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Full Length Research paper

Quality of buffalo meat keema at different storage temperature

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Buffalo meat from young male, spent male and spent female groups was evaluated to develop ready to eat keema. The keema prepared by standardized formulations and processing were stored at ambient (37±1°C) and refrigeration (4±1°C) temperature and the quality was compared on day 3. The physicochemical characteristics showed significantly higher energy and fat content in keema prepared from spent buffalo groups compared to young males. The pH and moisture were significantly higher in young male buffalo meat keema than spent group. Buffalo meat keema from young male groups were significantly higher in tenderness and connective tissue residue scores than the spent groups. Ambient temperature stored buffalo meat keema resulted in significant deterioration of quality parameters compared to the refrigeration storage. The buffalo meat keema could be stored up to 2 days at ambient temperature with an overall acceptability in the range of extremely acceptable to moderately acceptable.

Key words: Buffalo meat, keema, quality, storage, temperature.

INTRODUCTION

Traditional buffalo meat products are highly relished in all buffalo rearing countries of Asia, Africa, Australia, Europe and South America. Buffalo meat keema is an indigenous and delicious meat product of India prepared by cooking comminuted meat with spices and seasonings. Due to great regional diversity, this product display variations in processing method and sensory quality. Although increasing urbanization and change in lifestyle have changed in the past few years, the liking for traditional meat products still exists among 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 cater for the needs of the increasing working population, the demand for ready-to-eat meat products is growing in Indian and overseas markets.

The quality of keema is determined by the food animal source, raw materials and the method of processing. The packaging and storage condition does influence the shelf life of buffalo meat keema. Papadima and Bloukas (1999) indicated that storage conditions affected the microflora, pH, weight losses and water activity but had no effect on composition, colour and sensory attributes of traditionally processed Greek sausages. Karthikeyan et al. (2000) analysed physicochemical, microbiological and sensory attributes of hurdle treated chevon keema and indicated that it was fairly acceptable for up to 5 days at ambient temperature. Boles and Swan (2002) found that age/gender often significantly influenced the processing characteristics but storage regime affected the sensory attributes of beef roasts. Investigation by Diana and Iciar (2004) proved that vacuum packaging of the dry fermented sausages was the best method to prevent formation of lipid oxidation volatile compounds. As per Capillas et al. (2007), the storage temperature affected microbial development and production of biogenic amines. Thomas et al. (2008) found that changes in pH, TBARS, tyrosine value and microbial counts influenced the quality of hurdle treated pork sausages stored at ambient temperature.

Refrigerators are not commonly available in every household in developing countries. In addition, frequent interrupted power supply is a day to day problem in

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Table 1. Composition of spice mix for buffalo meat keema.

S/No.	Ingredients	% in the mix
1	Coriander powder *(Dhania)*	25.0
2	Cumin seeds *(Zeera)*	12.0
3	Dried ginger *(Sont)*	10.0
4	Aniseed *(Soanf)*	10.0
5	Black pepper *(Kali mirch)*	10.0
6	Capsicum *(Mirch powder)*	5.0
7	Degi mirch	5.0
8	Turmeric *(Haldi)*	5.0
9	Caraway seed *(Ajowain)*	2.5
10	Cardamom *(Bada elaichi)*	2.5
11	Cinnamom *(Dal chini)*	2.5
12	Cloves *(Laung)*	2.5
13	Nutmeg *(Jaiphal)*	2.5
14	Split bengal gram *(Channa dal)*	1.5
15	Mace *(Javithri)*	1.0
16	Curry leaves	1.0
17	Bay leaf *(Tej patha)*	1.0
18	Poppy seeds *(Kaskas)*	1.0
	Total	100.0

countries like India. Under these situations, people process the buffalo meat keema and eat it fresh. Some people store the surplus, reheat and consume on the next day or within 24 h of preparation. Scientific processing, accompanied by good manufacturing practices and suitable packaging would improve the shelf life of the buffalo meat keema without refrigerator storage. But the quality of keema stored at ambient temperature till an acceptable storage period would definitely differ with the quality of keema stored at refrigerator for the same period. But there is no scientific evidence on this hypothesis. Moreover the scientific information on processing and quality of traditional buffalo meat keema from different groups of buffaloes is not available. Considering the above points, a study was undertaken to develop processed keema from different buffalo groups and to compare their quality during storage at ambient (37±1°C) and refrigeration (4±1°C) temperature for a specific storage period.

MATERIALS AND METHODS

Raw materials

Buffalo meat from young male (about 18 months old), aged/spent male (culled buffalo bullock) and spent female (culled buffalo female) buffaloes (>10 years) were procured from Bareilly meat market, to use in the experiments of keema. The meat samples were collected from the longissimus dorsi muscle of the carcasses of almost similar conformation from each group of buffaloes slaughtered according to the traditional halal method. The temperature of meat at sampling was around 27°C. 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 about 24 h. Later, the separable fat and connective tissue were removed. The meat was portioned, packed in LDPE bags and transferred to the 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 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 a 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 (Table 1).

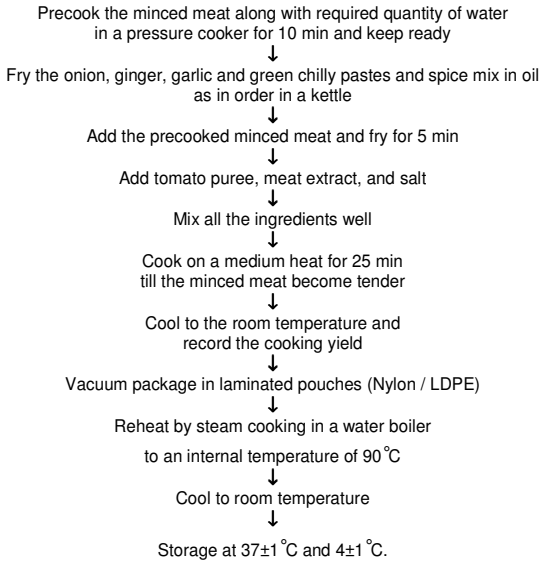

Figure 1. Flow diagram for processing of buffalo meat keema.

Processing of buffalo meat keema

About 1500 g of meat each from different groups of buffaloes was ground in a meat mincer with 4 mm plate. The following standardized formulation and procedure was used for the processing of buffalo meat keema from different groups (Table 2 and Figure 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 to reach an internal core temperature of 90°C in the keema. The buffalo meat keema was cooled to the room temperature and the cooking yield was recorded. The experiment was repeated three times.

Reheating of packaged buffalo meat keema

The ready to eat buffalo meat keema were vacuum packaged in

Table 2. Standardized formulation for processing of buffalo meat keema.

S/No.	Ingredients	% of meat
1	Minced meat (g)	100
2	Water (mL)	50.0
3	Onion paste (g)	12.0
4	Oil (mL)	10.0
6	Tomato puree (g)	6.0
5	Garlic paste (g)	4.0
7	Spice mix (g)	2.0
8	Salt (g)	2.0
9	Green chilly paste (g)	1.2
10	Ginger paste (g)	1.0
11	Degi mirch powder (g)	0.2

laminated pouches (Nylon / LDPE) 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 thermo-meter (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.

Comparison of quality of buffalo meat keema

The buffalo meat keema processed as per the standardized formulation were stored at ambient temperature (37 ± 1°C) in an incubator (Bharat Instruments and Chemicals, New Delhi, India) and refrigerator (Godrej Cold Gold, India). To evaluate the effect of different groups of meat samples on the quality of keema, product yield, pH, proximate composition, water activity (a_w), thiobarbituric acid reacting substances (TBARS), calorific value, sensory attributes and microbiological assay were determined on the day of processing.

Buffalo meat keema for both ambient and refrigeration storage were processed in the same batch, having same quality characteristics on day one in each group. The comparison of quality changes in buffalo meat keema stored at ambient (37 ± 1°C) and refrigeration (4 ± 1°C) temperature was evaluated on the alternate day (day 3). The product was compared for its various physicochemical, microbiological and sensory quality attributes.

Analytical procedures

Physicochemical properties

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 to bomb 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. The distillation method of Tarladgis et al. (1960) was followed to estimate TBARS value. 2-Thiobarbituric acid mixed in glacial acetic acid was used to develop a pink colour in the distillate of buffalo meat keema. The absorbance of the colour developed was recorded at 538 nm using a spectrophotometer (Scanning mini SPEC, model SL 177, Elico Ltd, Hyderabad). The absorbance was multiplied by a factor 7.8 and TBARS value was expressed as mg malonaldehyde/kg of sample (Koniecko, 1979).

Microbiological quality

All the microbiological parameters of buffalo meat keema were determined as per the methods described by APHA (2001). Ready made media from Hi-Media Laboratories (P) Ltd, Mumbai, India were used for the enumeration of different microbes. Preparation of samples and serial dilution of buffalo meat keema were done near the flame in a horizontal laminar flow unit (Model YSI-188, Yarco Sales (P) Ltd., New Delhi, India) which was pre-sterilized by ultra-violet 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 $\log_{10}$ cfu/g. Although plates showing colonies ranging from 30 - 300 were set as limit of detection for microbiological methods, plates showing below this limit were also recorded to interpret the difference in counts between groups and days.

Plate count agar (M091) was used to enumerate total plate count. The plates were incubated at 37 ± 1°C for 48 h and plates showing 30 - 300 colonies were counted. Violet red bile agar (VRBA, M049A) was used as the media for coliform count. The plates were incubated at 37 ± 1°C for 48 h. The number of red purple/pink colony was counted. Anaerobic agar (M 228) media was used to enumerate the anaerobes present in the vacuum packaged sample. The plates were incubated at 37 ± 1°C for 48 h and the colonies with white colour were counted. About 20 ml of 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 the colonies with white colour were counted. Baired Parker agar with sulpha (M 1140) cooled to 50°C and 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 that appeared on the plates were counted.

Sensory evaluation

Standard sensory evaluation method using 8-point descriptive scale was followed, where 8 = excellent; 1 = extremely poor. The experienced sensory panel consisted of scientists and post graduate students of Division of Livestock Products Technology, Indian

Table 3. Physicochemical characteristics of keema prepared from different groups of buffaloes.

Parameters	Groups		
	Young male	Spent male	Spent female
Physicochemical characteristics			
Meat pH	5.66±0.03^{a}	5.57±0.01^{b}	5.52±0.01^{b}
Keema pH	6.04±0.02^{a}	5.98±0.01^{b}	5.95±0.01^{b}
Yield (%) $^{\#}$	58.60±2.31	59.72±1.71	57.99±1.65
Moisture (%)	63.17±0.63^{a}	59.92±0.75^{b}	61.24±0.31^{b}
Protein (%)	19.47±0.18^{b}	20.31±0.27^{a}	19.15±0.34^{b}
Fat (%)	9.86±0.48^{b}	13.74±1.01^{a}	12.65±0.71^{a}
Energy (Kcal/100g DM)	520.05±6.40^{b}	549.56±2.11^{a}	541.98±6.87^{a}
Water activity	0.94±0.01	0.94±0.01	0.94±0.01

N = 6, $^{\#}$n = 3
Means with different superscripts in the same row indicate significant difference (P < .05).

Veterinary Research Institute, Izatnagar, India, which judged the samples. The panelists were trained according to the guidelines of American Meat Science Association, 1995 and well acquainted with different sensory attributes during their post graduate/doctoral programme.

They were briefly explained to about the nature of the experiment without disclosing the identity of samples. The final product of ready to eat buffalo meat keema prepared fresh was evaluated for sensory attributes on the same day. Whereas, buffalo meat keema held at ambient (37 ± 1°C) and refrigeration (4 ± 1°C) temperature was evaluated on day 3. The stored samples were warmed (40 - 45°C) using microwave oven (LG electronics India (P) Ltd., Mumbai) for 1 min and served to the panelists. The panelists evaluated the 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 the experiments for different quality characteristics were compiled and analyzed using SPSS (version 10.0 for Windows; SPSS, Chicago, III., U.S.A.) with randomized block design. The data were subjected to analysis of variance, least significant difference and paired t-test (Snedecor and Cochran, 1995) for comparing the means to find the difference between groups and storage period. The smallest difference ($D_{5\%}$) for two means to be significantly different (P < 0.05) was reported.

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 3). 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 (Karthikeya 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. The moisture content of keema was similar to the findings of Karthikeyan et al. (2000) in chevon keema.

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 the difference in protein content of the meat. The higher level of protein in the final product was as a result of 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. The level of a_w observed in the present study substantiated the findings of Karthikeyan et al. (2000) in chevon keema.

As observed in the present study, the products in the vicinity of 0.94 a_w were preferred by the sensory panel.

Quality comparison of buffalo meat keema at ambient (37 ± 1°C) and refrigerated (4 ± 1°C) storage

Physicochemical characteristics

The buffalo meat keema stored at ambient temperature for 3 days showed a lower pH compared refrigerated storage. The higher TBARS value to their refrigerated counterparts (Table 4). This was due to the production of acidic metabolites by proteolytic degradation (Leistner et al., 1981).

TBARS value of young male buffalo meat keema increased significantly ($P < 0.05$) at 3 days ambient storage compared to refrigerated storage. The higher TBARS value during ambient storage was due to increased lipid oxidation at low a_w values (Labuza et al., 1972).

It was also due to the higher microbial proliferation that occurred in the samples stored at ambient temperature. A positive correlation between microbial load and TBARS values was reported in ground buffalo meat (Sahoo and Anjaneyulu, 1997).

Microbiological characteristics

The total plate count was significantly ($P < 0.01$) higher in 3 days ambient stored buffalo meat keema compared to refrigeration storage (Table 4). A high aerobic plate count in keema was mainly due to the conducive a_w and pH (Smolka et al., 1974; Leistner et al., 1981).

Coliforms were not noticed in both ambient and refrigerator stored buffalo meat keema on day 3. The absence or low count of coliforms and yeast and mold 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, total anaerobes and *lactobacillus* count were significantly ($P < 0.01$) higher in 3 days ambient stored buffalo meat keema compared to refrigeration storage. Microbial spoilage of meat at higher temperatures was mainly due to the growth of mesophilic organisms such as *S. aureus* and *E. coli* (Narasimha and Ramesh, 1988). A high *S. aureus* count was related to the conducive pH and a_w (Leistner et al., 1981). Vacuum packaging enhanced the growth of gas forming anaerobes and *lactobacillus* in the ambient stored buffalo meat keema. A significant increase in *lactobacillus* count was reported in cooked pork sausages at 10°C compared to 4°C (Pexara et al., 2002). Yeast and molds were noticed on day 3 of ambient stored buffalo meat keema.

Sensory attributes

The appearance, flavour, juiciness, tenderness and overall acceptability scores of keema declined significantly ($P < 0.01$) during 3 days ambient storage compared to refrigeration storage (Table 5). The connective tissue residue scores of young male and spent female buffalo meat keema also decreased significantly ($P < 0.05$) during day 3 of ambient storage compared to their refrigerated counterparts.

The decline in colour scores during ambient storage was due to lipid oxidation and subsequent oxidized compounds reacting with amino acids during non-enzymatic browning of the product (CheMan et al., 1995). Flavour changes were more in case of room temperature stored mutton curry compared to refrigerated product (Himanish and Radhakrishna, 2001). The steep decline in flavour scores was attributed to the liberation of fatty acids (Branen, 1979), oxidation of fat (Santamaria et al., 1992) and increased microbial load (Sahoo and Anjaneyulu, 1997). The significant decrease in texture during ambient storage was due to changes in the disulphide bond and contents of amino acid (Santamaria et al., 1992). An abrupt reduction in overall acceptability during ambient storage was mainly attributed to decline in flavour in chevon keema (Karthikeyan et al., 2000) in chevon keema.

Conclusions

Scientific processing by adopting good manufacturing practices and suitable packaging helped greatly to improve the shelf life of the ambient temperature stored buffalo meat keema. The product characteristics and overall acceptability of keema from spent buffalo groups was better than young male group. The rate of quality deterioration was faster in ambient temperature stored keema than refrigerated group.

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Table 4. Comparison of physicochemical and microbiological characteristics of buffalo meat keema at refrigerated (4±1°C) and ambient (37±1°C) storage on day 3.

Parameters/Groups	Storage temperature (°C)		
	4±1°C	37±1°C	t-value
	pH		
YM	6.17±0.04	6.13±0.02	1.230
SM	5.99±0.01	5.97±0.02	1.148
SF	5.97±0.01	5.96±0.04	0.193
	TBARS value (mg malonaldehyde/kg)		
YM	0.18±0.02	0.23±0.02	6.325**
SM	0.16±0.01	0.24±0.04	2.284
SF	0.17±0.01	0.27±0.05	2.086
	Total plate count (log cfu/g)		
YM	1.29±0.10	3.64±0.43	4.890**
SM	1.35±0.09	3.74±0.16	13.433**
SF	1.35±0.12	3.57±0.19	12.069**
	***Staphylococcus aureus* count (log cfu/g)**		
YM	1.78±0.10	3.25±0.09	58.649**
SM	2.07±0.14	3.82±0.14	9.876**
SF	1.35±0.14	3.83±0.25	15.798**
	Total anaerobic count (log cfu/g)		
YM	1.26±0.16	3.11±0.07	8.474**
SM	1.20±0.13	3.19±0.20	6.113**
SF	1.23±0.08	2.89±0.27	7.047**
	***Lactobacillus* count (log cfu/g)**		
YM	2.23±0.08	3.66±0.12	7.110**
SM	2.09±0.35	3.88±0.15	5.371**
SF	2.75±0.10	3.52±0.12	31.650**
	Yeast and mold count (log cfu/g)		
YM	ND	1.05±0.05	20.932**
SM	ND	1.10±0.06	17.338**
SF	ND	ND	

N = ; *P < .05;** = P < 0.01.
YM = Young male; SM = Spent male; SF = Spent female; ND = Not detected.

Table 5. Comparison of sensory attributes of buffalo meat keema at refrigerated (4±1°C) and ambient (37±1°C) storage on day 3.

Parameters/Groups	Storage period (days)		
	4±1°C	37±1°C	t-value
	Appearance		
YM	7.19±0.05	6.45±0.09	7.183**
SM	7.06±0.02	6.55±0.08	6.124**
SF	7.06±0.02	6.57±0.09	5.499**

Table 5. Contd.

	Flavour		
YM	7.04±0.03	5.71±0.16	7.772**
SM	7.18±0.05	5.26±0.19	10.004**
SF	7.24±0.04	5.48±0.14	12.253**
	Juiciness		
YM	7.12±0.05	5.98±0.08	14.974**
SM	6.99±0.04	5.86±0.13	8.562**
SF	6.99±0.04	5.90±0.09	11.043**
	Tenderness		
YM	7.24±0.06	6.79±0.11	3.581**
SM	6.86±0.05	6.50±0.09	3.573**
SF	7.05±0.06	6.50±0.09	5.246**
	Connective tissue residue		
YM	7.12±0.05	6.81±0.11	2.805*
SM	6.76±0.06	6.74±0.08	0.234
SF	7.06±0.06	6.79±0.06	2.726*
	Overall acceptability		
YM	7.05±0.10	5.69±0.15	7.656**
SM	7.26±0.10	5.31±0.20	9.790**
SF	7.25±0.07	5.65±0.15	10.644**

n = 6; *P < 05;** = P < 0.01.
*Based on 8 point descriptive scale,
YM = Young male; SM = Spent male; SF = Spent female; ND = Not detected.

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