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Full Length Research Paper

Use of hurdle techniques to maintain the quality of vacuum packed buffalo meat during ambient storage temperatures

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The shelf stable buffalo meat chunks processed with an infusion solution formulation containing humectants such as glycerol 6.0%, sodium chloride 6.0% and propylene glycerol 1.0% along with sodium nitrite 0.01% and Sorbic acid 0.2% were vacuum packaged in multilayered nylon barrier laminates and stored at 30±3°C for 6 weeks. During the storage period the water activity was significantly affected whereas the moisture content and the shear force value were not significantly affected. The pH, residual nitrite, total haempigments and protein solubility decreased while the thiobarbituric acid (TBA), free fatty acids (FFA) and soluble hydroxyproline increased. Overall mean TBA value for storage was 0.98 mg malonaldehyde/kg. At the end the microbial counts of the product were TPC log 4.18, *Staphylococcus aureus* log 3.50, yeasts and moulds log 1.89 and anaerobic counts log 2.61 and sensory rating was maintained between good to very good. Thus the shelf life of the meat chunks at temperature (30±3ºC) was six weeks.

Key words: Shelfstable, buffalo meat, vacuum package, ambient temperature storage, hydroxyproline, free fatty acids (FFA), thiobarbituric acid (TBA) value, Microbes.

INTRODUCTION

India produces 1.9 million metric tones of buffalo meat out of which 21% (0.399 million tones is exported (Anonymous, 2008) Meat is an important national resource and has good domestic and export market. Meat being perishable commodity needs preservation till it is transported and distributed to retailers and actual meat consumers. Refrigeration is a common method of meat preservation. However, refrigeration is an energy consuming and costly process which is not always practicable in many developing countries like India which is having enormous geographical area with tropical climate. Therefore it becomes imperative to find some cheaper alternative for preservation of meat. Hurdle technology can be effectively utilized to store and transport meat and meat products at ambient temperatures while maintaining good microbiological quality, ensuring proper safety with acceptable sensory and nutritional properties. A hurdle treated product can be made available conveniently at cheaper and affordable price. Hurdle technology, also referred to as combination preservation technique, uses the parameters which are hurdles for microbial growth and 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 of

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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.

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 defense personnel especially as combat ration with light weight. It has been possible to produce various types of shelf stable and intermediate moisture meat products with hurdle technology. Some of the popular meats produced using this processing technology all over the world as per Liestner (1985) are Raw ham, Fermented sausage (Europe), Mortadella (Italy), Bruhdauerwurst (Germany), Gelderse Rook worst (Netherlands), Charque (Brazil), Beef Jerky (North America), Pemmican (North America), Biltong (South Africa), Kundi (West Africa), Dendeng Giling (Indonesia) and Tsusousan (China). In view of the growing need for shelf stable meat and meat products and the economic importance of buffalo meat, it was proposed to undertake the study with the objective to evaluate the storage quality of vacuum packaged shelf stable buffalo meat chunks at ambient temperature. The purpose was to assess the feasibility for transportation of the product to long distances and its subsequent storage without refrigeration during marketing, besides the aim was that the shelf stable meat chunks could be processed and cooked by consumers as per their convenience and taste in to a variety of meat products.

MATERIALS AND METHODS

Buffalo meat 5.5 kg was cut to 2.5 × 1.25 × 1.25 cm size were desorbed in the infusion solution formulation containing humectants such as glycerol 6.0%, sodium chloride 6.0% and propylene glycerol 1.0% along with sodium nitrite 0.01% and Sorbic acid 0.2% and processed as reported earlier (Malik and Sharma, 2010). The chunks were packed under vacuum in nylon laminates and stored at 30±3°C. Samples were taken out for physico-chemical, microbiological and sensory analysis on 0, 1, 2, 3, 4, 5th and 6 weeks of storage period as per the standard procedures outlined earlier (Malik and Sharma, 2010).

The procedures of Soluble hydroxyproline, FFA and TBA residual nitrite, haempigments and anaerobic count is given as under. Fresh buffalo meat chunks of same size were packed and frozen stored and after thawing were cooked in the same way as treated samples and served as control samples for shear force value and sensory evaluation at each storage interval.

Free fatty acids

The method as described by Koniecko (1979) was followed with slight modifications. Exactly 10 g of meat sample was blended for 2 min with 60 ml of chloroform in the presence of about 10 g anhydrous sodium sulphate. Then it was filtered through Whatman No.1 filter paper. 20 ml of chloroform extract was dried in an oven to determine the fat weight. Another 20 ml extract was taken into a 150 ml conical flask. About 4 to 5 drops of 0.2% phenolphthalein indicator were added to the chloroform extract, which was titrated against 0.1N alcoholic potassium hydroxide to get the pink colour end point. The quantity of potassium hydroxide consumed during titration was recorded. FFA as percent of oleic acid was calculated as follows:

$$\text{FFA \% (\% of oleic acid)} = \frac{0.1 \times \text{ml 0.1N alcoholic KOH} \times 0.282}{\text{Weight of fat (g)}} \times 100$$

Soluble hydroxyproline

For preparation of soluble fraction of hydroxyproline, procedure of Okonkwo et al. (1992a) was used with slight modification. 4 g 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 and 40 ml of concentrated hydrochloric acid was added to it 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 through filter paper 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 0.01M 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 ml of 3 N sulphuric acid and 2 ml of 5% p-dimethlaminobenzaldehyde 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 percent soluble hydroxyproline was calculated as:

$$\text{Percent soluble hydroxyproline} = \frac{\text{Soluble hydroxyproline of supernatant fraction}}{\text{Soluble hydroxyproline of supernatant} + \text{Insoluble hydroxyproline of residue fraction}} \times 100$$

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

Residual 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°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 Tarladgis 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. 10 g 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.

Anaerobic plate count

58 g Anaerobic agar was suspended in 1 liter 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 ml 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.

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, 1960) for comparing the means to find the effects between treatments, storage periods and their interaction for various parameters in different experiments.

RESULTS

The mean values for various quality parameters of vacuum packaged shelf stable buffalo meat chunks (SBM) as affected by storage at ambient temperature are presented in Tables 1 to 4. The ANOVA indicated no significant (*P>0.05*) effect on moisture content, a significant effect (*P<0.05*) on water activity (a_w) and a highly significant (*P<0.01*) effect of ambient temperature storage on all other parameters (Table 1). There was no significant (*P>0.05*) change in a_w up to 4th week of storage. However, a significant decrease was observed at 5th week which did not change up to 6th week of storage. The mean pH values did not change significantly during 1st week of storage.

A significant (*P<0.01*) decrease in pH was observed at each storage interval up 5th week of storage and did not change thereafter till the end of storage period The residual nitrite content showed a decreasing trend during the entire period of storage. The decrease was significant on second week and on successive storage intervals up to 4th week. Thereafter, a significant decrease was observed only on 6th week of storage. Haempigments decreased significantly (*P<0.01*) at each storage interval during the entire period of storage. The values ranged from 387.55 to 187.89 ppm. The mean value for entire storage period was 301.92 ppm.

Table 1. Effect of storage on physico-chemical characteristics of vacuum packaged shelf-stable buffalo meat chunks at ambient temperature (30±3°C).

Parameter	n	Storage period (weeks)							
		0	1	2	3	4	5	6	Overall mean±SE
Water activity (a_w)	3	0.913^{b}±0.0007	0.912^{b}±0.0006	0.912^{b}±0.0004	0.912^{b} ±0.0004	0.911^{b}±0.0004	0.910^{a}±0.0006	0.910^{a} ±0.0006	0.911±0.0003
Moisture (%)	6	52.57±0.18	51.54±0.33	51.93±0.22	51.51±0.35	51.79±0.22	51.82±0.18	51.81±0.07	51.85±0.10
pH	6	5.53^{e}±0.02	5.54^{e}±0.04	5.41^{d}±0.02	5.29^{c}±0.03	5.22^{b}±0.01	5.03^{a}±0.02	5.03^{a}±0.02	5.29±0.03
Nitrite (ppm)	6	20.43^{f} ±0.85	19.52^{f}±0.76	16.07^{e}±1.07	13.87^{d}±0.52	9.32^{c}±0.29	8.53^{abc}±0.18	7.19^{a}±0.19	13.56±0.81
TBA (mg malonald ehyde/value kg meat)	6	0.36^{a}±0.02	0.46^{b}±0.03	0.68^{c}±0.02	0.77^{d}±0.06	0.85^{d}±0.01	1.81^{e}±0.04	1.93^{f}±0.01	0.98±0.09
Total haempigments (ppm)	6	387.55^{f} ±3.04	387.94^{f} ±5.68	347.70^{e} ±4.33	312.70^{d}±1.67	281.30^{c}±2.34	208.32^{b}±2.07	187.89^{a}±2.87	301.92±11.74
Free fatty acids (%)	6	0.61^{a} ±0.04	0.68^{a}±0.07	0.70^{a}±0.01	1.46^{b}±0.20	1.64^{b}±0.11	2.04^{c}±0.02	2.40^{d}±0.04	1.36±0.12
Soluble hydroxyproline (%)	6	9.04^{a}±0.04	9.25^{a} ±0.05	9.84^{b}±0.14	9.82^{b}±0.11	9.92^{b}±0.20	9.93^{b}±0.06	15.25^{c}±0.17	10.44±0.31
Protein solubility (%)	6	82.62^{a}±0.29	81.77^{b}±0.18	80.86^{c}±0.09	79.47^{d}±0.07	77.98^{e}±0.18	77.70^{e}±0.12	69.36^{f}±0.28	78.54±0.65
Protein (%)	6	32.42±0.	-----	-----	-----	-----	-----	32.79 ±0.13	32.60±0.11
Fat (%)	6	8.39 ±0.13	-----	-----	-----	-----	-----	8.49±0.06	8.44±0.07
Ash (%)	6	5.26±0.002	------	------	------	------	------	5.27±0.003	5.27±0.002

Means±SE bearing different superscripts in each row differ significantly (P<0.05).

Table 2. Effect of storage on shear force values of vacuum packaged shelf-stable buffalo meat chunks at ambient temperature (30±3°C).

Sample	Storage period (weeks)							Treatment
	0	1	2	3	4	5	6	Mean±SE
Control	3.43±0.16	3.55±0.19	3.51±0.18	3.79±0.15	3.87±0.11	3.7±0.175	3.86±0.10	3.68^{a}±0.06
Shelf-stable chunks	4.19±0.18	4.17±0.18	4.35±0.15	4.38±0.12	4.18±0.10	4.22±0.11	4.15±0.12	4.23^{b}±0.05
Weekly mean±SE	3.81±0.13	3.86±0.14	3.93±0.13	4.08±0.11	4.03±0.08	3.99±0.11	4.01±0.08	

n=24, Means±SE bearing different superscripts row wise and column wise differ significantly (P<0.05).

TBA value, free fatty acids and soluble hydroxyproline protein solubility

The TBA value of SBM ranged from 0.36 to 1.93 mg malonaldehyde/kg with a mean value of 0.98 during the 6 weeks of storage. The increase in TBA values was found to be statistically significant at each storage interval except at 3rd and 4th weeks of storage. In general, FFA depicted an increasing trend during the entire period of storage. However, there was no significant (*P>0.05*) increase up to 2nd week of storage. At 3rd

Table 3. Effect of storage on microbial growth (log_{10}cfu/g) of vacuum packaged shelf-stable buffalo meat chunks at ambient temperature (30±3°).

Storage Period (Weeks)	Total plate count	*Staphylococcus aureus* count	Yeast and mold count	Anaerobic plate count
0	ND	ND	ND	ND
1	2.45±0.05^{a}	ND	ND	ND
2	2.71±0.04^{b}	1.48±0.08^{a}	1.36±0.02^{a}	2.12±0.06^{a}
3	2.79±0.05^{b}	1.72±0.06^{a}	1.73±0.05^{b}	2.26±0.04^{a}
4	2.93±0.03^{b}	1.85±0.04^{a}	1.82±0.11^{b}	2.45±0.08^{b}
5	3.72±0.16^{c}	2.40±0.28^{b}	2.29±0.10^{c}	2.98±0.08^{c}
6	4.18±0.08^{d}	3.50±0.06^{c}	2.28±0.16^{c}	3.23±0.06^{d}
Mean ±SE	2.92±0.12	2.19±0.20	1.89±0.1	2.61±0.12

Mean±SE bearing different superscripts column wise differ significantly (P<0.05).

week, there was significant (*P<0.01*) increase in values which did not differ significantly up to 4th week of storage. A significant increase was observed during subsequent storage intervals. The soluble hydroxyproline content increased with each storage interval. But statistically, the values from 0 day to 1st week were same and after registering a significant increase (*P<0.01*) at 2nd week did not change significantly up to 5th week of storage until a significant increase was again recorded at 6th week of storage. The protein solubility of meat chunks ranged from 82.62 to 69.36% with a mean value of 78.54% for the entire storage period. Storage had a highly significant (*P<0.01*) effect on protein solubility and the values decreased significantly at each successive storage interval except being comparable at 4 and 5th weeks of storage.

Proximate composition, shear force value and sensory attributes

The storage had no significant (P>0.050 effect on moisture, protein, fat and ash content of vacuum packaged shelf stable buffalo meat chunks during the entire storage period. A highly significant (*P<0.01*) difference in shear force values of control and treated meat chunks was observed, the values being higher for shelf stable chunks. However, storage had no significant (*P>0.05*) effect on shear force value of SBM (Table 2). The results of sensory attributes of stored vacuum packaged shelf stable buffalo meat chunks are presented in Table 4. The ANOVA indicated that there was no significant effect on any of the sensory attributes of meat chunks due to hurdle treatment. However, storage had a highly significant (*P<0.01*) effect on appearance, juiciness and overall palatability and a significant (*P<0.05*) effect on texture of the product. Score values for flavour and saltiness of the product were very slightly affected due to storage.

Appearance

The scores for appearance, in general, remained statistically same from 0 day to 3rd week and from 4 to 6th weeks of storage were comparable and significantly lower than 0 day.

Flavour, saltiness, juiciness and texture

The sensory scores for flavour and saltiness were not significantly different (*P>0.05*) from control and were not significantly affected due to storage whereas juiciness and texture scores did not decline significantly up to 5th week of storage.

Overall palatability

Overall palatability of shelf stable meat chunks ranged between 6.81 and 6.24 during storage. The palatability scores remained unaffected up to 3rd week followed by a significant decrease at 4th week as compared to first week and then did not change significantly up to 6th week.

Microbiological quality

The effect of ambient temperature storage on the

Table 4. Effect of storage on sensory attributes of vacuum packaged shelf-stable buffalo meat chunks at ambient temperature (30±3°C).

	Storage period (weeks)							
Samples	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**Treatment mean ±SE**
				Appearance				
Control	6.86±0.10	6.81±0.10	6.86±0.08	6.62±0.14	6.48±0.13	6.57±0.11	6.52±0.13	6.67±0.04
Shelf-stable chunks	7.05±0.08	6.71±0.14	6.81±0.11	6.76±0.12	6.43±0.13	6.48±0.11	6.19±0.15	6.63±0.05
Mean±SE	6.95^{d}±0.07	6.76^{cd}±0.09	6.83^{d}±0.07	6.69^{bcd}±0.09	6.45^{ab}±0.07	6.52^{abc}±0.08	6.36^{a}±0.10	
				Flavour				
Control	6.67±0.16	6.81±0.11	6.52±0.13	6.57±0.13	6.48±0.15	6.52±0.13	6.48±0.11	6.58±0.05
Shelf-stable chunks	6.67±0.16	6.38±0.16	6.29±0.14	6.48±0.13	6.67±0.17	6.48±0.13	6.38±0.13	6.48±0.05
Mean±SE	6.67±0.11	6.60±0.10	6.40±0.10	6.52±0.09	6.57±0.11	6.50±0.09	6.43±0.08	
				Juiciness				
Control	6.67±0.17	6.76±0.15	6.48±0.15	6.67±0.14	6.57±0.15	6.57±0.15	6.23±0.17	6.56±0.06
Shelf-stable chunks	6.76±0.14	6.67±0.14	6.43±0.13	6.48±0.11	6.71±0.14	6.43±0.13	6.14±0.16	6.52±0.05
Mean±SE	6.71^{b}±0.10	6.71^{b}±0.10	6.45^{ab}±0.10	6.57^{b}±0.09	6.64^{b}±0.10	6.50^{ab}±0.10	6.19^{a}±0.11	
				Texture				
Control	6.81±0.11	6.71±0.12	6.57±0.13	6.62±0.13	6.67±0.14	6.57±0.15	6.19±0.18	6.59±0.05
Shelf-stable chunks	6.67±0.14	6.48±0.15	6.43±0.11	6.52±0.13	6.67±0.16	6.52±0.13	6.43±0.13	6.53±0.05
Mean±SE	6.74^{b}±0.09	6.59^{b}±0.09	6.50^{ab}±0.08	6.57^{b}±0.09	6.67^{b}±0.10	6.55^{ab}±0.10	6.31^{a}±0.11	
				Saltiness				
Control	7.00±0.12	6.81±0.11	6.57±0.11	6.81±0.15	6.76±0.13	6.81±0.13	6.43±0.16	6.74±0.05
Shelf-stable chunks	6.81±0.15	6.67±0.13	6.57±0.11	6.71±0.14	6.86±0.13	6.81±0.15	6.62±0.14	6.72±0.05
Mean±SE	6.90±0.11	6.74±0.08	6.57±0.08	6.76±0.10	6.81±0.08	6.81±0.10	6.52± 0.11	
				Overall palatability				
Control	6.86±0.14	6.90±0.10	6.62±0.13	6.62±0.13	6.43±0.11	6.52±0.15	6.24±0.14	6.60±0.05
Shelf-stable chunks	6.76±0.14	6.57±0.15	6.67±0.17	6.48±0.11	6.48±0.13	6.43±0.13	6.24±0.15	6.52±0.05
Mean±SE	6.81^{c}±0.10	6.74^{c}±0.09	6.64^{bc}±0.11	6.55^{abc}±0.08	6.45^{ab}±0.08	6.48^{ab}±0.10	6.24^{a}±0.10	

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

microbial counts (Table 3) was highly significant (*P<0.01*). On 0 day there was no detectable growth of any of the microbial types that is, total plate counts, *Staphylococcus aureus*, Yeast and mould and Anaerobic plate counts. The growth of total plate count organisms was detected on 1st week whereas the other three types of organisms studied could not be detected even up to 1st week of storage. Thereafter, total plate counts increased significantly touching log 4.18 at 6th weeks of storage. *S. aureus* counts did not increase significantly (*P>0.05*) from 2nd to 4th week of storage. Microbial counts increased significantly at 5th and 6th week of storage. Yeast and mould counts were detected at 2nd week and increased significantly with subsequent storage intervals. However, the counts could reach only log 2.28 at the end of 6th week of storage. Anaerobic plate counts were detected at 2nd week and increased significantly with subsequent storage intervals. However, the counts could reach only log 3.23 at the end of 6th week of storage.

DISCUSSION

Physico-chemical characteristics

Water activity

The water activity (Table 1) did not change up to 4th week as there was no significant loss of moisture. However, after 4th week, very little, yet significant decrease in water activity might be due to some water having been taken in some chemical reactions. A little decrease in moisture content might have also contributed to this change. Some decrease in water activity in intermediate moisture beef packed in cryovac bags and stored at room temperature (30°C) for 12 weeks was also reported by Okonkwo et al. (1992b). In fact, decrease in water activity may lead to increased shelf stability of the product; hence it may be called a desirable change.

pH

The significant decrease in pH values during storage could be due to glycerol oxidative products (Obanu and Ledward, 1986). It is believed that cross linking reactions, by removing amino groups from the system, cause a decrease in pH (Okonkwo et al., 1992b) but hydrolysis of the collagen molecules releases amino groups which will increase the pH (Webster et al., 1982a,b); the resultant pH being governed by the relative rates of these two reactions (Okonkwo et al., 1992b). Similar decrease in pH of intermediate moisture meats from beef during storage was observed by Webster et al. (1986) and Okonkwo et al. (1992b).

Nitrite

There residual nitrite content (Table1) was 20.43 ppm on 0 day which got further depleted to 7.19 ppm at the end of 6 weeks of storage period. A significant depletion at each week was recorded. The depletion of nitrite content during storage has been explained by Sadler and Swan (1997) on the basis of the 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 been observed by Cassens et al. (1979) in cooked meats and Sadler and Swan (1997) in chilled stored salted minced beef.

TBA value

The lipid oxidation which produces peroxides and malonaldehyde is not a problem in intermediate moisture meat products (Ledward, 1985). Increase in TBA values of glycerol desorbed and dried pork during 42 days storage at 37°C were observed by Jo and Kwon (1972) and in buffalo meat patties stored under refrigeration by Kandeepan et al. (2009) but that did not damage the organoleptic quality of these meat products. Vacuum packaging was reported to give low TBA values at 37°C in intermediate pork than the same stored at 4 or 21°C (Sahoo, 1972). However, TBA values in this study were lower than reported for similar type of hurdle treated goat meat by Karthikeyan (1997) and did not cross the threshold limit of 1 to 2 mg/kg for rancidity (Watts, 1962). The lower TBA values may be due to heating at 80°C (Verma et al., 1984), antioxidant effect of nitrite and vacuum packaging (Ockerman and Kuo, 1982). Another reason that has been put forward for lower TBA values in such products is the antioxidant effect of premelanoidins formed during desorption process (Obanu, 1980).

Total haempigments

The significant decrease in total haempigments (Table 1) during the entire period of storage was similar to the findings of Prabhakar et al. (1992) and Okonkwo et al. (1992b). However, the breakdown in their case was less. Different authors have given an explanation for the decrease in concentration of haematin complex, such as

the breaking of haematin into pyrrole fragments (Lawrie, 1979), non-haematin iron (Obanu et al., 1976) and certain complexes (Ledward et al., 1980), the formation of insoluble complexes with denatured proteins as in the case of cooked meat haemproteins (Obanu and Ledward, 1975) or conversion of myoglobin to bile pigments (Lawrie, 1974), while Ledward (1982) says that decrease may be due to glycerol oxidation product of muscle lipid oxidation products. Reyes-Cano et al. (1995) suggested that the decrease in haematin concentration is possible due to the modification of the fifth and sixth coordination position of iron in haematin complex cooked meat haemproteins.

Free fatty acids

The FFA increased during the storage (Table 1) showing that some kind of lipolysis occurred during this period. The FFA value did not increase above 2.40%. Even though 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 onset of spoilage. They concluded that FFA value was not a reliable quality indicator of vacuum packaged beef. Further, Modi et al. (1998) recorded increase in initial FFA level of 1.83 to 5.14% in shelf stable spiced chicken bits stored at 20±2°C under vacuum for 60 days.

Soluble hydroxyproline

The breakdown of collagen resulted in increase of soluble hydroxyproline content during storage which was evident on first week but remained fairly under check from 2nd to 5th week. Similar breakdown in collagen has been reported by Obanu et al. (1975a), Webster et al. (1986) and Okonkwo et al. (1992b) in intermediate moisture beef stored at 30° and 38°C for 12 weeks. However, the values of soluble hydroxyproline in this study were far less than that observed by these workers suggesting that collagen breakdown did not occur to a great extent in this product as was also clear from the shear force values.

Protein solubility

The protein solubility in 3% SDS and 1% β-mercaptoethanol decreased from initial value of 82.62 to 69.36% at the end of 6 weeks of storage (Table 1). It suggests that same insolublisation reactions like hydrolysis and denaturation might have been occurring during this period (Garcia et al., 1997). The insolubilisation of proteins in intermediate moisture meats (IMM) has been observed by Madovi (1980), Webster et al. (1986) and Okonkwo et al. (1992b) in intermediate moisture beef stored at 30 to 38°C in cryovac bags. Reyes-Cano et al. (1995) studied protein insolublisation in Cecina (Mexican beef IMM) during storage at 4, 25 and 35°C under vacuum. However, the insolubility of proteins was comparatively less in this study at 6th week as against the reports of only 60% soluble protein at 6th week by above authors.

Proximate composition

The proximate composition did not show significant change during storage. Due to vacuum packaging of the product in multilayered nylon pouches, the loss of moisture was not significant. Similar findings were reported by Sahoo (1995) in vacuum packaged buffalo meat nuggets stored at 4±1°C for 1 month.

Shear force value

The shear force value was significantly higher in SBM than frozen buffalo meat. Similar finding has been reported by Okonkwo et al. (1992a). This may be the effect of desorption, pasteurization and drying of SBM. The shear force value of the product did not change significantly in this study which may be an advantage. During storage, texture is thought to be changed by collagen breakdown on one hand leading to soft texture and cross linking reactions on other hand leading to toughness and a balance between the two determines the textural (Obanu et al., 1975b) characteristics of the product.

Microbiological quality

The ambient temperature (30±3°C) storage of vacuum packaged SBM recorded significant increase in the microbial counts during a period of 6 weeks (Table 3). Similar increase in microbial counts was reported by Kandeepan et al. (2009) in refrigerated buffalo meat patties. On 0 day none of the microbes could be detected. *S. aureus*, Yeast and moulds and Anaerobic plate counts could not be detected even on completion of 1st week of storage because the cells might have suffered heat injury and spores might have been sub lethally

injured due to heating effect coupled with Perigo factor during heating with nitrite (Perigo and Roberts, 1968; Leistner, 1985).

Total plate counts

Total plate counts showed a significant increase by 2nd week and the counts remained fairly stable up to 4th week. This may be due to combined hurdle effect. Thereafter, the counts increased significantly up to the end of storage. However, the counts remained well below the spoilage limit of log 7.0. Vacuum packaged meats may favour the growth of Lactobacilli and *Brochothrix. thermosphecta* (Seideman et al., 1976; Seideman and Durland, 1983). Since *B. thermosphecta* is sensitive to nitrite, the growth could essentially be that of lactic acid bacteria (Jay, 1986). Preservative effect of lysozyme and EDTA in refrigerated stored patties was reported recently by Cannarsi et al. (2008). Meat with higher count of lactic acid bacteria for example, vacuum packaged luncheon meat having dominant flora as lactobacilli and count of 10^8/g were considered acceptable (Egan et al., 1980). *S. aureus* count were first detected at 2nd week and remained fairly stable up to 4th week. This is because firstly they were heat injured and once they brought metabolic adjustment the count remained static due to unfavourable conditions of hurdles, but at 5th week the counts increased significantly touching a count of log 3.50 at 6th week. The inhibition of *S. aureus* at water activity of 0.91 in anaerobic conditions has already been reported by Scott (1957) and Leistner et al. (1981). Further, counts required to produce harmful effects have been reported to be 10^7/g (Troller, 1976). *S. aureus* in our experiment did not reach such a high level.

Nitrite has been reported to effectively control growth of *S. aureus* in both aerobic and anaerobic environment below the level associated with enterotoxin production (Barber and Deibel, 1972). Yeast and mould counts remained very low during the entire period of storage and did not grow more than log 2-2.8. Their ability to grow in acidic environment and at low water activity might have allowed them to grow to some extent (Johanstan and Thompkin, 1984). Such low counts may not pose any threat to human health. Anaerobic plate counts were low. Although in vacuum packaged meats, *Lactobacilli* and *Clostridia* could pose some problem. Since water activity was low in this product (0.913 to 0.910) and *Clostridium botulinum* and *C. perfringens* are not expected to grow below water activity 0.94 (Jackobsen, 1985) and increase during subsequent storage intervals might be due to growth of Lactobacilli only. In Indian conditions, meat is consumed after proper cooking. Therefore, counts at such low levels during 6 weeks of storage seem to be safe.

Sensory attributes

The shelf stable buffalo meat chunks when cooked were as accepted as frozen stored buffalo meat. During entire storage period, there was no significant difference (Table 4) between the two which reflects the good acceptance of the SBM. The scores for appearance were significantly decreased at 4th week as compared to 2nd week but later on scores remained unchanged from 4th to 6th week. Similar result of decreasing sensory attributes of buffalo meat patties during refrigeration storage were reported by Kandeepan et al. (2009). The decrease in appearance scores may be due to colour lightening processes such as haemprotein degradation and depletion of nitrite. However, appearance scores still remained between good and very good. The flavour of the product did not show any difference between the frozen stored meat and SBM. It could be possible as increase in TBA value was below the threshold value of detectable rancidity. Flavour scores remained between moderately desirable to very desirable.

The scores for saltiness were almost similar as salt concentration did not change during the period of storage. Juiciness and texture scores of SBM also remained similar to frozen meat control as did the other sensory parameters. Although the scores for both the sensory attributes decreased significantly during storage, still they remained between moderate to very desirable. In fact, juiciness and texture rating was not much affected because rehydration of SBM for 2 h increased the moisture per cent by nearly 15% which improved juiciness as well as texture. The overall palatability scores for SBM and frozen stored meat did not show significant variation during the storage like other sensory attributes. Though significant decrease in overall palatability scores of SBM was noticed between storage period of first week and 4, 5 and 6th week yet sensory ratings remained between moderately palatable to very palatable throughout the storage period.

Conclusion

The meat chunks maintained fair physico-chemical characteristics, safe microbiological load and good to very good sensory acceptability at the end of 6 weeks of storage at 30±3°C.Thus it can be concluded that hurdle treatment can be used satisfactorily to produce shelf

stable meat chunks for further use into meat products as per consumer convenience.

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