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Effect of Water Activity on the Physico-chemical, Microbiological and Sensory Qualities of Buffalo Meat Sausage Stored under Ambient Temperature†

[1]S. Sureshkumar, [2]V. Venkataramanujam, [3]K. Dushyanthan and [4]A. Kalaikannan
[1]Central Sheep and Wool Research Institute,
Avikanagar, Rajasthan-304501, India
[2,3] Department of Meat Science and Technology,
Madras Veterinary College, Chennai-600 007, India
[4]Department of Animal Husbandry Statistics and Computer Applications,
Madras Veterinary College, Chennai-600 007, India

Abstract: The study was undertaken to find the effect of water activity on buffalo sausage quality and storage stability at ambient temperature. Water activity (a_w), temperature and pH have been identified as the three primary factors controlling microbial growth. The water activity of the buffalo meat sausage (treated) was adjusted to 0.88 by addition of humectants viz., salt, sugar, ISP, HVP, sodium lactate and subsequent heat treatment while the a_w of the untreated sausage was 0.932. Tyrosine value showed a significant increase throughout the storage periods. There was a marked but not significant decrease in the TBARS number of the treated samples. On 5th day, the TBARS value of the treated sausages exceeded the threshold limit of 2 mg kg^{-1} resulting in the spoilage. The treatment had a significant inhibitory effect on the TVC, Staphylococcal count and Streptococcal count and anaerobic count. Whereas in case of coliform and yeast mould count it had no significant effect. On the third day of storage the flavour and texture, juiciness and over acceptability scores were well within the acceptable range. Whereas in the case of untreated samples there was a slime formation and off odour development on the 3rd day of storage. Although the sensory score (over all acceptability) of the treated samples were scored less ($p>0.05$), the product was acceptable upto 3rd day storage whereas the untreated samples spoiled after 1st day of storage.

Key words: Water activity, shelf life, buffalo meat sausage, ambient temperature

Introduction

Most of the meat produced in India is being sold in the form of fresh meat, only less than 3% is being sold as processed meat. Because of the rapid urbanization and hurried way of life, there is a spurt in the consumption of processed foods and a shift in the consumption pattern. Buffalo meat being comparatively cheaper, will have additional advantages over other meats. Sausage is a popular and highly relished meat product world over. The present low level processing of meat is mainly because of the difficulties in marketing by maintaining the cold chain of the products. To overcome this problem a technology has to be developed to store the meat products under ambient temperature without recourse to refrigeration. Hurdle technology (also called combined methods, combined processes, combination preservation, combination techniques or barrier technology) advocates the

Corresponding Author: S. Sureshkumar, Central Sheep and Wool Research Institute,
Avikanagar, Rajasthan-304501, India Tel: +9144-25381506277 Fax: +9144-25362787

deliberate combination of existing and novel preservation techniques in order to establish a series of preservative factors (hurdles) that present should not be able to overcome by any microorganisms. These hurdles may be temperature, water activity (a_w), pH, preservatives and so on (Leistner and Gorris, 1995). Water activity (a_w), temperature and pH have been identified as the three primary factors controlling microbial growth in many foods. However, most investigators have not directly considered aw. Instead, they have modeled the effect of sodium chloride concentration, the humectants used to modify a_w (McMeekin *et al*., 1987). Even though there are several studies on Intermediate Moisture Foods (IMFs), relatively scant information is available on the Hurdle Technology Foods (HTFs). Recently, the storage life of tandoori chicken with a_w 0.86 and pH 5.3 was extended to 2 days (Manish Kumar and Berwal, 1996) by the application of hurdle technology. The present study was undertaken to investigate the effect of this important hurdle (water activity-a_w) on the physico-chemical, microbiological and sensory qualities of the buffalo meat sausage stored under ambient temperatures.

Materials and Methods

Hot boned meat and fat from buffalo carcasses were collected from local corporation slaughter house, Chennai and were cleaned with potable water. They were chilled for 24 h at 4±1°C and then frozen to -18±1°C. On the day of experiment the frozen meat was thawed before being minced through a 4.5 mm plate of a meat mincer (OMAS, Model No. 169789, Electrolux food service, Italy).

Preparation of Buffalo Meat Sausage

The minced meat and fat were chopped in bowl chopper (Model MTK 662, Maschinen fabrik., Germany). The emulsion was prepared by adding minced meat and other ingredients of recipe (Table 1). The specified quantities of salt, sodium tri polyphosphate and sodium nitrite were premixed and added to the meat mix. The refined vegetable oil (sunflower) was cooled to 4°C before adding to the mixture. During chopping the temperature of the emulsion was maintained at 10-12°C by the addition of slushed ice. The emulsion was then stuffed into 18-20 mm diameter sheep casing using a sausage stuffer (Model MWF 591, Maschinen fabric., Germany), linked and cooked in hot air oven at about 100°C for 60 min so as to reach the internal core temperature of 72°C. The cooked sausages were vacuum packed in multilayer (Polyester/Polyethylene pouches of size 6"× 9") pouches to prevent post processing contamination. The vacuum packaged sausages were served warm to taste panelists on day zero and subjected to storage studies.

Trial for Optimum Level of Humectants

Different levels of humectants viz., sugar (1 and 2%); Isolated Soya Protein (ISP) (Supro 500-E1, Protein Technologies International, St. Louis, 3 and 5%), whole egg powder (SKM Foods, India, 1, 2 and 3%), hydrolysed vegetable protein (HVP) (Protchem India, 1 and 2 %) and sodium lactate (Lactochem, Chennai, 2 and 3%) were tried. Incorporation of sugar beyond 1% resulted in an undesirable sweet taste to the product. The product with ISP at 5% level resulted in the soya flavour masking the meat flavour, the optimum being at 3%. The whole egg powder at 1% was found to be optimum with good sensory qualities. Preliminary trials revealed that the HVP at 2% render a salty taste and 1% was found to be optimum. Sodium lactate at 3% level resulted in a sour taste and 2% level was found to be optimum. The levels of ingredients including preservatives used in the control and treated formulations are presented in Table 1.

Measurement of Water Activity (a_w)

The a_w of the sausage sample was determined by a slight modification of the procedure recommended by Lerici *et al.* (1983). An approximately 50 g sample was packed in a 60 mL glass

Table 1: Formulation of sausage

Ingredient levels (w/w)	Untreated sausage	Treated sausage
Buffalo meat	80.0	80.0
Buffalo fat	5.0	5.0
Oil (Refined sunflower oil)	15.0	15.0
Sodium tri ploy phosphate[a]	0.3	0.3
Sodium chloride[a]	2.0	2.0
Egg powder[a]	1.0	1.0
Added water[a]	7.0	7.0
Humectants[a]		
Sugar	-	1.0
Isolated soy protein	-	3.0
Hydrolysed vegetable protein	-	1.0
Preservatives[a]		
Sodium nitrite	0.012	0.012
Spice mix	1.5	1.5
Green condiments	4.0	4.0
Sodium lactate	-	2.0[a]

Added on meat and fat basis

tube and the mouth was corked air tight. Then it was immersed in a cooling chamber which had precooled ethanol at -30°C to -35°C in a glass beaker and kept in a deep freezer (Vertical type, Vest Frost, Denmark). A resistance thermometer probe was introduced inside the cork to monitor the temperature. The rate of decrease in temperature was observed which was constant up to a specific point and thereafter, the rate markedly decreased. The point, where the rate altered was indicated by the start of ice crystal formation, was taken as the freezing point of the sample. The freezing point was converted to water activity value using the formula developed by Lerici *et al*. (1983).

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

where ln is the natural logarithm, T is freezing point temperature in Kelvin.

Effect of Water Activity (a_w) on the Product Quality and Storage Stability

The buffalo meat sausage with water activity (a_w) 0.88 was achieved by the addition of humectants and by subsequent over cooking. The sausage prepared without humectants served as control. The data obtained from six trials were analysed. The products were stored at ambient temperature (35±2°C) and analysed for the following quality attributes; pH, tyrosine values (Strange *et al*., 1977), thiobarbituric acid reactive substances (TBARS) (Tarladgis *et al*., 1976), moisture (APHA, 1976) and water activity. Total viable count, coliform count, staphylococcal count, streptococcal count, yeast and mould count and anaerobic count were enumerated (APHA, 1984). Sensory attributes were evaluated by semi-trained panelists using the 9 point hedonic scale. The results obtained from the above experiments were statistically analysed (Snedecor and Cochran, 1989).

Results and Discussion

Physico-chemical Qualities

Table 2 shows that the pH of the untreated samples were significantly ($p<0.01$) lower (6.36±0.03) than the treated samples (6.44±0.02). This may be due to the addition of HVP, ISP and sodium lactate, which was appreciated in the 0 day samples. This was in agreement with Karthikeyan *et al*. (2000). The addition of humectants like sugar, HVP, ISP and sodium lactate significantly ($p<0.01$) reduced the product water activity (0.884±0.001). Upon storage at ambient temperatures, the a_w showed a significant ($p<0.01$) linear increase which was in agreement with Prabhakar and Ramamurthi (1990). The moisture content of the treated samples were significantly ($p<0.01$) lower (47.50±0.45) than the untreated samples (60.85±0.56). The moisture content of all the samples were significantly ($p<0.01$) increased from 52.17±1.96 on day 0 to 57.28±1.98 on day 5.

Table 2: Effect of a_w of physico-chemical properties of buffalo meat sausage

Parameters	Storage periods (days) 0	1	3	5	Overall treatment mean
pH					
Untreated sausage	6.40±0.05	6.48±0.04	6.43±0.03	6.14±0.02	6.36±0.03^{x}
Treated sausage	6.44±0.02	6.50±0.02	6.52±0.04	6.31±0.03	6.44±0.02^{y}
Overall day's mean	6.42±0.03^{b}	6.49±0.02^{b}	6.47±0.03^{b}	6.23±0.03^{a}	
Water activity (a_w)					
Untreated sausage	0.932±0.001	0.934±0.001	0.937±0.001	0.943±0.001	0.936±0.001^{y}
Treated sausage	0.880±0.001	0.882±0.000	0.884±0.001	0.890±0.001	0.884±0.001^{x}
Overall day's mean	0.906±0.008^{a}	0.908±0.008^{b}	0.910±0.008^{c}	0.916±0.008^{d}	
Moisture (%)					
Untreated sausage	58.56±0.73	59.81±0.90	61.31±0.94	63.72±0.74	60.85±0.56^{y}
Treated sausage	45.78±0.27	46.07±0.26	47.31±0.36	50.85±0.43	47.50±0.45^{x}
Overall day's mean	52.17±1.96^{a}	52.94±2.12^{a}	54.31±2.16^{b}	57.28±1.98^{c}	
Tyrosine value (mg kg^{-1})					
Untreated sausage	9.25±0.73	9.46±0.60	11.04±0.91	32.96±2.18	15.68±2.17^{x}
Treated sausage	12.17±0.62	13.75±0.65	25.08±1.70	40.08±1.26	22.77±2.39^{y}
Overall day's mean	10.71±0.63^{a}	11.60±0.77^{a}	18.06±2.31^{b}	36.52±1.61^{c}	
TBARS Number (mg malonaldehyde kg^{-1})					
Untreated sausage	0.74±0.04	0.75±0.02	2.04±0.22	2.76±0.15	1.57±0.19
Treated sausage	0.77±0.08	0.74±0.03	1.36±0.06	2.98±0.14	1.46±0.19
Overall day's mean	0.75±0.04^{a}	0.75±0.02^{a}	1.70±0.15^{b}	2.87±0.10^{c}	

Means bearing same superscripts do not differ significantly

Similar changes in the a_w and moisture were also observed in intermediate moisture deep fried mackerel (Che Man *et al*., 1995) and Sudanese dry meat (Gailani and Fung, 1989). The tyrosine value of the treated samples were significantly ($p<0.01$) higher (22.77±2.39) over the untreated (15.68±2.17) samples (Table 2). This may be due to the denaturation and subsequent proteolysis of ISP during heating which was accentuated by addition of lactic acid (Syed *et al*., 1995). Throughout the storage periods the TV showed a significant ($p<0.01$) increase in all the samples which may be due to the protein breakdown by chemical and microbial actions. Karthikeyan *et al*. (2000) also observed higher protein degradation during storage in caprine keema at ambient temperatures. There was a marked but not significant decrease in the TBARS number of the treated samples conversely Karthikeyan *et al*. (2000) found a significantly higher TBARS number of hurdle treated caprine keema. This may be due to variation in production presentation, comminuted non-emulsion product, which may facilitate the fat oxidation. Upon storage, all the samples showed a significant increase in the TBARS number whereas between 0 and 1 day of storage the differences were not significant. In the treated samples the TBARS number were slightly decreased from 0 and 1st day of storage. The decrease in the TBARS number upon storage was in agreement with Kalaikannan *et al*., (2005). The TBARS values of sausage beyond the threshold limit of 2 mg kg^{-1} (Watts, 1962) on 3rd and 5th day for untreated and treated sausage, respectively, which may be due to increase in oxidation upon storage.

Microbiological Qualities

The TVC of the treated samples were significantly ($p<0.01$) lower than the untreated samples (Table 3). Upon storage, the untreated samples exceeded (log 7.65±0.40) the spoilage limit of log 7 (Hytiaineu *et al*., 1975) on 5th day of storage while in the treated samples, the TVC was well within the acceptable limit. Irrespective of the treatment, all the samples showed a steady increase in the TVC during storage periods. Sahoo and Anjaneyulu (1997) also observed a gradual increase in TVC of the buffalo meat nuggets during the storage periods. There was no marked difference between the treatments on the coliforms count, whereas during the storage periods, it

Table 3: Effect of a_w on the microbiological qualities of buffalo meat sausage

Parameters	Storage periods (days) 0	1	3	5	Overall Treatment mean
TVC (log cfu g^{-1})					
Untreated sausage	2.49±0.13	3.22±0.14	4.70±0.13	8.95±0.14	4.84±0.53^{y}
Treated sausage	1.69±0.11	2.06±0.08	3.35±0.10	6.35±0.11	3.36±0.38^{x}
Overall day's mean	2.09±0.14^{a}	2.64±0.19^{b}	4.02±0.22^{c}	7.65±0.40^{d}	
Coliform counts (log cfu g^{-1})					
Untreated sausage	0.33±0.21	0.67±0.21	0.50±0.22	2.08±0.05	0.89±0.17
Treated sausage	0.00±0.00	0.33±0.21	1.13±0.09	2.14±0.05	0.90±0.18
Overall day's mean	0.17±0.11^{a}	0.50±0.15^{ab}	0.82±0.15^{b}	2.11±0.04^{c}	
Staphylococcal count (log cfu g^{-1})					
Untreated sausage	2.26±0.05	2.43±0.05	3.46±0.07	5.58±0.16	3.43±0.28^{y}
Treated sausage	1.28±0.10	1.34±0.08	1.51±0.05	3.53±0.10	1.92±0.20^{x}
Overall day's mean	1.77±0.16^{a}	1.89±0.17^{a}	2.48±0.30^{b}	4.56±0.32^{c}	
Streptococcal count (log cfu g^{-1})					
Untreated sausage	1.23±0.11	1.96±0.05	2.82±0.09	5.15±0.12	2.79±0.31^{y}
Treated sausage	1.13±0.09	1.36±0.15	1.73±0.13	3.48±0.14	1.92±0.20^{x}
Overall day's mean	1.18±0.07^{a}	1.66±0.12^{b}	2.27±0.18^{c}	4.31±0.27^{d}	
Yeast and mould count (log cfu g^{-1})					
Untreated sausage	1.58±0.11	1.75±0.07	1.93±0.03	2.25±0.09	1.88±0.06
Treated sausage	1.36±0.09	1.61±0.03	1.95±0.04	2.18±0.13	1.78±0.08
Overall day's mean	1.47±0.08^{a}	1.68±0.04^{b}	1.94±0.02^{c}	2.21±0.08^{d}	
Anaerobic counts (log cfu g^{-1})					
Untreated sausage	2.54±0.14	3.48±0.20	4.89±0.13	8.66±0.20	4.89±0.49^{y}
Treated sausage	1.98±0.04	2.87±0.07	3.65±0.09	5.87±0.11	3.59±0.30^{x}
Overall day's mean	2.26±0.11^{a}	3.18±0.13^{b}	4.27±0.20^{c}	7.26±0.43^{d}	

Means bearing same superscripts do not differ significantly

it showed a significant ($p<0.01$) increase from log 0.17±0.11 on day 0 to 2.11±0.04 on day 5. The treated samples had a significant ($p<0.01$) lower *staphylococcal* and *streptococcal* counts. Althought the soya protein tends to increase the microbial load (Chowdhury *et al*., 1994), the reduced a_w lowered the microbial growth (Karthikeyan *et al*., 2000) in treated samples. The staphylococcal and streptococcal counts showed a significant ($p<0.01$) increase during the storage periods. A logarithmic increase in TVC and gradual increase in staphylococcal count of the untreated sausage might be due to the conducive a_w (Leistner *et al*., 1981). The was no significant difference between the treated and untreated samples in the yeast and mould count. The yeast and mould counts were significantly ($p<0.01$) increased over the storage periods irrespective of all treatments. The anaerobic counts of treated sausage samples were significantly ($p<0.01$) lower than the untreated sausage samples. In the present study, both the samples were vacuum packed which might have facilitated the growth of anaerobic organisms. Fu *et al*. (1992) also reported that the anaerobes and facultative anaerobes are the most important spoilage bacteria in vacuum packaged meats. In case of treated samples, the reduction in the a_w might be attributed to the destruction of vegetative cells (Leistner and Gorris, 1995). Hence the overall reduced level might be noticed in the anaerobic counts of the treated samples.

Sensory Attributes

Both the treated and untreated samples were spoiled on 5th day, hence only the appearance scores were recorded (Table 4). Upto 3rd day of storage, the appearance scores for both the samples were highly acceptable whereas it was moderately acceptable on 5th day. This may be due to the slime formation and discoloration due to microbial activity. In the treated samples, the flavor scores were well with in the acceptable range upto 3rd day of storage whereas in case of untreated samples it was only upto 1st day of storage. A sudden decrease in the flavor scores for untreated samples on the 3rd day and subsequent spoilage might be due to increased liberation of fatty acids and oxidation of fat (Santamaria *et al*., 1992). Due to the development of off flavor and slime formation in the untreated samples on the 3rd day of storage the texture, juiciness and overall acceptability scores were not recorded. The treated sausage samples were scored lesser than the untreated samples for

Table 4: Effect of a_w on the sensory qualities* of buffalo meat sausage

Parameters	Storage periods (days) 0	1	3	5	Overall treatment mean
Appearance					
Untreated sausage	8.84±0.07	8.56±0.11	7.06±0.10	3.72±0.16	7.04±0.43
Treated sausage	8.67±0.15	8.39±0.06	7.56±0.14	4.28±0.16	7.22±0.37
Overall day's mean	8.75±0.08^c	8.47±0.06^c	7.31±0.11^b	4.00±0.14^a	
Flavour					
Untreated sausage	8.06±0.16	6.89±0.22	2.28±0.16	Spoiled	5.74±0.61
Treated sausage	8.06±0.10	7.06±0.13	5.78±0.11	1.78±0.14	5.67±0.50
Overall day's mean	8.06±0.09^d	6.97±0.13^c	4.03±0.54^b	1.78±0.14^a	
Texture					
Untreated sausage	8.06±0.10	7.28±0.26	Spoiled	Spoiled	7.67±0.18^y
Treated sausage	7.61±0.10	6.95±0.10	6.11±0.16	Spoiled	6.89±0.16^x
Overall day's mean	7.83±0.10^c	7.11±0.14^b	6.11±0.16^a	Spoiled	
Juiciness					
Untreated sausage	7.28±0.10	6.50±0.08	Spoiled	Spoiled	6.89±0.13
Treated sausage	6.94±0.16	6.50±0.14	5.94±0.16	Spoiled	6.46±0.13
Overall day's mean	7.11±0.10^c	6.50±0.08^b	5.94±0.16^a	Spoiled	
Overall acceptability					
Untreated sausage	8.39±0.10	6.94±0.16	Spoiled	Spoiled	7.67±0.24
Treated sausage	8.28±0.22	7.61±0.26	6.28±0.18	Spoiled	7.39±0.24
Overall day's mean	8.33±0.12^c	7.28±0.18^b	6.28±0.18^a	Spoiled	

* 9 point hedonic scale. Means bearing same superscripts do not differ significantly

texture, This might be due to the adition of humectants which imparts the rubberiness to the product. The juiciness score of the treated samples were lesser than the untreated samples which may be due to the reduction in the moisture content. Even though there was a decrease in the juiciness score, the difference were not significant. There was no significant difference between the treatments in the overall acceptability scores because the overall acceptability scores of the untreated samples were not recorded after one day of storage. Incase of treated samples the overall acceptability scores were 7.39±0.24. Upon storage, all the sensory scores showed a significant ($p<0.01$) decrease. Based on the sensory quality studies the treated samples were acceptable upto 3rd of storage whereas in case of untreated samples it was only upto 1 day of storage.

Conclusions

The water activity was adjusted by employing the humectants viz., salt, sugar, ISP, HVP, sodium lactate and subsequent heat treatment. The product was acceptable upto 3rd day storage whereas the untreated samples spoiled after 1st day of storage. Further extension of the shelf life of the sausage can be explored by the use of antioxidants in the formulation in order to prevent the rapid lipid oxidation.

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