Characterization of fractions from the recovery of bovine lung protein using pH shift processing

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Abstract

Protein was recovered from bovine lung using a pH shift process involving alkaline solubilisation and acid precipitation. Results show that protein can be successfully recovered using the pH shift process without generating salt in the final product. Bovine lung has a high protein and connective tissue content; while collagenous connective tissue was not solubilized, it was concentrated in the insoluble material and is recoverable by centrifugation. SDS PAGE showed similar protein profile for lung tissue, solubilized, and recovered protein but the precipitation process was not effective in recovering all protein ≤ 66Kda.

Key Words – Bovine lung, pH shift, collagen, SDS PAGE

I. INTRODUCTION

Bovine lung is a low value co-product of the meat processing industry, with protein content comparable to meat. A potential way to increase the value is to recover the protein for use as a functional food ingredient. Due to the high connective tissue content in lung [1] extraction of protein is more challenging than other offal; it is therefore an excellent model on which to optimise protein extraction methods. The pH shift method is a non-thermal stepwise process based on protein solubilisation using acid or alkaline conditions, followed by a targeted pH shift to precipitate the proteins and has been used with some success in the recovery of functional protein from seafood and poultry sources.

II. MATERIALS AND METHODS

Protein was recovered from bovine lung using conditions previously optimised for maximum protein yield: pH 10.8, ratio of solvent to sample of 13.02, temperature 19°C and extraction time of 140 minutes. Soluble and insoluble material was separated by centrifugation (10,000 g 20 minutes 4°C). The supernatant containing soluble protein was decanted and chilled to 4°C; precipitation was achieved by pH adjustment to 5.03 (using NaOH 5 M or HCl 3.7%). Recovered (precipitated) protein was collected following centrifugation (10,000 g, 20 minutes, 4°C). Subsamples of supernatants and pellets as well as the starting material were taken for characterization. Protein content was determined according to the AOAC method [2] using a factor of 6.25 to convert nitrogen to crude protein per cent. Fat and moisture content were measured using AOAC methods for moisture [3] and fat [4]. Ash content was measured using a dry ashing method [5], NaCl content was determined by titration with silver nitrate following ashing, and total collagen was calculated from hydroxyproline content according to the method of Kolar 1990 [6]. SDS PAGE using denaturing conditions was used to determine the protein profile of the fractions.

III. RESULTS AND DISCUSSION

The composition of minced bovine lung and the fractions generated during the pH shift process are shown in Table1. Bovine lung has protein content of 17.64%, and with a collagen content of 3.8%, almost one quarter of bovine lung protein is accounted for as collagen. Protein solubilisation in alkaline conditions has resulted in this collagen being concentrated in the insoluble material after the first centrifugation step, where collagen accounts for nearly 70% of the protein present in the pellet, similar results were seen by [7]. The remaining protein in the pellet is likely to contain elastin and some soluble protein trapped during the sedimentation process. Collagen was not detected in the supernatant containing protein solubilized under alkaline conditions. Determination of salt (NaCl) content of the fractions showed that salt was not detected in the pellet containing precipitated protein and appears to have been concentrated in the supernatant 2 containing soluble protein after acid precipitation. As the recovered protein appears not to contain NaCl its potential as a food ingredient in processed meats is enhanced as it will not contribute to the salt content of the product.

The protein profile of bovine lung and the fractions from pH shift processing characterized by SDS PAGE under denaturing conditions are shown in Figure 1. Equal quantities of proteins from each fraction were loaded in duplicate lanes and the molecular weight standard is shown in lane 11. While differences in band intensity were obvious, there was commonality among the banding patterns for minced lung tissue, soluble protein, insoluble material and protein recovered by precipitation. The banding pattern for supernatant 2 (soluble protein not recovered by precipitation) had an absence of higher molecular weight bands with bands present

in the range 66-6.5 kDa. These represent alkaline solubilized proteins which were resistant to acid precipitation. This may in some part be due to the dilute salt concentration (0.04% NaCl) of the supernatant containing protein still soluble after precipitation.

Table 1 Composition (g/100g) of bovine lung tissue and fractions from ph shift process

Sample	**Protein**	**Fat**	**Moisture**	**Ash**	**Salt NaCl**	**Collagen**
Lung tissue	17.64 (0.08)	1.42 (0.21)	79.00 (0.58)	1.28 (0.35)	0.35 (0.040)	3.90 (0.26)
Supernatant 1 (Soluble protein)	0.91 (0.05)	ND	98.89 (0.56)	0.10 (0.03)	0.04 (0.020)	ND
Pellet 1(Insoluble material)	6.23 (1.32)	0.07 (0.1)	93.07 (0.89)	0.22 (0.03)	0.01 (0.002)	4.33 (0.59)
Supernatant 2 (Soluble protein)	0.56 (0.07)	ND	99.67 (0.03)	0.10 (0.01)	0.04 (0.100)	ND
Pellet 2 (Precipitated protein)	13.65 (0.27)	1.40 (0.49)	84.63 (1.87)	0.42 (0.10)	ND	ND

*ND None detected (mean SD on 3 replicates)

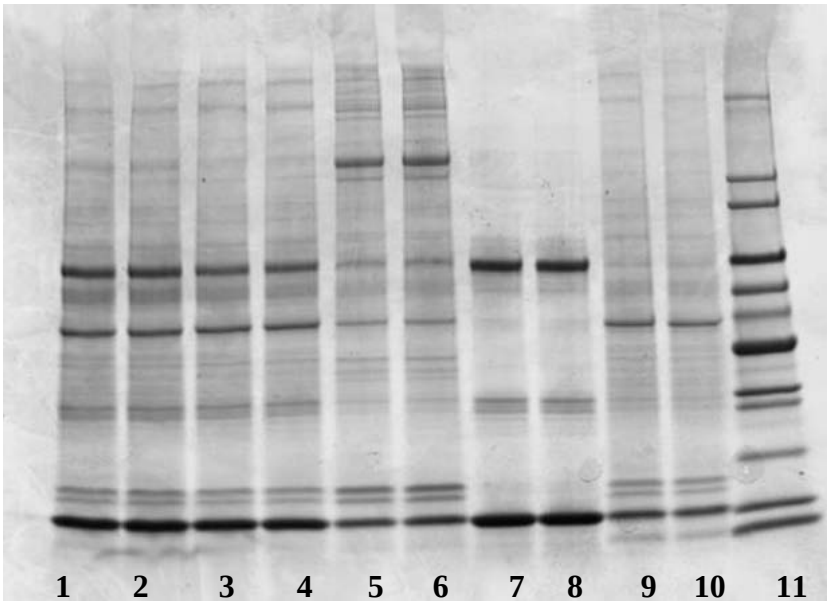

Figure 1 SDS-PAGE (4-20% linear gradient) of bovine lung and fractions from ph shift process. Lane 1 & 2 Bovine lung tissue, lane 3 & 4 soluble protein, lane 5 & 6 insoluble material , lane 7 & 8 soluble protein remaining after precipitation , lane 9 & 10 Protein recovered by precipitation, lane 11 molecular weight standards (200, 116 ,97, 66, 55, 45, 36, 29, 24, 20, 14 and 6.5kDa)

IV. CONCLUSION

Protein recovery using pH shift is a suitable method to solubilize and recover myofibrillar and sarcoplasmic protein while concentrating the collagenous connective tissue in the insoluble material. The pH shift process does not appear to generate NaCl in the recovered protein.

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