Shelf life study of hurdle treated ready-to-eat spiced buffalo meat product stored at 30 ± 3 °C for 7 weeks under vacuum and aerobic packaging

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Abstract Shelf stable ready to eat spiced pickle type buffalo meat product was prepared after desorbing in infusion solution (glycerol 3.5%, sodium chloride 5.0%, honey 2.0%, mango powder 2.2%, spices 1.0%, sodium nitrite 0.015%, phosphate 0.2%, Sorbic acid 0.2% and acetic acid 1%), pressure cooking of meat in infusion solution for 20 min followed by frying for 2 min in mustard oil and mixing with prefried condiments and spices. The physico-chemical properties i.e. pH, water activity, proximate composition, FFA, Soluble hydroxyproline, TBA values, nitrite content, protein solubility, shear force value, haempigments, microbiological and sensory quality of the product remained good and hygienically safe and almost comparable in aerobic PET jars and multilayered nylon barrier pouches stored at 30 ± 3 °C for 7 weeks. It can be suggested that storage of such product may be conveniently done even in food grade PET jars without going for vacuum packaging which is a bit costly.

Keywords Ready-to-eat · Shelf stable · Buffalo meat · Water activity · Hurdle · Protein solubility · TBA · Staphylococcus · Spices · Meat pigment · Vacuum packaging · PET jars · Nylon laminates · Aerobic packaging

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Introduction

Meat products form the essential component of the non vegetarian diet. Meat is liked for its unique taste and is rich in nutrients, providing best quality of protein, essential fatty acids, essential amino acids and a number of minerals and vitamins particularly the B12 group. Meat is converted into a number of products all over the world depending upon the consumer liking and desirability. Meat is an easily spoilable commodity therefore, requiring some preservation. Various methods of meat preservation are applied in the world depending upon the consumer liking, availability, shelf life extensibility and safety of the methods. Refrigeration is a common method of preservation of meat. However, refrigeration is an energy consuming and costly process which is not always practicable in the remote areas of the world and also the higher costs of energy has made us to think of some cheaper alternative methods. It therefore, becomes imperative to find some cheaper alternative to refrigeration for preservation of meat. Hurdle technology can be utilized to store and transport meat at ambient temperatures with convenience at cheaper and affordable price while maintaining the safe microbiological quality, good sensory and nutritional properties (Sharma 1997). Hurdle technology, also referred to as combination preservation technique (Leistner 1994), uses the parameters which are hurdles for microbial growth (Bogh Sorensen 1994) enzymatic deterioration. The hurdles and their intensities depend upon the type of food, its natural micro flora, chemical composition and climatic conditions of handling and storage. The purpose is to disturb the homeostasis (Leistner and Gorris 1995) of microbes in order to render them inactive. The hurdles generally used are water activity, pH, redox potential, mild heat treatment, refrigeration, preservatives and competitive flora etc. (Leistner 1978).

Hurdle technology has made it possible to devise some semi-moist, ready-to-eat, shelf stable, sound and convenient meat and meat products to meet the requirements of a special class of people like space scientists, mountaineers, and defence personnel especially as combat ration with light weight. A review of literature on the subject reveals that technologists have prepared intermediate moisture meat products in various countries using sugar and salt desorption process at high concentrations. Such levels of humectants may produce disagreeable taste (Ledward 1981). Also there is a desire for newer products from consumers owing to changing food habits and life style. In view of the growing need for shelf stable ready to eat meat products this study was conducted to evaluate the physico chemical, microbiological and sensory quality of shelf stable ready-to-eat meat product at ambient temperature under aerobic and vacuum packaging systems.

Materials and methods

Buffalo meat required for the experiments was procured from a selected retail meat shop located in Bareilly meat market. The meat samples strictly belonged to round (consisting mostly of semimembranosus, semitendinosus, biceps, femoris and quadriceps muscles) of carcasses of almost similar conformation of spent adult female Murrah buffaloes slaughtered according to traditional Halal method at buffalo slaughterhouse of Bareilly Municipal Corporation. Boneless meat cuts of required weight were purchased within 4 to 5 h of slaughter packed in low density polyethylene (LDPE) bags and brought to the laboratory within 30 min. The meat chunks were kept for conditioning in a refrigerator at 4 ± 1 °C for about 24 h till further use.

The chemicals and ingredients used as humectants and preservatives in the experiments were all food grade and from standard companies.

Double refined mustard oil conforming to Agmark standard was purchased from local market and used for cooking of the products.

Formulation of infusion solutions The infusion solution formulation was devised and ingredients were accurately weighed according to formulation (glycerol 3.5%, sodium chloride 5.0%, honey 2.0%, mango powder 2.2%, spices 1.0%, sodium nitrite 0.015%, phosphate 0.2%, Sorbic acid 0.2% and acetic acid 1%,) and added to potable sterile water thoroughly stirring with a sterilized glass rod till all the ingredients got mixed or dissolved in water.

Preparation of the ready to eat spiced buffalo meat products In the preliminary trials the quantity of acetic

acid required for pH adjustment, pressure cooking and frying times were standardized with infusion solution.

Chilled meat was cleared of the visible and separable connective tissue and excess fat. The meat was then cut into two cubic centimeter pieces and equal quantities of these meat cubes were desorbed in the infusion solutions in the ratio of 1:1 (by weight). Acetic acid 0.1% on meat weight basis was added to infusion solution formulation and was kept under refrigerated conditions for 24 h.

The meat cubes along with the infusion solution after 24 h desorption were pressure cooked for pre standardized time of 20 min.

Condiments 5% on meat weight basis were fried to brown colour in 1/3 mustard oil out of a total of 15% oil on meat weight basis. Rest of the mustard oil was kept for frying of meat. Later 1.5% spice mix on meat weight basis was added towards the end of frying to avoid charring.

The pressure cooked meat cubes were separated from the cookout and subjected to frying with mustard oil heated to 150 °C for 2 min.

The fried condiments and spice paste was added to cook out. This mixture was simmered in a pan to a thick consistency for about 20–25 min till much of the moisture got evaporated.

The fried meat cubes were added to simmering thick slurry of condiments and cook-out and mixed with a spoon. The product thus prepared was cooled to room temperature. Three trials were conducted. The samples collected for physico chemical, microbiological and sensory analysis were analyzed for various parameters by standard methods as described below.

Packaging materials and packaging of the product

Multi-layered nylon barrier film pouches of 105 μ thickness in natural colour procured from M/s Hitkari Industries Ltd., New Delhi were used for vacuum packaging of the meat products. While polyethylene terephthalate (PET) jars (Pearl make) were used for aerobic packaging of ready-to-eat spiced buffalo meat product. The product was divided in two parts One half of the product was evenly packed in food grade polyethylene terephthalate (PET) jars and the other half was packed in multilayered nylon laminates under vacuum. Both types of packages were kept at 30 ± 3 °C in an incubator and samples were taken out at weekly intervals for analytical purpose.

Cooking yield

The weight of meat before desorption together with the weight of humectants, oil, spices and condiments was taken

as initial weight while weight of the finished product was recorded as final weight. Cooking yield was expressed in percentage.

Physico-chemical properties

pH

pH of fresh buffalo meat and meat products was determined by the method of Keller et al. (1974). 10 g meat sample was blended for 1 min with 100 ml of freshly distilled water in a homogenizer. The pH of the homogenate was recorded by dipping combined glass electrode of digital pH meter (Century India, Model CP 901). pH of infusion solutions was recorded by dipping electrode in the infusion solutions.

Proximate composition

The proximate composition of fresh buffalo meat and meat products was determined by following the procedure laid down by AOAC (1995).

Water activity (a_w)

The water activity (a_w) of meat samples was determined by the procedure recommended by Lerici et al. (1983), with slight modification (Malik and Sharma 2010). Approximately 50 g minced sample was tightly packed inside a 60 ml glass tube and mouth was corked air-tight. Then it was immersed in a cooling chamber. The cooling chamber had precooled ethanol at $-30\text{ }^{\circ}\text{C}$ to $-35\text{ }^{\circ}\text{C}$ in 1 l glass beaker and kept in deep freezer (Vertical type, Vest frost, Denmark). Resistant thermometer probe (Century, CT809, and S.No. 101) was introduced inside the cork to monitor the temperature of the product. The rate of decrease of temperature was monitored by looking at electronic digital display (Century) which was constant up to a specific point. After that the rate markedly decreased. The point at which the rate markedly altered was taken as freezing point of the sample. The freezing point was converted to a_w value using

$$-\ln a_w = 27.622 - 528.373(1/T) - 4.579 \ln T$$

Where, T = freezing point temperature in Kelvin.

Shear force value

The objective texture measurement of shelf stable meat products was done using Warner Bratzler shear press (Model No. 81031307, G.R. Elect. Mfg. Co., USA).

Muscle cores with cross-sections of $1.27\text{ cm} \times 1.27\text{ cm}$ were prepared by cutting the meat pieces through their longitudinal axis. Maximum force (kg) required to shear meat cores along the transverse axis was recorded and expressed as the force (kg) required to shear meat.

Sodium chloride (salt)

Method described by Koniecko (1979) was followed. Duplicate 5 g sample per treatment per trial was placed in 250 ml conical flasks and moistened with 10 ml of 0.5 N silver nitrate followed by addition of 15 ml of conc. nitric acid. The flask contents were brought to boil on a plate heater and boiling continued for few minutes until sample was dissolved. Few drops of conc. potassium permanganate (10%) solution were added and boiling continued until permanganate colour disappeared after which 25 ml of distilled water was added and contents again boiled for 5 min. The flasks were cooled, volume made to about 150 ml with distilled water. Then 5 ml of ferric ammonium sulphate indicator solution and 10 ml of diethyl ether were added in succession, the contents mixed well and titrated against 0.1 N ammonium thiocyanate to the end point (brown colour). Results were expressed as per cent salt and calculated as follows:

$$\text{Salt}(\%) = (A - 0.2B) \times 2.92 / \text{sample weight, in g}$$

Where A = ml of 0.5 N silver nitrate added and B = ml of 0.1 N ammonium thiocyanate used.

Nitrite

The method outlined by AOAC (1995) was adopted for nitrite estimation. 5 g finely comminuted and thoroughly mixed sample was taken in 50 ml beaker. About 40 ml distilled water was added and heated to $80\text{ }^{\circ}\text{C}$. It was mixed thoroughly with glass rod to break up all lumps and transferred to 500 ml volumetric flask. The beaker and glass rod were thoroughly washed with hot distilled water adding washing to the flask. The flask was filled with hot distilled water to bring volume to about 300 ml and subsequently transferred to steam bath and allowed to stand for 2 h with occasional shaking. After heating for 2 h in steam bath the flask was removed from it and allowed to cool to room temperature, volume made with distilled water to 500 ml and remixed. The contents of the flask were filtered through nitrogen free filter paper. 30 ml aliquot containing 5–50 μg NaNO_2 was taken in a 50 ml volumetric flask. To this 2.5 ml sulphanilamide reagent was added, mixed followed by addition of 2.5 ml

NED reagent after 5 min. The contents of the flask were well mixed and diluted with distilled water to make volume to 50 ml. After keeping for 15 min developing colour the absorbance of 1 ml aliquot was read at 540 nm in Beckman (Model DU 640) Spectrophotometer against blank of 45 ml distilled water, 2.5 ml sulphanilamide reagent and 2.5 ml NED reagent. The content of nitrite in the sample was determined from the standard curve for nitrite prepared according to the same method and results expressed in ppm.

Thiobarbituric acid (TBA) value

The method of Taladgis et al. (1960) with slight modifications was followed. 10 g sample was blended with 49 ml distilled water and 1 ml of sulphanilamide reagent in a homogenizer. The mixture was quantitatively transferred into a Kjeldahl flask. Another 48 ml of distilled water was used for rinsing the blender and poured into the flask followed by addition of 2 ml of hydrochloric acid solution (1 volume with 2 volume of water). Few chips of paraffin wax were added and the flask heated at high heat and 50 ml of distillate collected into a graduated cylinder. The distillate was mixed well, 5 ml was pipetted into test tubes to which 5 ml of TBA reagent was added and mixed. The tubes on a stand were immersed in boiling water bath for 35 min followed by 10 min cooling in tap water. The OD was read at 538 nm against reagent blank in a Beckman (Model DU 640) Spectrophotometer. The OD was multiplied by the factor 7.8 and results expressed as mg malonaldehyde/kg meat.

Total haempigments

The method used by Hornsey (1956) was adopted for measurement of total pigments. The meat was trimmed of the excess fat tissue, cut with scissors and then minced thoroughly using a pestle and mortar. Ten grams of minced sample was first mixed to a smooth paste with approximately 10 ml of a acetone water acid mixture containing 40 ml of acetone and 2 to 4 ml of water (water calculated on the basis of moisture content of meat sample) and 1 ml of concentrated hydrochloric acid (1 ml acid replaced 1 ml water in acetone water ratio of 4:1). The remainder of the acetone solution was then added and after mixing the solution was kept for 1 h. After 1 h the solution was filtered through Whatman filter paper No.1 and OD was recorded at 640 nm against acetone water acid mixture of same ratio as for the sample in a Beckman (Model DU 640) spectrophotometer. The OD recorded was multiplied by 680 to give total haempigments as ppm of haematin.

Protein solubility

The method used by Okonkwo et al. (1992a) was adopted. Meat sample was cleared of any visible fat and minced thoroughly using a pestle and mortar. The ground meat (0.5 g) was dispersed in 50 ml of 3% SDS plus 1% beta mercaptoethanol. After standing for 30 min the suspension was heated in boiling water bath for 30 min and centrifuged for 30 min at $5000 \times g$ as per Madovi (1980) in a Remi T8 centrifuge whilst still warm. The nitrogen content of the supernatant and residue were determined by the micro kjeldahl method. Nitrogen was converted to protein by multiplying with 6.25 and the per cent soluble protein calculated.

Per cent soluble protein

$$= \frac{\text{Protein in supernatant}}{\text{Protein in supernatant} + \text{Protein in residue}} \times 100$$

Soluble hydroxyproline

For preparation of soluble fraction of hydroxyproline, procedure of Okonkwo et al. (1992a) was used with slight modification. Four grams of the ground sample was dispersed in 12 ml of a quarter strength Ringer's solution, heated in a water bath for 70 min at 77 °C followed by centrifugation at 4000 rpm in a Remi T8 centrifuge for 20 min. The supernatant was decanted and the residue was washed with 12 ml of quarter strength Ringer's solution and recentrifuged at 4000 rpm for 10 min. The supernatants were combined. Forty milliliters of concentrated hydrochloric acid was added to filtrate and 40 ml of 6 N HCl added to the residue in conical flasks. After covering with watch glass these were placed in hot air oven at 105 °C for 16 h. For determination of hydroxyproline in the hydrolysates procedure for Neumon and Logan (1950) was followed. The hydrolysates were filtered and volume adjusted to 100 ml with water. Suitable aliquots were neutralized with 40% NaOH to pH 7.0. And volume made to 100 ml with distilled water. To 1 ml aliquots in test tubes 1 ml each of 0.01 M copper sulphate, 2.5 N sodium hydroxide and 6% hydrogen peroxide solution were added in succession. The contents were mixed and tubes kept at room temperature for 5 min with occasional shaking. The tubes were then placed in a water bath at 80 °C for 5 min with frequent vigorous shaking and then chilled in ice. Four milliliters of 3 N sulphuric acid and 2 ml of 5% p-dimethylaminobenzaldehyde in n-propanol were added and mixed thoroughly. The tubes were placed in a water

bath at 70 °C for 16 min, cooled in tap water and OD was read at 540 nm. Suitable standards and distilled water blank were carried through similarly. The hydroxyproline

concentration in the samples was determined from the standard curve. The per cent soluble hydroxyproline was calculated as:

$$\text{Per cent soluble Hydroxyproline} = \frac{\text{Soluble hydroxyproline of supernatant fraction}}{\text{Soluble hydroxyproline Of supernatant} + \text{Insoluble hydroxyproline of residue fraction}} \times 100$$

Microbiological quality

All the microbiological parameters were determined following the APHA (1984) as outlined earlier (Malik and Sharma 2010)

Preparation of serial dilution

Ten grams of meat sample was taken near flame in a sterile mortar with the help of sterilized forceps and scissors. Ninety milliliters of sterile 0.1% peptone water was added to it and homogenized using a sterile pestle for 2 min for uniform dispersion to get 10^{-1} dilution. One milliliter of this dilution was transferred to 9 ml of sterile 0.1% peptone water in a test tube and mixed uniformly to get 10^{-2} dilution. Again 1 ml of 10^{-2} dilution was added to 9 ml peptone water and mixed to obtain 10^{-3} dilution and so on. Serial dilutions were made as per requirement.

Total plate count

Plate count agar 23.5 g was suspended in 1 l distilled water, boiled to dissolve completely and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min. Final pH was 7.0 ± 0.2 . About 20 ml of sterilized media at 45 °C was poured to each sterile petridish in duplicate after dropping of 1 ml inoculums of suitable dilutions. The plates were incubated at 35 °C for 24 h. Plates showing 25 to 250 colonies were counted. The number of colonies were multiplied by the reciprocal of the dilution and expressed as $\log_{10}$ cfu/g.

Staphylococcus aureus count

Sixty-three grams Baird Parker Agar base was suspended in 950 ml distilled water and boiled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min, cooled to 50 °C, added aseptically 50 ml concentrated egg yolk emulsion and 3 ml sterile 3.5% potassium tellurite solution and mixed well before pouring. Final pH of the medium was 7.0 ± 0.2 . Now,

0.2 ml of suitable dilutions in duplicate was spread onto the surface of the medium. The petridishes were incubated at 35 °C for 48 h. The number of intensely dark, shiny, regularly shaped colonies surrounded by clear holes was counted and expressed as $\log_{10}$ cfu/g.

Yeast and mold count

Thirty-nine grams of potato dextrose agar was suspended in 1 l distilled water, boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min. To obtain pH 3.5 acidified the sterile cooled medium with 10 ml of 10% tartaric acid. Precaution was taken not to heat the medium after addition of the acid. One milliliter in duplicate of suitable dilution was inoculated into sterile petriplates and molten medium at 45 °C was poured over the plates. The petridishes were incubated at 25 °C for 5 days. Black, white, red, greenish black coloured colonies appeared on the plates were counted and expressed as $\log_{10}$ cfu/g.

Anaerobic plate count

Fifty-eight grams anaerobic agar was suspended in 1 l distilled water, boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min. Final pH of the medium was 7.2 ± 0.2 . One milliliter of suitable dilutions in duplicate was inoculated to the sterile petriplates and molten growth medium at 45 °C was poured over the plates. The petridishes were put into the anaerobic jar and incubated at 35 °C for 48 h. White colonies on the surface of the medium were counted and expressed as $\log_{10}$ cfu/g.

Sensory evaluation of buffalo meat product

A semi trained experienced taste panel (Seman et al. 1987) members consisting of scientists and postgraduate students of the Division and Institute evaluated the sensory attributes of buffalo meat product. The 8-point descriptive scale was used (wherein value of 8 is extremely desirable and 1 is

extremely undesirable). The product was served to the panelists as such without warming.

Statistical analysis

The data obtained from the various trials under each experiment was pooled and processed at the Institute's Computer Centre. The data was subjected to analysis of variance, least square difference and critical difference (Snedecor and Cochran 1967) and Duncan's multiple range tests (Steel and Torrie 1982) for comparing the means to find the effects between treatments, storage periods and their interaction for various parameters in different experiments.

Results and discussion

To evaluate the shelf life of ready-to-eat spiced buffalo meat cubes, the product was packed in two types of packaging materials PET jars (aerobic) and in multilayered nylon barrier film pouches (vacuum) stored at 30 ± 3 °C in an incubator for 7 weeks and evaluated for physico-chemical, sensory and microbial quality at weekly intervals. The mean values of various quality parameters of shelf stable ready-to-eat spiced buffalo meat (SRBM) as affected by packaging and ambient temperature storage are presented in Tables 1, 2, 3 and 4.

Physico-chemical characteristics

The ANOVA indicated highly significant ($P < 0.01$) effect of packaging type on TBA values and moisture content (Table 1) and significant ($P < 0.05$) effect on soluble hydroxyproline content whereas other parameters viz. pH, water activity, residual nitrite, haempigments, FFA, protein solubility, protein, fat, ash and shear force value were not significantly ($P < 0.05$) affected. Storage had a highly significant ($P < 0.01$) effect on pH, nitrite, TBA values, total haempigments, free fatty acids, soluble hydroxyproline, protein solubility and moisture content and a significant effect on water activity and fat content whereas protein, ash and shear force values of the product were not significantly ($P > 0.05$) affected. The effect of interaction of storage and packaging was highly significant ($P < 0.01$) on TBA values with no effect on all other parameters.

pH pH values (Table 1) decreased significantly from 0 day to end of 7 weeks storage period. A significant decline in pH was noticed at 2 weeks as compared to 0 day which did not change significantly up to 5 weeks. pH value was not significantly affected during storage from 5 to 7 weeks of storage. Packaging (aerobic or vacuum) had no influence

on the pH of SRBM product. Similar findings were also recorded by Babji and Murthy (2000) in goat meat stored under aerobic and vacuum conditions at refrigeration temperature. The pH of the SRBM product significantly decreased (Table 1) during 7 weeks storage from initial 5.3 to 5.0. Penetration of acetic acid into the musculature may be responsible for such decrease (Singh and Panda 1984). Another reason for pH decrease may be some cross linking reactions involving removal of certain amino groups (Okonkwo et al. 1992b). Further, reduction of honey sugars by some microbes into acids may have also contributed to pH decline (Kaushik et al. 1993). Reddy and Rao (1997) had similar findings in chicken pickle stored for 80 days at ambient temperature.

Water activity (a_w) The water activity (Table 1) increased significantly during first week and later remained stable with no significant change up to the end of 7 weeks storage period. The increase in first week may be due to equilibration of water activity between meat pieces and the slurry of cookout, spices, solutes etc. Once the equilibrium was reached, the water activity values remained constant till the end. However, packaging type had no significant effect on the water activity of the SRBM product.

TBA values The TBA values had not changed significantly during first week of storage. A significant rise in TBA values was observed at each subsequent storage interval up to 5 weeks. TBA values decreased significantly at 6 weeks of storage and were not significantly different from those at 7 weeks of storage. Aerobically packaged product in PET jars had significantly ($P < 0.01$) higher TBA values than vacuum packaged product in laminates. A highly significant effect of interaction of packaging and storage was also observed on TBA values of the finished product. The thiobarbituric acid is an index of rancidity and is measured by malonaldehyde concentration/kg of meat tissue. The TBA value was significantly higher (Table 1) in aerobically packaged SRBM than the vacuum packaged product. This is because in the former case oxygen becomes available for oxidation of meat lipids. Similar results were recorded by Ockerman and Kuo (1982). A significant increase in TBA values was recorded during the storage up to 5 weeks. Thereafter, the values started declining. This decline is considered due to the decomposition of lipid hydroperoxides during storage to carbonyls. These carbonyls react with proteins to form premelanoidins which are antioxidants (El-Zeany et al. 1973). During storage of intermediate moisture meats, Obanu et al. (1975) also observed a decrease in TBA at a later stage. However, TBA values were below 1–2 considered to be the threshold limit for perception of rancidity (Watts 1962).

Table 1 Effect of storage and packaging on physico-chemical characteristics of shelf-stable ready-to-eat spiced Buffalo meat at ambient temperature (30±3 °C)

Packaging type	Storage period (weeks)							Treatment
	0	1	2	3	4	5	6	7
pH								
Aerobic	5.3±0.03	5.3±0.06	5.2±0.05	5.1±0.02	5.1±0.03	5.1±0.02	5.0±0.03	4.9±0.09
Vacuum	5.3±0.03	5.3±0.07	5.2±0.08	5.1±0.01	5.1±0.06	5.0±0.02	5.0±0.03	5.1±0.03
Mean±SE	5.3±0.02 ^d	5.3±0.04 ^{cd}	5.2±0.04 ^{bc}	5.1±0.01 ^b	5.1±0.03 ^b	5.1±0.01 ^{ab}	5.0±0.02 ^a	5.0±0.05 ^a
Water activity (a_w)*								
Aerobic	0.91±0.003	0.91±0.001	0.91±0.001	0.91±0.001	0.91±0.000	0.91±0.000	0.91±0.000	0.91±0.000
Vacuum	0.91±0.003	0.91±0.001	0.91±0.001	0.91±0.000	0.91±0.000	0.91±0.000	0.91±0.000	0.91±0.000
Mean±SE	0.91±0.002 ^a	0.91±0.000 ^b	0.91±0.000 ^{ab}	0.91±0.000 ^{ab}	0.91±0.000 ^{ab}	0.91±0.000 ^{ab}	0.91±0.000 ^{ab}	0.91±0.000 ^{ab}
Nitrite (ppm)								
Aerobic	20.3±0.10	19.1±0.05	18.4±0.05	16.1±0.04	15.6±0.09	14.4±0.06	13.0±0.08	12.2±0.04
Vacuum	20.3±0.10	19.2±0.06	18.4±0.04	16.0±0.04	15.6±0.08	14.4±0.06	13.0±0.08	12.2±0.04
Mean±SE	20.3±0.07 ^b	19.2±0.04 ^g	18.4±0.03 ^f	16.1±0.02 ^e	15.6±0.06 ^d	14.4±0.04 ^c	13.0±0.05 ^b	12.20±0.02 ^a
TBA (mg malonaldehyde/kg meat)								
Aerobic	0.59±0.01	0.62±0.03	0.74±0.03	0.83±0.01	0.92±0.01	1.1±0.04	0.93±0.02	0.83±0.02 ^b
Vacuum	0.59±0.01	0.59±0.02	0.61±0.003	0.64±0.01	0.74±0.01	0.70±0.02	0.65±0.01	0.64±0.01 ^a
Mean±SE	0.59±0.01 ^a	0.61±0.02 ^a	0.67±0.02 ^b	0.74±0.03 ^c	0.83±0.03 ^c	0.90±0.06 ^f	0.79±0.04 ^{de}	0.77±0.04 ^{cd}
Total haemipigments (ppm)								
Aerobic	429.2±8.0	406.4±5.9	378.1±3.7	355.6±3.6	345.0±4.2	305.4±3.1	241.5±3.2	206.0±2.1
Vacuum	429.2±8.0	407.2±6.0	377.6±4.4	354.8±3.1	344.6±4.6	311.9±2.1	227.3±7.9	206.9±2.4
Mean±SE	429.2±5.4 ^b	406.8±4.0 ^g	377.8±2.7 ^f	355.2±2.3 ^e	344.8±3.0 ^d	308.7±2.1 ^c	234.4±4.7	204.4±2.5 ^a
Free fatty acids (%)								
Aerobic	1.0±0.03	1.9±0.34	2.7±0.05	2.9±0.03	2.9±0.03	2.8±0.03	2.9±0.03	2.8±0.04
Vacuum	1.0±0.03	1.7±0.31	2.8±0.03	2.9±0.05	2.9±0.05	3.0±0.02	2.8±0.11	2.7±0.20
Mean±SE	1.0±0.02 ^a	1.8±0.22 ^b	2.8±0.03 ^c	2.9±0.03 ^c	2.9±0.03 ^c	2.9±0.03 ^c	2.9±0.06 ^c	2.7±0.11 ^c
Soluble hydroxyproline (%)								
Aerobic	48.1±0.32	48.7±0.40	48.4±0.29	49.1±0.54	46.6±0.21	49.0±2.4	46.5±1.1	42.1±0.68
Vacuum	48.1±0.32	47.3±0.25	47.9±0.38	47.7±0.78	46.6±0.94	44.7±0.21	46.3±1.33	39.2±1.01
Mean±SE	48.1±0.22 ^b	48.0±0.32 ^b	48.1±0.24 ^b	48.4±0.50 ^b	46.6±0.46 ^b	46.8±1.6 ^b	46.4±0.81 ^b	40.6±0.72 ^a
Protein solubility (%)								
Aerobic	92.6±0.22	87.6±0.3	87.7±0.52	87.3±0.41	86.0±0.40	83.6±0.51	83.2±0.65	78.9±0.25
Vacuum	92.6±0.22	87.7±0.67	87.3±0.64	86.8±0.33	84.8±0.19	83.5±0.63	81.9±0.80	79.9±0.55
Mean±SE	92.6±0.14	87.6±0.34 ^d	87.5±0.38 ^d	87.0±0.27 ^d	85.4±0.34 ^c	83.5±0.36 ^b	82.5±0.54 ^b	79.4±0.35 ^a

Means (±SE) bearing different superscripts row-wise and column wise differ significantly ($P<0.05$)* $n=3$

Table 2 Effect of storage and packaging on shear force value (kg/1.27 cm²) of shelf-stable ready-to-eat spiced buffalo meat at ambient temperature (30±3 °C)

Packaging type	Storage period (weeks)								Treatment
	0	1	2	3	4	5	6	7	Mean±SE
Aerobic	5.7±0.11	5.8±0.11	5.7±0.09	5.7±0.12	5.6±0.1	5.6±0.10	5.8±0.09	5.7±0.12	5.7±0.04
Vacuum	5.7±0.11	5.7±0.15	5.7±0.14	5.7±0.12	5.6±0.12	5.7±0.09	5.7±0.11	5.8±0.12	5.7±0.04
Mean±SE	5.7±0.08	5.7±0.09	5.7±0.08	5.7±0.08	5.6±0.08	5.7±0.07	5.8±0.07	5.7±0.08	

Means±SE bearing different superscripts row-wise and column wise differ significantly ($P<0.05$)

$n=24$

In general, it has been reported that lipid oxidation is not a problem in intermediate moisture meats (Ledward 1985). Antioxidant activity of spices (Al-Jalay et al. 1987) and nitrite also play a role in checking the rancidity development.

Storage and packaging type interaction also had significant effect on the TBA values. This may be because lipid oxidation takes place in presence of oxygen and there is a progressive increase during storage in aerobically packed than vacuum packed product.

Nitrite Storage had a highly significant ($P<0.01$) effect on nitrite content as it decreased significantly at each storage interval during the entire storage period (Table 1). The residual nitrite content was not affected by packaging type during storage period. This may be because the measurable levels of nitrite decrease during heating in meats and also during storage depending on the temperature and pH (Jay 1986). Further, microbial count in both types of packaging

were very low and similar, their role in depletion of nitrite (Nordin 1969) also remained almost same.

The residual nitrite content was 20.3 ppm on 0 day which got further depleted at each storage interval and recorded 12.2 ppm at the end of 7 weeks storage (Table 1). The depletion of nitrite content during storage has been explained by Sadler and Swan (1997) as due to reaction of nitrite with meat components to form a range of compounds such as protein complexes, which are not detected in the analysis. Similar depletion in nitrite content during storage has also been observed by Cassens et al. (1979) in cooked meats.

Total haempigments Total haempigment content (Table 1) decreased significantly at each storage interval, the concentration of total haempigments being highest on 0 day (429.2 ppm) and lowest at the end of 7 weeks storage period (204.4 ppm). Packaging type did not cause any significant effect on total haempigments (Table 1). However, significant

Table 3 Effect of storage and packaging on microbiological characteristics of shelf-stable ready-to-eat spiced buffalo meat at ambient temperature (30±3 °C)

Packaging type	Storage period (weeks)							Treatment
	1	2	3	4	5	6	7	Mean±SE
Total plate count (log cfu/g)								
Aerobic	2.1±0.02	2.2±0.05	2.6±0.06	2.6±0.10	2.9±0.14	3.6±0.07	3.7±0.09	2.8±0.13 ^b
Vacuum	2.1±0.02	2.2±0.04	2.2±0.12	2.3±0.19	2.7±0.14	3.4±0.09	3.4±0.06	2.6±0.12 ^a
Mean±SE	2.1±0.02 ^a	2.2±0.03 ^{ab}	2.4±0.10 ^b	2.5±0.12 ^b	2.8±0.10 ^c	3.5±0.08 ^d	3.5±0.08 ^d	
Yeast and mould count (log cfu/g)								
Aerobic	1.9±0.07	2.3±0.01	2.0±0.12	2.6±0.30	2.9±0.10	2.9±0.07	2.9±0.10	2.5±0.01 ^b
Vacuum	1.7±0.01	1.7±0.01	1.9±0.14	2.3±0.30	2.5±0.11	2.8±0.05	2.6±0.13	2.2±0.10 ^a
Mean±SE	1.8±0.06 ^a	2.0±0.13 ^{ab}	2.0±0.08 ^{ab}	2.4±0.21 ^b	2.7±0.11 ^{bc}	2.9±0.05 ^c	2.7±0.11 ^c	
Anaerobic plate count (log cfu/g)								
Aerobic	1.3±0.15	1.2±0.10	1.6±0.06	1.7±0.05	1.7±0.07	2.2±0.13	1.9±0.29	1.7±0.09 ^a
Vacuum	1.6±0.06	1.7±0.11	1.6±0.07	2.1±0.06	2.0±0.02	2.3±0.07	2.3±0.09	2.0±0.10 ^b
Mean±SE	1.4±0.10 ^a	1.4±0.13 ^a	1.6±0.04 ^{ab}	1.9±0.10 ^b	1.9±0.08 ^b	2.2±0.07 ^c	2.1±0.27 ^c	

Means±SE bearing different superscripts row-wise and column wise differ significantly ($P<0.05$)

Table 4 Effect of storage and packaging on sensory attributes of shelf-stable ready-to-eat spiced buffalo meat at ambient temperature (30±3 °C)

Packaging type	Storage period (weeks)							Mean±SE
	0	1	2	3	4	5	6	7
Appearance								
Aerobic	7.1±0.11	7.0±0.10	7.1±0.05	6.9±0.10	6.8±0.10	6.6±0.14	7.0±0.05	6.8±0.11
Vacuum	7.1±0.11	7.1±0.12	7.1±0.05	7.0±0.08	6.8±0.10	6.8±0.11	6.9±0.07	6.8±0.10
Mean±SE	7.1±0.08 ^b	7.1±0.08 ^b	7.1±0.03 ^b	7.0±0.07 ^a	6.8±0.07 ^a	6.7±0.09 ^a	6.9±0.04 ^a	6.79±0.07 ^a
Flavour								
Aerobic	6.8±0.10	7.0±0.11	6.8±0.10	6.8±0.09	6.8±0.12	6.5±0.13	6.9±0.07	6.7±0.13
Vacuum	6.8±0.10	7.0±0.11	6.8±0.10	6.8±0.09	6.8±0.13	6.7±0.11	6.8±0.10	6.9±0.10
Mean±SE	6.8±0.07	7.0±0.08	6.8±0.07	6.8±0.06	6.8±0.09	6.6±0.08	6.8±0.06	6.8±0.08
Juiciness								
Aerobic	6.7±0.11	6.8±0.14	6.8±0.10	6.7±0.10	6.8±0.09	6.7±0.13	6.6±0.11	6.7±0.13
Vacuum	6.7±0.11	6.9±0.12	6.8±0.10	6.6±0.15	6.8±0.10	6.7±0.11	6.8±0.10	6.8±0.12
Mean±SE	6.7±0.07	6.8±0.09	6.8±0.07	6.7±0.09	6.8±0.06	6.7±0.08	6.7±0.07	6.7±0.09
Texture								
Aerobic	6.8±0.11	7.0±0.10	6.8±0.09	6.7±0.10	6.7±0.10	6.6±0.11	6.7±0.10	6.8±0.17
Vacuum	6.8±0.11	7.2±0.10	6.9±0.08	6.8±0.10	6.7±0.10	6.7±0.10	6.7±0.10	6.8±0.13
Mean±SE	6.8±0.08 ^{ab}	7.1±0.07 ^b	6.8±0.07 ^{ab}	6.7±0.07 ^a	6.7±0.07 ^a	6.6±0.07 ^a	6.7±0.07 ^a	6.8±0.09 ^a
Saltiness								
Aerobic	6.9±0.07	6.8±0.14	7.1±0.08	6.8±0.14	6.8±0.11	6.4±0.13	7.0±0.12	6.7±0.1
Vacuum	6.9±0.07	6.9±0.14	7.0±0.05	6.8±0.13	6.9±0.10	6.5±0.13	7.1±0.11	6.9±0.13
Mean±SE	6.9±0.05	6.8±0.10	7.0±0.05	6.8±0.09	6.8±0.08	6.5±0.09	7.0±0.08	6.8±0.10
Sourness								
Aerobic	6.9±0.08	7.0±0.11	7.1±0.11	6.8±0.14	6.8±0.12	6.6±0.13	7.0±0.12	6.9±0.14
Vacuum	6.9±0.08	7.1±0.08	7.1±0.11	6.8±0.13	6.8±0.11	6.6±0.13	7.0±0.13	6.8±0.14
Mean±SE	6.9±0.05	7.0±0.07	7.1±0.08	6.8±0.09	6.8±0.08	6.6±0.09	7.0±0.09	6.8±0.10
Overall palatability								
Aerobic	6.9±0.07	6.9±0.07	6.8±0.0	6.7±0.10	6.8±0.09	6.5±0.13	6.8±0.09	6.8±0.09
Vacuum	6.9±0.07	7.00±0.08	6.8±0.10	6.8±0.10	6.8±0.09	6.5±0.11	6.7±0.10	6.7±0.10
Mean±SE	6.9±0.05 ^b	6.9±0.05 ^b	6.8±0.06 ^b	6.8±0.07 ^b	6.8±0.06 ^b	6.5±0.09 ^b	6.8±0.07 ^b	6.8±0.07 ^a

Means±SE bearing different superscripts row-wise and column wise differ significantly ($P<0.05$) $n=21$

decrease in total haempigments during entire period of storage may be explained on the basis of breakdown of haempigments in complexes (Ledward et al. 1980). This breakdown may be caused by glycerol or lipid oxidation products (Ledward 1981). Reyes-Cano et al. (1995) suggested that the decrease in haematin concentration of cooked meat haemprotein is possible due to modification of the 5th and 6th coordination positions of iron.

Free fatty acids (FFA) The FFA increased significantly up to 2 weeks (Table 1) but thereafter values remained fairly stable with no significant change up to 7 weeks of storage. The free fatty acid per cent was not significantly affected by the type of packaging. Lipolysis is not solely dependent on oxygen but several other factors also, which remained similar in both the cases. The free fatty acids significantly increased during storage (Table 2) showing that some kind of lipolysis occurred during the storage period. However, free fatty acids did not increase above 2.7. This value is higher than the maximum acceptable level of 1.8% prescribed by Pearson (1968). However, Bell and Garout (1994) found higher FFA in unspoiled samples than the samples at the onset of spoilage. They concluded that FFA value was not a reliable quality indicator of vacuum packaged beef. In this case also, there was no perception of spoilage.

Soluble hydroxyproline Soluble hydroxyproline did not show any change up to 6 weeks of storage. However, a significant decrease was recorded at 7 weeks. The aerobic packaged samples had significantly ($P<.05$) higher values than the vacuum packaged product. Packaging had a significant effect on the solubility of hydroxyproline (Table 1). The aerobically packaged SRBM product in PET jars had higher soluble collagen per cent than the vacuum packaged SRBM product in multilayered nylon barrier pouches. It has been reported that oxygen enhances breakdown of collagen to soluble hydroxyproline although oxygen is not essential for breakdown (Webster et al. 1982).

The soluble hydroxyproline content in the SRBM product remained unchanged up to 6 weeks storage. This may occur due to two reasons. Firstly already enough of hydroxyproline was solubilised by 0.1% acetic acid (Verzar 1964) due to pressure cooking (Macfarlane and McKenzie 1976) and as frying as heating causes increased solubilisation of collagen (Laakhonen et al. 1970; Marjorie and Bernadine 1975). Secondly, sugars (specially glucose) has been reported to be responsible for inhibition of formation of soluble hydroxyproline by way of forming additional cross linkages of collagen molecules (Webster et al. 1986). Thus, honey could also contribute to above results to some extent. Significant decrease in soluble hydroxyproline at 7 weeks may be due to increased cross linking of collagen after certain period.

Protein solubility Storage had a highly significant ($P<0.01$) effect on protein solubility since significant decrease in protein solubility was observed at 1, 4, 5 and 7 weeks of storage (Table 1). The protein solubility in 3% SDS and 1% β -mercaptoethanol were significantly affected by the storage period and initial value of 92.6% was reduced to 79.4% at the end of 7 weeks. It suggests that some insolubilisation reactions, hydrolysis and denaturation might have been taking place during this storage period (Garcia et al. 1997). Similar, decrease in protein solubility upon storage has been reported in many intermediate moisture meats (Obanu et al. 1975; Madovi 1980; Webster et al. 1986; Okonkwo et al. 1992b; Reyes-Cano et al. 1995). However, there was remarkably less degree of decrease here suggesting that crosslinking reactions might be minimal in this type of product.

Proximate composition

Moisture The percent mean $\pm$ SE of moisture values of aerobic and vacuum packaged product on zero day was 51.4 ± 0.31 and which after 7 weeks had significantly got reduced to 49.2 ± 0.25 and $50.40\pm0.20\%$ for aerobic and vacuum packaged product respectively.

Packaging had a highly significant effect on moisture content ($P<0.01$). The product packaged under vacuum had significantly higher moisture content than the aerobically packaged in PET jars. Storage also brought about significant changes in moisture content. There was a significant ($P<0.01$) decrease in moisture content at 3 weeks as compared to 0 day. Though moisture showed a decline trend thereafter also but the difference was not found to be statistically significant.

The moisture content of the SRBM product was affected by packaging. So moisture was significantly less in the SRBM packaged in PET jars. This loss of moisture might have occurred through the lid of the jar. Similar decrease in moisture content of buffalo meat nuggets in permeable packs was recorded by Sahoo and Anjaneyulu (1997).

Protein: The percent mean $\pm$ SE of protein value of aerobic and vacuum packaged product on zero day was 28.6 ± 0.23 and which after 7 weeks had got increased to 29.5 ± 0.21 and $29.3\pm0.25\%$ for aerobic and vacuum packaged product respectively. **Ash:** The percent mean $\pm$ SE of Ash value of aerobic and vacuum packaged product on zero day was 3.85 ± 0.02 and which after 7 weeks were 3.9 ± 0.02 and $3.9\pm0.02\%$ for aerobic and vacuum packaged product respectively. Protein and ash content of SRBM were not significantly affected either due to storage or packaging during the period of 7 weeks. **Fat:** The percent mean $\pm$ SE of fat values of aerobic and vacuum packaged product on zero day were 14.7 ± 0.30 and 14.7 ± 0.30 respectively, which after 7 weeks had got significantly increased to

15.5±0.17 and 15.2±0.23% respectively. Fat content was not significantly affected by packaging but storage had a significant effect and thus the fat content at 7 weeks of storage was significantly higher than the fat content on 0 day. Packaging did not cause any significant effect on other constituents like fat, protein and ash. This may be because moisture loss was only about 1% and this effect got distributed to all other components eliminating the chances of significant difference in any single parameter. Further, protein and ash were neither significantly affected by packaging nor storage.

Shear force values Shear force values of SRBM product were neither affected significantly by storage nor packaging (Table 2). Texture of the product as evaluated by the Warner-Bratzler shear press did not show any significant effect either due to packaging or storage (Table 4). This is also reflected by the sensory evaluation which shows almost similar values except at first week. This may be due to the fact that pressure cooking had gelatinized the collagen so no further softening of collagen could occur during storage. Similar trend in shear force values of gizzard pickle was reported by Sachdev et al. (1994).

Sensory quality The sensory scores of vacuum and aerobically packaged shelf stable ready-to-eat buffalo meat (SRBM) product are presented in Table 4. The packaging had no significant ($P>0.05$) effect on sensory attributes, whereas storage had significant ($P<0.05$) effect only on appearance, texture and overall palatability of SRBM product. There was no interaction effect of packaging and storage on sensory attributes.

The sensory scores of appearance (Table 4) were not significantly affected up to 2 weeks of storage and there was a marginal, yet significant decline at 3 weeks which remained stable up to 7 weeks without any significant change. Flavour, saltiness, sourness and juiciness scores of the product were not significantly affected during the entire period of storage. Texture scores were significantly ($P<0.05$) lower at 3 weeks but thereafter these were not significantly affected during the entire period of storage. The scores for the overall palatability of the product were not significantly ($P>0.05$) affected up to 6 weeks although a marginal yet significant decline was observed at 7 weeks of storage.

The sensory quality of the product (Table 4) remained stable during the 7 weeks storage at ambient temperature. The packaging type did not cause any significant effect on any of the sensory attributes. The appearance scores ranged from very good to excellent on 0 day and remained stable up to 2 weeks. From third week onwards, sensory rating remained between good and very good till the end of storage. This may be due to the slight darkening of colour

due to spices, oil, condiments etc. which masked the cured meat colour to a certain extent. Such colour changes are not uncommon in pickled meats during storage. Similar findings in chicken pickle were reported by Puttarajappa et al. (1996).

Flavour of the SRBM product was evaluated between moderately desirable and very desirable throughout the storage. Juiciness rating also ranged from moderately juicy to very juicy throughout the storage. Pal and Agnihotri (1994) also had similar findings with goat meat pickle stored at ambient temperature for 2 months. The texture of the product was judged between moderately desirable and very desirable throughout the 7 weeks storage period, suggesting that there was no tenderisation of the product. This sensory rating correlated well with the collagen solubility and shear force values to a large extent. The saltiness and sourness scores reflect salt content and level of acidity of the product. The two may adversely affect the acceptability of the pickled meats above or below optimum level. The sourness was evaluated between moderately desirable and very desirable, suggesting no further development of acidity during the storage period.

The overall acceptability of the SRBM was judged between good and very good during 7 weeks storage period. It suggests that high quality shelf stable spiced pickle type buffalo meat product can be prepared with the application of hurdle technology which could retain high sensory acceptability at least up to 7 weeks storage at 30±3 °C.

Microbiological quality

The results of microbiology of shelf stable ready-to-eat spiced buffalo meat aerobically packaged in PET jars and vacuum packaged in laminates at ambient temperature (30±3 °C) are presented in Table 3. Packaging had a significant ($P<0.05$) effect on all the three types of microbes i.e. total plate count, yeast and mould count and anaerobic plate count in the product except *Staphylococcus aureus* which was not detected up to the 7 weeks of storage period. Storage had a highly significant ($P<0.01$) effect on all the three types of microbes detected during storage, whereas a highly significant ($P<0.01$) effect of interaction of packaging and storage was observed only on anaerobic plate counts. The microbes were detected only after first week of storage as no growth was detected on 0 day.

Total plate count ranged from log 2.1 to log 3.5 during the entire storage period of 7 weeks. Yeast and mold count increased from log 1.8 at 1 week to log 2.7 on 7 weeks of storage. Similarly, anaerobic plate count showed an increase from log 1.4 to log 2.2 at 6 weeks and then significantly decline at 7 weeks to log 2.1.

The aerobically packaged SRBM had significantly higher total plate count (log 2.8), yeast and mold count (log 2.5) and significantly lower anaerobic plate count (log 1.7) as against the corresponding values of log 2.6, 2.2 and 2.0 in the vacuum packaged SRBM.

The microbiological quality of the SRBM product remained good and fairly safe in both types of packaging materials. *Staphylococcus aureus* could not be detected at any storage intervals indicating that the hurdles employed were sufficient to check the organism in SRBM (Table 3)

Total plate count showed significant effect of packaging as aerobic packaging showed higher count than the anaerobic (Table 3). However, the difference was not much and the count in both types of packaging was below log 4.0. However, these counts were less than the count reported for similar type of products stored at ambient temperature such as gizzard pickle (Sachdev et al. 1994), goat meat pickle (Pal and Agnihotri 1994) and chicken meat pickle (Puttarajappa et al. 1996). This could be possible only because of to a balanced application of hurdles.

Yeast and Mould count were also significantly increased in aerobically packaged SRBM product in PET jars than the vacuum packaged. The reason is obvious as yeast and moulds are aerobic microorganisms. However, the count remained less than log 3.0 during 7 weeks of storage.

Anaerobic plate count was significantly higher in a vacuum packaged product. The count significantly increased up to 6 weeks and declined at 7 weeks. This may be due to germination of sub lethally damaged spores but the vegetative cells are not able to survive for long in a highly competitive environment and thus count decreased (Leistner et al. 1981). Similar findings have been reported by Puttarajappa et al. (1996) in chicken pickle stored at 26–28 °C for 6 months.

Conclusion

The shelf stable ready-to-eat spiced buffalo meat product retained good sensory, microbiological and physico-chemical properties during 7 weeks of ambient temperature storage, both in vacuum packaged multilayered nylon pouches and aerobically packaged in PET jars. Thus, either packaging system can be utilized for storage. However, PET jar is a handy, reusable and easily affordable container which can be adopted even in rural areas in cottage industry.

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