $\pm$ 0.18 ^c	6.1 $\pm$ 0.05 ^c	5.7 $\pm$ 0.08 ^d
SF	7.2 $\pm$ 0.04 ^{a1}	7.2 $\pm$ 0.04 ^{a1}	7.0 $\pm$ 0.06 ^{a12}	6.7 $\pm$ 0.09 ^{b2}	6.6 $\pm$ 0.12 ^b	6.4 $\pm$ 0.14 ^c	6.2 $\pm$ 0.05 ^c	5.9 $\pm$ 0.04 ^d
Juiciness								
YM	7.1 $\pm$ 0.05 ^{a1}	7.1 $\pm$ 0.05 ^{a1}	7.0 $\pm$ 0.04 ^a	6.9 $\pm$ 0.05 ^{ab1}	6.9 $\pm$ 0.04 ^{b1}	6.6 $\pm$ 0.05 ^{c1}	6.5 $\pm$ 0.11 ^c	6.0 $\pm$ 0.01 ^d
SM	6.9 $\pm$ 0.04 ^{a2}	6.9 $\pm$ 0.04 ^{a2}	6.9 $\pm$ 0.09 ^a	6.7 $\pm$ 0.10 ^{ab2}	6.5 $\pm$ 0.10 ^{bc2}	6.4 $\pm$ 0.09 ^{bc12}	6.2 $\pm$ 0.14 ^c	6.0 $\pm$ 0.10 ^d
SF	6.9 $\pm$ 0.04 ^{a2}	6.9 $\pm$ 0.04 ^{a2}	6.9 $\pm$ 0.07 ^a	6.8 $\pm$ 0.10 ^{a12}	6.5 $\pm$ 0.09 ^{b2}	6.3 $\pm$ 0.12 ^{b2}	6.3 $\pm$ 0.16 ^b	6.0 $\pm$ 0.07 ^c
Tenderness								
YM	7.2 $\pm$ 0.10 ^{a1}	7.2 $\pm$ 0.06 ^{a1}	7.2 $\pm$ 0.06 ^{a1}	7.2 $\pm$ 0.05 ^{a1}	7.0 $\pm$ 0.07 ^{a1}	7.0 $\pm$ 0.05 ^{a1}	6.7 $\pm$ 0.05 ^{b1}	6.2 $\pm$ 0.11 ^c
SM	6.8 $\pm$ 0.05 ^{a2}	6.8 $\pm$ 0.05 ^{a3}	6.7 $\pm$ 0.06 ^{a2}	6.7 $\pm$ 0.07 ^{a2}	6.6 $\pm$ 0.08 ^{a2}	6.4 $\pm$ 0.07 ^{b2}	6.2 $\pm$ 0.09 ^{b2}	6.2 $\pm$ 0.06 ^b
SF	7.0 $\pm$ 0.06 ^{a12}	7.0 $\pm$ 0.06 ^{a2}	6.8 $\pm$ 0.07 ^{a2}	6.7 $\pm$ 0.13 ^{ab2}	6.5 $\pm$ 0.07 ^{bc2}	6.4 $\pm$ 0.08 ^{cd2}	6.3 $\pm$ 0.15 ^{cd2}	6.2 $\pm$ 0.06 ^d
Connective tissue residue								
YM	7.2 $\pm$ 0.06 ^{a1}	7.1 $\pm$ 0.05 ^{ab1}	7.1 $\pm$ 0.05 ^{ab1}	7.0 $\pm$ 0.10 ^{ab}	7.0 $\pm$ 0.05 ^{ab1}	7.0 $\pm$ 0.01 ^{ab1}	6.5 $\pm$ 0.09 ^{c1}	6.1 $\pm$ 0.06 ^d
SM	6.7 $\pm$ 0.05 ^{a2}	6.7 $\pm$ 0.06 ^{a2}	6.7 $\pm$ 0.06 ^{a3}	6.7 $\pm$ 0.05 ^a	6.6 $\pm$ 0.10 ^{a2}	6.3 $\pm$ 0.07 ^{b2}	6.2 $\pm$ 0.08 ^{b2}	6.1 $\pm$ 0.17 ^b
SF	7.0 $\pm$ 0.06 ^{a1}	7.0 $\pm$ 0.06 ^{a1}	6.9 $\pm$ 0.04 ^{a2}	6.7 $\pm$ 0.13 ^{ab}	6.5 $\pm$ 0.06 ^{bc2}	6.4 $\pm$ 0.07 ^{c2}	6.2 $\pm$ 0.06 ^{c2}	6.2 $\pm$ 0.25 ^c
Overall acceptability								
YM	7.1 $\pm$ 0.03 ^a	7.0 $\pm$ 0.10 ^{ab}	7.0 $\pm$ 0.00 ^{ab2}	7.0 $\pm$ 0.00 ^{ab1}	6.8 $\pm$ 0.04 ^{b1}	6.6 $\pm$ 0.07 ^{c1}	6.0 $\pm$ 0.02 ^d	5.9 $\pm$ 0.07 ^d
SM	7.2 $\pm$ 0.10 ^a	7.2 $\pm$ 0.10 ^a	7.1 $\pm$ 0.06 ^{a1}	6.7 $\pm$ 0.08 ^{b2}	6.3 $\pm$ 0.12 ^{c2}	6.2 $\pm$ 0.05 ^{cd2}	6.0 $\pm$ 0.02 ^d	5.7 $\pm$ 0.08 ^e
SF	7.2 $\pm$ 0.07 ^a	7.2 $\pm$ 0.07 ^a	7.1 $\pm$ 0.08 ^{a1}	6.7 $\pm$ 0.09 ^{b2}	6.5 $\pm$ 0.11 ^{bc2}	6.3 $\pm$ 0.14 ^{c12}	6.0 $\pm$ 0.04 ^d	5.9 $\pm$ 0.04 ^d

n=21

Based on 8 point descriptive scale

YM Young Male, SM Spent Male, SF Spent Female

Means with different superscripts (letters in the same row and numbers in the same column) indicate significant difference ($P<0.05$)

dized flavour in ground beef was reported to be detected sensorily, if TBARS values were in the range of 0.6–2.0 mg malenaldehyde/kg (Greene and Cumuze 1982). A steep decline in flavour scores might be attributed to increased liberation of fatty acids (Brannen 1979), oxidation of fat (Santamaria et al. 1992) and increased microbial load.

Juiciness scores decreased significantly ($P<0.05$) with increase in storage period. Keema from young buffalo meat had a significantly higher juiciness score than spent buffalo groups. Juiciness was significantly higher in meat from younger than older ducks (Prabhakara Reddy 1990). The relative decline in juiciness was attributed to low water retention as the pH changed during refrigerated storage.

Tenderness scores of keema were significantly ($P<0.05$) higher in young male buffalo group compared to other groups. Meat from spent animals was less tender than young animals

(Cross et al. 1973; Naveena et al. 2004). The decrease in tenderness score was attributed to decreased activation of μ -calpain in older animals (Morgan et al. 1993). The tenderness of spent buffalo group keema was adjudged equally for both sexes. As storage period increased a significant ($P<0.01$) decrease in tenderness scores were observed in all groups. This was due to slow dehydration of refrigerated keema. The significant decrease in texture during storage might be due to changes in disulphide bond and contents of amino acid. This finding agreed with gradual texture decline in meat and meat products (Syed Ziauddin et al. 1995).

Almost no detectable connective tissue residue was realized while chewing keema from young male buffalo meat. Steaks from more mature animals had more detectable connective tissue. Higher amount of connective tissue in older animals resulted in decreased tenderness of meat (Huff and Parrish 1993). As storage period progressed,

spent buffalo group keema differed non-significantly for connective tissue residue scores.

Overall acceptability of refrigerated keema decreased significantly ($P < 0.05$) with progressing storage period. Keema from spent animal group had higher acceptability scores up to day 6 than young male group. This was due to better colour, flavour and fine texture of meat product from young males compared to spent buffaloes. The decline in scores during storage was due to lower moisture retention, decline in flavour and increase in microbial load. A gradual decline and abrupt reduction for all sensory attributes was mainly attributed to decrease in flavour.

Conclusions

The product characteristics and overall acceptability of keema from spent buffalo groups was better than young male group. The microbial load of processed buffalo meat keema was inconsistent showing little relevance to age or sex of buffalo meat source. The buffalo meat keema was well acceptable within 18 days of refrigerated storage.

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