

## USEFULNESS OF PHYSICO-CHEMICAL TESTS TO MONITOR CHANGES IN BUFFALO MEAT DURING REFRIGERATED STORAGE

M.K. Agnihotri<sup>1</sup>, H.B. Joshi<sup>2</sup> and R.C. Keshri<sup>2</sup>

### ABSTRACT

Meat samples from 8 to 10 year old Murrah buffaloes were subjected to refrigerated storage ( $5 \pm 1^\circ\text{C}$ ) for a period of 0, 3, 6 and 9 days and changes in moisture, pH, titrable acidity (pHt) and extract release volume (ERV) were monitored. Samples treated with antibiotics (oxytetracycline and mycostatin) to prevent microbial growth served as controls. In treated as well as untreated samples, moisture and ERV significantly increased and pH decreased until the third day of storage and later did not change significantly. pHt did not change significantly until the ninth day although meat spoilage could be detected earlier through standard plate counts and odour scores. Results indicate that changes in moisture, pH, pHt and ERV are not rapid enough to detect the incipient spoilage in buffalo meat during the refrigerated storage.

### INTRODUCTION

Undesirable physico-chemical changes occur in meat stored improperly, thus affecting its marketability and consumer acceptance. Changes in physico-chemical parameters like pH, titrable acidity (pHt) and extract release volume (ERV) during refrigerated storage of meat from various farm animals have been studied in depth for use as rapid tests for incipient spoilage (Jay 1968; Pearson 1968a, b; Shelef and Jay 1970; Strange et al., 1977; Jose et al., 1984). However, such information is not available on buffalo meat, thus warranting this investigation.

### MATERIALS AND METHODS

Meat samples from 8 to 10 year old Murrah buffaloes of either sex were collected from a local slaughterhouse. Changes in moisture, pH, pHt and ERV of buffalo meat during refrigerated

storage ( $5 \pm 1^\circ\text{C}$ ) were monitored on 0 (2.5 to 3.0 h post-slaughter), 3, 6 and 9 days in five separate trials. Samples treated with antibiotics to check microbial growth served as controls.

Thigh muscle (biceps femoris, semitendinosus and gastrocnemius) were excised from the carcasses within 2.5 to 3.0 h post-slaughter and brought to the laboratory in ice containers. The fatty layer, tendon and nerves were removed from the three muscles and they were cut into equal halves to form two lots, A and B. Into lot A, a solution containing oxytetracycline (100 ppm) and Mycostatin (60 ppm) was injected at the rate of 120 ml per kg muscle. The muscles were then cut into cubes of 5 to 6 cm size and divided into four sub-lots. Each sub-lot contained cubes of all the three muscles in equal proportions depending on their weight and size. Each was then dipped separately in the antibiotic solution for 30 min. at room temperature. After that, excess antibiotic solution was drained off and each sub-lot was finally wrapped in polyethylene bags. One sub-lot was retained for 0 day analysis of different parameters; the others were stored in a refrigerator ( $5 \pm 1^\circ\text{C}$ ). Muscle samples in lot B were similar to those of lot A in all aspects except that they were not treated with antibiotics.

For analysis, muscle samples were minced to get a uniform homogeneous material. Moisture content was estimated as per the AOAC (1975) procedure. ERV, pH and pHt were determined according to the procedures described by Strange et al. (1977). Changes in colour, odour, sliminess, and overall appearance were judged by a minimum of seven semi-trained panelists using a five-point scale. Sensitivity ratios, which indicate the extent of changes in values of each parameter as the meat passed from state of freshness to spoilage, were calculated. Data were analysed using standard statistical procedures (Snedecor and Cochran 1967).

<sup>1</sup> Central Institute for Research on Goats, Makhdoom, Farah, Mathura (U.P.) 281 122

<sup>2</sup> Division of Livestock Products Technology, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh

## RESULTS AND DISCUSSION

### Moisture

The initial moisture content in untreated meat samples ranged from 76.23 to 78.30 with a mean value of  $77.40 \pm 0.38$  percent and varied significantly between trials (Table 1). Higher moisture content in treated control (T1) was due to the dipping of the muscle cubes in the antibiotic solution. Irrespective of the treatment, moisture content was higher on the third day of storage. This could be due to attainment of ultimate pH resulting in an overall reduction of reactive groups available for water binding on the muscle proteins and thus making available a greater amount of free water (Forrest et al., 1975). During the later period of storage, muscle samples did not show appreciable change in moisture content.

### pH

The meat pH on 0 day was recorded as  $6.05 \pm 0.09$  (Table 1). Though the variation in meat pH between trials was statistically non-significant, the initial pH value of the fifth trial samples was lowest (pH 5.66) in comparison to the remaining four trials. Since the environmental temperature was  $41.0^\circ\text{C}$  when the meat samples were procured and processed, this might have favoured faster post-mortem glycolysis in muscles. Bate-Smith and Bendall (1949) and Marsh (1954) found that in beef longissimus dorsi muscle, pH reached below 5.5 at  $43.0^\circ\text{C}$  in less than 2 h. A significant decrease ( $P < 0.05$ ) in meat pH on the third day of storage in our study could be due to anaerobic glycolysis and lactate formation. The pH values showed an increasing but non-significant trend in both the treatments (T1 and T2) by the ninth day of storage. Such changes could be due to bacterial degradation of proteins and nonprotein nitrogenous substances in the untreated samples as well as enzymatic proteolysis in the treated samples. Increase in the volatile nitrogen content observed in meat juice from  $9.42 \pm 0.99$  mg on 0 day to  $16.57 \pm 0.13$  mg on the ninth day supported this contention (Agnihotri, 1988).

Sensitivity ratios, calculated on the basis of pH values at the onset of "slight strange odour" in untreated meat samples, indicated that change in pH value from state of freshness (pre-rigor state) to

incipient spoilage was only 0.08 unit. As "slight strange odour" was noticed at pH 5.8, it was concluded that the pH limit should not exceed this value. However, when the standard plate counts (SPC) in fresh and spoiling meat samples were also taken into consideration for calculating the ratio, the unit change in pH value went on to the negative side (Table 2) instead of showing increase during incipient spoilage. The changes in pH value in untreated samples during storage up to 9 days were not appreciable even in samples with higher bacterial counts (log 7.0 to 7.15/g meat) and almost similar pH values were recorded in samples with low microbial load. Further, there was no significant difference in pH values between the antibiotic treated and untreated samples throughout the storage period and pH values varied considerably at the time of onset of incipient spoilage (ninth day of storage) in different samples. Therefore, after achieving ultimate pH, further increase in pH can only be expected at an advanced stage of spoilage and measurement of pH alone cannot be considered as a reliable index of incipient spoilage of buffalo meat. Similar observations were made in beef (Wolf 1971; Strange et al., 1977).

### pHt

The pHt value of fresh buffalo meat samples (0 day) was  $4.48 \pm 0.05$  (Table 1). Irrespective of the treatment, values did not change significantly throughout the storage period. Furthermore, values varied significantly in different trials ( $P < 0.05$ ). The relationship of pHt to the increase in bacterial counts also was poor. The sensitivity ratio was very low. Therefore, the pHt value, similar to pH, appeared to be an ineffective monitor for detecting microbial spoilage in buffalo meat during refrigerated storage.

### ERV

The average ERV value on 0 day was  $29.1 \pm 4.8$  ml, but values as low as 19.0 ml were recorded in some of the meat samples (Table 1). Pearson (1968 a) also recorded abnormally low ERV values in fresh pre-rigor beef. ERV values significantly increased on the third day irrespective of the treatment. Samples treated with antibiotics showed steady increase in ERV reaching as high as  $47.2 \pm 4.6$  ml on the ninth day, whereas in untreated samples, the ERV values showed slight increase up to the sixth day followed by a gradual decline.

However, the difference between the treatments was not significant. The significant increase in ERV on the third day of storage could be due to a fall in muscle pH closer to the isoelectric point of principal muscle proteins (Shelef 1973) resulting in their decreased hydration capacity.

Irrespective of the treatment, ERV varied significantly between trials in both the treated and untreated samples ( $P < 0.05$ ) and increase in bacterial numbers did not result in a corresponding increase of ERV. Lower ERV values could be observed only when the meat was in an advanced state of spoilage. These results are in agreement with the observations of Daly et al. (1976) and Strange et al. (1977) in beef. Further, values obtained with fresh samples showed very wide variation as indicated by higher C.V.(%) values and low sensitivity ratio (Table 2). Therefore, under the conditions of the current study, ERV was also observed to be unreliable for detection of bacterial spoilage during refrigerated storage.

From the results of this study, it is concluded that changes in moisture, pH, pH<sub>t</sub> and ERV are not rapid enough to detect the incipient spoilage in buffalo meat during refrigerated storage. Appreciable changes in all these parameters occur at an advanced stage of spoilage which could otherwise be detected through sensory evaluation.

## ACKNOWLEDGEMENT

The senior author thanks the Indian Council of Agricultural Research, New Delhi, for providing financial assistance in the form of ICAR senior fellowship.

## REFERENCES

- Agnihotri, M.K. (1988). Studies on keeping quality and spoilage pattern of buffalo meat. Ph.D. Thesis IVRI, (Deemed University), Izatnagar.
- AOAC. (1975). Official Methods of Analysis. 12th Ed. Association of Official Analytical Chemists, Washington, DC.
- Bate-Smith, E.C. and Bendall, J.R. (1949). Rigor-mortis and adenosine triphosphate. *Journal of Physiology* 106: 177-185.
- Daly, M.C., Morrissey, P.A. and Buckley, D.J. (1976). Quality of raw minced beef. *Irish Journal of Agricultural Research* 15: 283-289.
- Forrest, J.C., Aberle, E.D., Hedrick, H.B., Judge M.D. and Merkel, R. A. (1975). *Principles of Meat Science*. WH Freeman, San Francisco.
- Jose, M.T., Padmanabha, Iyer R. and Prabhakaran, P. (1984). Influence of pH on the keeping quality of beef. *Kerala Journal of Veterinary Science* 15: 135-139.
- Marsh, B.B. (1954). Rigor mortis in beef. *Journal of the Science of Food and Agriculture* 5: 70-75.
- Pearson, D. (1968 a) Assessment of meat freshness in quality control employing chemical techniques: A review. *Journal of the Science of Food and Agriculture* 19: 357-363.
- Pearson, D. (1968 b) Application of chemical methods for assessment of beef quality. IV. Physico-chemical and miscellaneous methods. *Journal of the Science of Food and Agriculture* 19: 556-559.
- Shelef, L.A. (1973). Hydration of fresh meat undergoing microbial spoilage. Abstracts of the Annual Meeting of the American Society for Microbiology 73: 24.
- Shelef, L.A. and Jay, J.M. (1970). Use of titrimetric method to assess the bacterial spoilage of fresh beef. *Applied Microbiology* 19: 902-905.
- Snedecor, G.W. and Cochran, W.G. (1967). *Statistical Methods*. 6th Ed. Allied Pacific, Bombay.
- Strange, E.D., Benedict, R.C., Smith, J.L. and Swift, C.E. (1977). Evaluation of rapid tests for monitoring alterations in meat quality during storage. I. Intact meat. *Journal of Food Protection* 40: 843-847.
- Wolf, G. (1971). Quality of beef of Hungarian pied cattle. *Husipar* 20: 86-92.

Table 1. Mean values of moisture content, pH, pHt and ERV of buffalo meat in refrigerated storage

Parameter	Treatment	Days of storage				Overall treatment means
		0	3	6	9	
Moisture (%)	T1	78.55±0.42 <sup>a</sup>	79.53±0.44 <sup>a</sup>	79.17±0.42 <sup>a</sup>	79.30±0.40 <sup>a</sup>	79.14
	T2	77.39±0.38 <sup>a</sup>	77.50±0.30 <sup>a</sup>	76.83±0.50 <sup>a</sup>	77.12±0.40 <sup>a</sup>	77.20
pH	T1	6.05±0.10 <sup>a</sup>	5.72±0.03 <sup>a</sup>	5.70±0.03 <sup>a</sup>	5.75±0.03 <sup>a</sup>	5.81
	T2	6.05±0.10 <sup>a</sup>	5.68±0.05 <sup>a</sup>	5.71±0.02 <sup>a</sup>	5.80±0.02 <sup>a</sup>	5.81
pHt	T1	4.48±0.05	4.47±0.09	4.38±0.09	4.44±0.09	4.44
	T2	4.48±0.05	4.52±0.06	4.50±0.08	4.54±0.08	4.51
ERV	T1	29.10±4.82 <sup>a</sup>	44.10±3.56 <sup>a</sup>	46.10±0.58 <sup>a</sup>	47.20±0.46 <sup>a</sup>	41.60
	T2	29.10±4.82 <sup>a</sup>	43.40±2.92 <sup>a</sup>	44.30±1.43 <sup>a</sup>	42.70±1.30 <sup>a</sup>	39.87

T1 = antibiotic treated control ; T2 = untreated sample.

Means bearing common superscripts in a row (small letters) and column (capital letters) within the parameters do not differ significantly (P > 0.05)

Table 2. Comparative evaluation of the reliability of various tests used for detecting fresh meat spoilage

Test criterion	Tests	Values obtained with fresh samples(1)				Mean values at the time of incipient spoilage (2)	Change	Sensitivity ratio(2/1)
		Min.	Max.	Mean	CV(%)			
SPC	pH	5.66	6.20	6.05	3.30	5.84	-0.21	1.04
	ERV	19.00	47.00	29.10	33.10	41.00	11.90	1.41
	pHt	4.34	4.60	4.48	2.20	4.67	0.19	1.04
Odour score	pH	5.68	5.75	5.71 <sup>a</sup>	0.54	5.79 <sup>a</sup>	0.08	1.01
	ERV	40.00	48.50	44.30 <sup>b</sup>	6.45	42.64 <sup>a</sup>	-1.67	0.96
	pHt	4.38	4.79	4.49 <sup>b</sup>	3.37	4.55 <sup>a</sup>	0.06	1.01

a = Values presented are from samples which showed SPC 10g <sup>-3</sup> 7.00/g meat on ninth day of storage

b = Values obtained from samples giving 'no off odour' i.e. on sixth day

c = Values obtained from samples giving 'slightly strange odour'(appeared in four samples on ninth day of storage)

d = not corrected for autolysis (2-1)