



American Journal of **Food Technology**

ISSN 1557-4571



Academic
Journals Inc.

www.academicjournals.com

Effect of Extraction Conditions and Post Mortem Ageing Period on Yield of Salt Soluble Proteins from Buffalo (*Bubalus bubalis*) Lean Meat

¹R. Thomas, ¹Y.P. Gadekar, ¹G. Kandeepan, ²S.K. George and ³M. Kataria

¹Division of Livestock Products Technology,

²Division of Animal Nutrition,

³Division of Animal Biochemistry Indian Veterinary Research Institute,
Izatnagar, Bareilly, U.P., 243 122, India

Abstract: Buffalo lean meat was used to evaluate the effects of pH of the salt solution (5.0, 5.5, 6.0, 6.5 and 7.0), dilution volume (4, 8, 12, 16 and 20 X sample weight), blending time (30, 60, 90, 120 and 150 sec) and post mortem ageing period (0, 5, 10 and 15 days) on the yield of Salt Soluble Proteins (SSP). Maximum yield of SSP was obtained at pH 6.0. SSP yield increased with increase of dilution volume and blending time. Ageing buffalo lean meat for 5, 10 and 15 days postmortem reduced the yield of SSP. Thus, the extraction conditions and post mortem ageing period have significant effect on the yield of SSP from buffalo (*Bubalus bubalis*) lean meat.

Key words: Buffalo lean meat, salt soluble protein, dilution volume, blending time, ageing period

INTRODUCTION

Extractable Salt Soluble Protein (SSP) content is widely used as a better index of lean meat quality. The amount of SSP is the major factor affecting the quantity of fat, which may be emulsified during the processing of comminuted meat products. Solubilized muscle protein encapsulates insoluble lipid and other components to form a coherent matrix (Kotter and Fischer, 1975). The amount of meat protein that can function in a coherent matrix is determined by the amount of insoluble connective tissue and the amount of myofibrillar protein that can be solubilized. Protein extractability of salt solutions mainly depends on pH, ionic strength and type of salt solutions (Franks, 1993). In addition, protein extractability depends on extracting procedures that includes volume of extraction solution, duration of blending/homogenization, centrifugal force and time etc. Lan *et al.* (1993) investigated the optimum protein extracting procedure for beef and pork muscles. Similarly, Munasinghe and Sakai (2004) investigated the protein extractability of NaCl, KCl and LiCl in pork lean meat. However, to the best of our knowledge, no investigations have been done to establish effects of pH of salt solution, dilution volume, blending time and post mortem ageing period on extractability of SSP from buffalo lean meat. In this context, we investigated the SSP extractability under above-mentioned conditions, keep in line with the Knipe *et al.* (1985) extraction procedure.

MATERIALS AND METHODS

Sample Preparation

About 1 kg of meat (mainly *biceps femoris*, *quadriceps*, *semimembranosus* and *semitendinosus* muscles) from round portion of adult female buffalo carcass of good finish were obtained within 5 h

Corresponding Author: R. Thomas, Division of Livestock Products Technology, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P., 243 122, India

of slaughter. The meat was chilled at $4\pm1^{\circ}\text{C}$ for 20 h. The separable fat and connective tissue were removed and the meat was minced coarsely and then more finely by passing through 8 mm and 4 mm plates, respectively using a meat mincer (Seydelman, Model WD 114, Germany). Samples were packaged in LDPE bags using a Roschermatic packaging machine (Model A-NG 91173, Germany), then frozen ($-18\pm2^{\circ}\text{C}$) and held for subsequent analysis. Four muscle samples were cut and individually vacuum packaged in laminated pouches. They were aged at $4\pm1^{\circ}\text{C}$ for 5, 10 and 15 days. Similar meat samples from a single carcass were utilized for each trial of the experiment.

Estimation of Salt Soluble Protein

The SSP content was determined by a slight modification of the method of Knipe *et al.* (1985). Finely minced 20 g meat sample was blended with chilled 50 mL 0.6M NaCl in laboratory blender (Ultra Turrex T25) at high speed for 1 min. and then added 50 ml chilled 0.6 M NaCl and blended for 1 min. (except for finding out the effect of blending time, where specific time frames of 30, 60, 90, 120 and 150 sec were used). The homogenate was quantitatively transferred with two rinsings to 250 mL polycarbonate centrifuge bottles and the final volume was made 200 mL. The samples were stirred on a mechanical shaker for 2 min and centrifuged at 5°C and 5500 rpm for 15 min in MSE coolspin centrifuge. After centrifugation, the fat layer floating on the surface was gently moved to one side with a stainless steel spatula and 1 mL aliquots in duplicate were drawn from the clear salt solubilized protein solution. To each 1 mL solution, 5 mL Biuret reagent (Gornall *et al.*, 1949) was added. This mixture was stirred and allowed to stand for 15 min for optimum colour development. Optical density was determined with a thomospectronic photometer (Model Genesys 10 UV, USA) at 540 nm and converted by using bovine serum albumen standard curve to mg protein per mL solution. Yield of SSP was calculated as percent sample weight.

Experimental Design

Three trials each were conducted to evaluate the effects of different extraction conditions on yield of SSP from buffalo lean meat. Extraction conditions that were studied included pH of extraction solution. A solution of 0.6 M NaCl was adjusted to pH (5.0, 5.5, 6.0, 6.5 and 7.0) with 1 M NaOH solution before blending. Other variables were dilution volume (4, 8, 12, 16 and 20 X sample weight), blending time (30, 60, 90, 120 and 150 sec) and postmortem ageing period (0, 5, 10 and 15 days at $4\pm1^{\circ}\text{C}$).

Statistical Analysis

The data generated from the study were pooled and evaluated statistically (Snedecor and Cochran, 1994) at the institute's computer centre. The figures describe the mean values, standard error and the level of significance of the effect of pH, dilution volume, blending time and post mortem ageing period on the yield of SSP.

RESULTS AND DISCUSSION

Effect of pH on Extraction of SSP

SSP extraction was significantly higher ($p < 0.05$) at pH 6.0 (Fig. 1). Lan *et al.* (1993) found similar protein extractability for beef *longissimus dorsi* and *semimembranosus* muscles at pH 6.0. In contrast, Munasinghe and Sakai (2004) observed highest protein extractability for pork lean meat at pH 6.5. Toorop *et al.* (1997) found significant variation of protein extractability for various meats between pH 6.0 and 7.0. Richardson and Jones (1987) reported that the total amount of protein extracted from the homogenates of either post rigor white or dark turkey muscle in 0.5 to 1.0 M NaCl increased as pH increased from 5.0 to 7.0. Their results suggest that the greatest pH effect was in the pH range 5.0 to

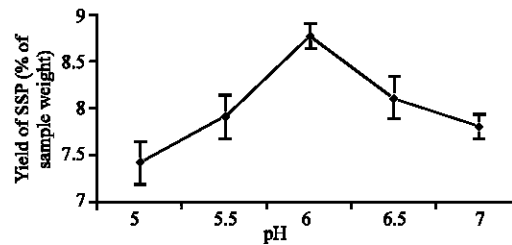


Fig. 1: Effect of pH of salt solution on extraction of SSP

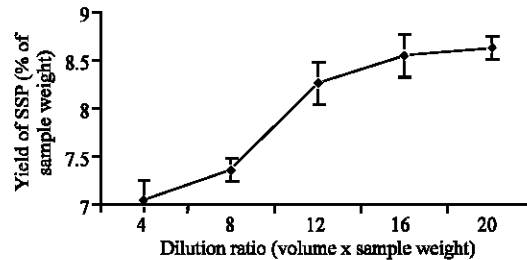


Fig. 2: Effect of dilution ratio on extraction of SSP

5.5 and above pH 5.75 increases in protein extractability were not observed. Samejima *et al.* (1992) reported that the solubility of rabbit skeletal muscle myofibrillar protein in 0.6 M NaCl buffer was always greater than that of pig cardiac myofibrils at pH>5.5 and maximal solubility was obtained at pH> 6.0, with little change up to pH 7.0. However, Kelleher and Hultin (1991) observed protein extraction from fish muscle increased with increasing pH between 6.0 and 8.5. Discrepancies between different studies may be related to different species and different protein samples prepared.

Effect of Dilution Ratio on Extraction of SSP

Results (Fig. 2) indicated that dilution ratio had a significant ($p<0.05$) effect on amount of muscle protein solubilized. Increases in volume from 4 to 20 X sample weight increased extraction of SSP from buffalo lean meat. It reveals that at higher dilution ratios, more muscle protein was solubilized. However, Lan *et al.* (1993) reported that at a dilution ratio 20 X sample weight, the yield of SSP for beef longissimus dorsi and semimembranosus muscles did not differ from that of 15 X sample weight dilution. Also, Regenstien and Rank Stamm (1979) observed that higher dilution ratios (1:20 to 1:40) had little or no effect on protein extraction.

Effect of Blending Time on Extraction of SSP

As blending time increased (Fig. 3) from 30 to 150 sec, the yield of SSP of buffalo lean meat increased ($p<0.05$). Similarly, Lan *et al.* (1993) observed an increased yield of SSP of beef up to 120 sec of blending. They also observed that the yield of SSP of pork increased up to 90 sec of blending time, but did not increase after 120 sec of blending. In contrast, Kelleher and Hultin (1991) observed that extraction of protein by NaCl from fish muscle decreased above 60 sec of blending time. The discrepancies between different studies might be due to difference in particle size. Buffalo lean meat has larger particle size than pork and fish muscles. Therefore, the particle size of pork and fish muscles might decreased rapidly with increased blending time, while the particle size of buffalo meat decreased linearly until 150 sec, but reduction rate was much lower. This may also be related to the higher amount of collagen cross linking in buffalo lean meat, as we used samples from spent/aged animals. The increased amount of collagen cross linking may have made the muscle fibers more difficult

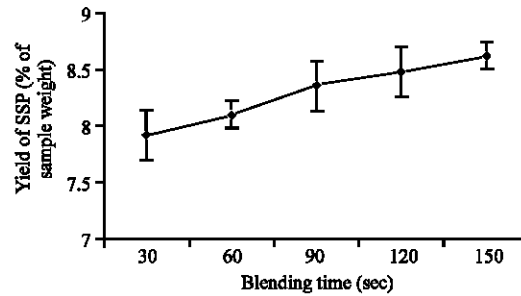


Fig. 3: Effect of blending time on extraction of SSP

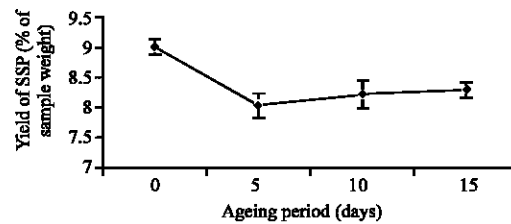


Fig. 4: Effect of postmortem ageing period on extraction of SSP

to break at lower blending time and as the blending time increased the degree of muscle cell or myofibril disruption increased, which would enhance protein extraction.

Effect of Postmortem Ageing Period on Extraction of SSP

Results (Fig. 4) indicated that yield of SSP decreased significantly ($p < 0.05$) with the elapse of ageing period at $4 \pm 1^\circ\text{C}$. Lan *et al.* (1993) demonstrated that SSP of beef *longissimus dorsi* and *semimembranosus* muscles decreased significantly during 14 day ageing period. However, they also reported a non-significant ($p > 0.05$) reduction of SSP in pork *longissimus dorsi* muscle. Li-Chan *et al.* (1985) reported that pre-rigor muscle proteins of beef muscle decreased in protein extractability with postmortem storage. Cheng and Parrish (1978) also reported that changes occurred in the SSP of myofibrils during postmortem storage (1 to 10 days) of beef *longissimus dorsi* muscle at 2°C . Their results suggested that the decrease in protein extractability might have been due to protein denaturation with postmortem storage. The ultimate pH of the muscle had an effect on protein properties. Those extracted from pre rigor muscle (high pH) had less denaturation and better functional properties. In contrast, Li-Chan *et al.* (1986) reported that extractable SSP content of chicken breast was higher at 7 days than at zero or 2 days postmortem. Also, Xiong and Brekke (1991) reported that post rigor breast myofibrils had higher protein extraction than pre rigor breast myofibrils, which indicated that muscle fibre type may have effects on protein extraction.

CONCLUSIONS

Maximum yield of SSP from buffalo lean meat was obtained at pH 6.0. SSP yield increased with increase of dilution volume and blending time. Ageing buffalo lean meat for 5, 10 and 15 days post mortem reduced the yield of SSP. Thus, the extraction conditions and post mortem ageing period have significant effect on the yield of SSP from buffalo lean meat.

REFERENCES

- Cheng, C.S. and F.C. Parrish, 1978. Molecular changes in the salt-soluble myofibrillar proteins of bovine muscle. *J. Food Sci.*, 43: 461-463.
- Franks, F., 1993. Solution properties of protein. In: Franks, F. (Ed.), *Protein Biotechnology*. New Jersey, Humana Press, pp: 133-190.
- Gornall, A.G., C.J. Bardwill and M.M. David, 1949. Determination of serum protein by means of the biuret reaction. *J. Biol. Chem.*, 177: 751-766.
- Kelleher, S.D. and H.O. Hultin, 1991. Lithium chloride as a preferred extractant of fish muscle proteins. *J. Food Sci.*, 56: 315-317.
- Knipe, C.L., D.G. Olson and R.E. Rust, 1985. Effect of selected inorganic phosphates, phosphate levels and reduced sodium chloride levels on protein solubility, stability and pH of meat emulsion. *J. Food Sci.*, 50: 1010-1013.
- Kotter, L. and A. Fischer, 1975. The influence of phosphates or polyphosphates on the stability of foams and emulsions in meat technology. *Die Fleischwirtschaft*, Nr., 3: 365.
- Lan, Y.H., J. Novakofski, T.R. Carr and F.K. McKeith, 1993. Assay and storage conditions affect yield of salt soluble protein from muscle. *J. Food Sci.*, 58: 963-966.
- Li-Chan, E., S. Nakai and D.F. Wood, 1985. Relationship between functional (fat binding, emulsifying) and physicochemical properties of muscle proteins. Effects of heating, freezing, pH and spices. *J. Food Sci.*, 50: 1034-1040.
- Li-Chan, L. Kwan and S. Nakai, 1986. Physicochemical and functional properties of salt-extractable proteins from chicken breast muscle deboned after different post-mortem holding times. *Canadian Inst. Food Sci. Technol. J.*, 19: 241-248.
- Munasinghe, D.M.S and T. Sakai, 2004. Sodium chloride as a preferred protein extractant for pork lean meat. *Meat Sci.*, 67: 697-703.
- Regenstein, J.M. and J. Rank Stamm, 1979. Factors affecting the sodium chloride extractability of muscle proteins from chicken breast, trout white and lobster tail muscles. *J. Food Biochem.*, 3: 191-204.
- Richardson, R.I. and J.M. Jones, 1987. The effect of salt concentration and pH upon water binding, water-holding and protein extractability of turkey meat. *Intl. J. Food Sci. Technol.*, 22: 683-692.
- Samejima, K., N.H. Lee, M. Ishioroshi and A. Asghar, 1992. Protein extractability and thermal gel formability of myofibrils isolated from skeletal and cardiac muscles at different post mortem periods. *J. Sci. Food Agric.*, 58: 385-393.
- Snedecor, G.W. and W.G. Cochran, 1994. *Statistical Methods*. 8th Edn. IOWA State University Press, Ames, Iowa.
- Toorop, R., S.J. Murch and R.O. Ball, 1997. Development of a rapid and accurate method for separation and quantification of myofibrillar proteins in meat. *Food Res. Intl.*, 30: 619-627.
- Xiong, Y.L. and C.J. Brekke, 1991. Protein extractability and thermally induced gelation properties of myofibrils isolated from pre and post rigor chicken muscles. *J. Food Sci.*, 56: 210-215.