Food Engineering Progress
Vol. 5, No. 4. pp. 253~257 (2001년 11월)

Effect of Recycling on the Protein Recovery Process using Ultrafiltration

Linus G. Fonkwe[1], Rakesh K. Singh[2] and Jun Ho Lee*

[1]*Department of Food Science, Purdue University, West Lafayette, IN 47907-1160, USA*
[2]*Department of Food Science & Technology, Univ. of Georgia, Athens, GA 30602-7610, USA*
**Division of Food, Biological and Chemical Engineering, Taegu University, Kyungsan, Kyungpook 712-714, Korea*

Abstract

During the concentration of proteins using an ultrafiltration process, permeate was used as the solvent for a second extraction process. The first permeate had a bacterial load of 7×10^7 cfu/mL while the second permeate had a load of 8×10^7 cfu/mL. The final permeate had a protein content and soluble solids content of 0.26 and 10 g/L, respectively, compared to 0.15 and 9 g/L, respectively for the first permeate. Treatment of the permeates with 1 N HCl and sodium hypochlorite reduced the bacterial loads of the first permeate to 5×10^7, and 4×10^7 for the final permeate. Soluble solid content was reduced in the first permeate but increased in the final permeate. The treatment of the permeates did not affect the amount of proteins in them.

Key words: protein, recovery, recycling, ultrafiltration

Introduction

It has been already recognized that poultry deboner residues are potentially valuable source of protein for human nutrition (Young, 1976; Wallace and Froning, 1979; Fonkwe and Singh, 1996a). A bony residue (waste material) that still contains valuable myofibrillar and sarcoplasmic proteins can be produced from deboning of poultry meat (Fonkwe and Singh, 1994a). This residue contains approximately 15 to 20% proteins of which about 18% are extractable myofibrillar and sarcoplasmic proteins that can be used for human consumption (Kijowski and Niewiarowicz, 1985; McCurdy *et al.*, 1986).

Protein recovery from mechanically deboned turkey residue (MDTR) is a single process involving two distinct steps; (1) an extraction step to extract the proteins from MDTR into an appropriate solution, (2) a process to precipitate and recover the extracted proteins from the solution (Fonkwe and Singh, 1994). Several researchers studied various aspects of recovery of myofibrillar and sarcoplasmic proteins from poultry bone residue (Young, 1976; Kijowski and Niewiarowicz, 1985; Ozimek *et al.*, 1986; McCurdy *et al.*, 1986; Opiacha, 1989; Opiacha *et al.*, 1991; Chi and Chen, 1994; Fonkwe and Singh, 1994a,b; 1996a,b; 1997).

It is often not an easy process to recover all the muscle proteins in solutions obtained from the extraction process using muscle tissues. Common and inexpensive methods of recovering food grade proteins from solutions include isoelectric precipitation, reduction of ionic strength, ultrafiltration and precipitation with polysaccharides. Work in our laboratory obtained conditions leading to the maximum precipitation of turkey muscle proteins using polysaccharides: carrageenan, caboxymethyl cellulose (Fonkwe and Singh, 1994a) and chitosan. These polysaccharides precipitated 92%, 83% and 74% of turkey proteins, respectively, from solution.

The extraction of proteins from animal tissue necessitates the use of large volumes of water. Usually, three to five volumes of water are used per unit mass of the tis-

Corresponding author: Jun Ho Lee, Division of Food, Biological and Chemical Engineering, Taegu University, Kyungsan, Kyungpook 712-714, Korea

sue. The protein solution obtained is thus dilute. After the recovery of the protein from solution, a large volume of wastewater is generated for disposal.

Disposal of the wastewater from such a protein recovery process could be cumbersome and expensive because of the volume of the wastewater. Also important things are the protein and soluble solids content, and the bacterial load of the wastewater. A common method of reducing the bacterial load of wastewaters involves the addition of chlorine or chlorine compounds such as sodium hypochlorite (bleach).

The objectives of this study were: (1) to effectively use an ultrafiltration process in recovering turkey muscle proteins from protein solutions, (2) to reuse the first ultrafiltration permeate as the solvent for a second protein extraction process, and (3) to reduce the bacterial load, the protein content and the soluble solids content of the final permeate prior to disposal.

Materials and Methods

Materials

Mechanically deboned turkey residue was obtained from Farbest Foods Inc. (Huntingburg, IN, USA). The sample was received frozen, cut into blocks (c. 300 g) and stored frozen at 20 until used. The frozen samples were thawed overnight at 8 to 10 in a refrigerator prior to use.

Extraction of protein

The experimental procedure for the extraction of proteins from MDTR was described by Fonkwe and Singh (1994a). MDTR was mixed with water in a 1:3 (wt/vol) ratio in a commercial Waring blender. A 3 M sodium hydroxide solution was used to raise the pH of the slurry to pH 10.6-10.7. The slurry was then blended for 2 min at low speed and held in the blender for 25 min at intermittent blending. The pH of the slurry was checked every 5 min and adjusted to pH 10.6-10.7 with 3 M sodium hydroxide solution.

Filtration

Following the extraction, the protein solution was filtered through several layers of cheese cloth to eliminate insoluble solids and fat particles. This filtration was repeated until no insoluble material was visible at the bottom of the protein solution.

Ultrafilration

The filtered solution was then pumped through a hollow fiber ultrafiltration system (Model S1Y10, AMICON, Danvers, MA) with nominal molecular weight cutoff (MWCO) point of 10,000 Daltons for three and half hours. The flow rate was about 2 L/min and nominal surface area of the membranes was about 0.09 m^2. The system was operated at the inlet pressure of 137.9 kPa. The protein concentrate and the ultrafiltration permeate were collected and their bacterial loads, protein content and soluble solids content were determined.

The permeate from above was used as a solvent in another extraction of proteins from mechanically deboned turkey residue. The protein concentrate and ultrafiltration permeate were again collected and their bacterial load, protein content and soluble solids content were also determined. The final permeate was then treated with 1N hydrochloric acid (to pH 5.3) to precipitate any myofibrilar and sacoplasmic proteins, and then filtered. This was followed by treatment with sodium hypochlorite to reduce the microbial load.

Analysis

The protein content of each ultrafiltration fraction was determined using the bicinchoninic acid method (Smith *et al.*, 1985). The bacterial load was determined using the standard plate count method (APHA, 1965). The soluble solids contents were determined using methods of the AOAC (1990).

Results and Discussion

Performance of ultrafiltration

The flux of water (amount of water that permeates across the ultrafiltration membrane per unit area per unit time) during the ultrafiltration process is shown as a function of time in Fig. 1. The flux, however, did not decrease significantly after about 2.5 hr of operation. Clogging of the membrane was not observed, indicating that the filtration process through several layers of

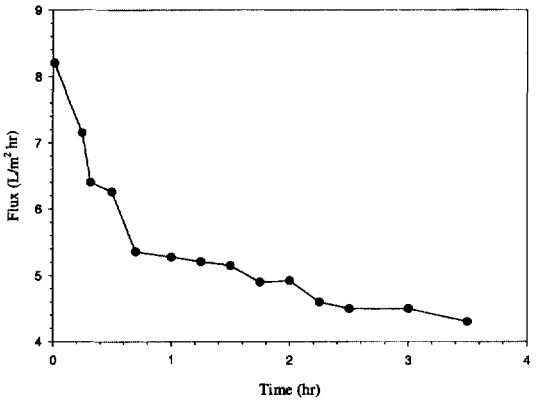

Fig. 1. Flux changes during the ultrafiltration process of MDTR.

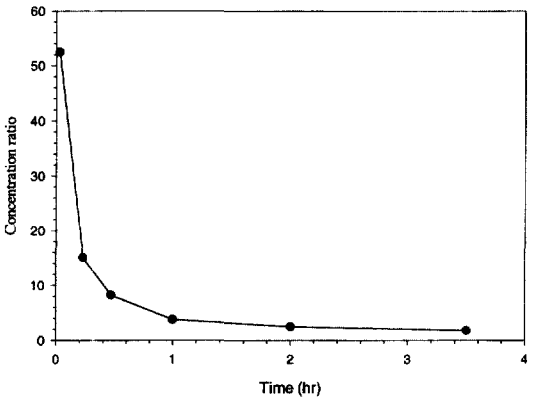

Fig. 2. Concentration ratio changes during the ultrafiltration process of MDTR.

cheesecloth was effective in removing insoluble solids and fat particles.

Figure 2 shows the concentration ratio (ratio of the initial volume of the protein solution to the volume of permeate at a given time) of the membrane plotted against time. This plot shows that the rate at which the proteins were being concentrated was high at first, and then exponentially decreased with time. The concentration ratio (like the water flux) did not decrease significantly after about 2.5 hours of operation.

Quality of protein solutions

The bacterial loads, protein content and soluble solids of the various ultrafiltration fractions are shown in Table 1. The tap water protein extract had about twice the bacterial load of the reused water protein extract. The ultrafiltration permeates had higher bacterial loads than the original protein extracts. This may be explained by following reason. It is possible that the higher protein content in the protein extracts and concentrate had some inhibitory effect on bacterial growth.

The protein content of the reused water extract and permeate were significantly higher than the protein content of the tap water extract and permeate. The permeated that was reused for extraction contained some proteins. These proteins added to those that were actually extracted caused the significant increase in the protein content of the reused water extract and permeate.

The protein extracts had more soluble solids than the permeates. The major proportion of the soluble solids in these solutions is mainly composed of proteins. Therefore, the protein concentrate had the highest amount of soluble solids followed by the protein extracts. The permeates with the least amount of proteins and other soluble solids which were less than 10 kD in size had the lowest soluble solid content. The amount of soluble solids in the reused water permeate was not significantly higher than the tap water permeate.

Treatment of the permeates with 1 N hydrochloric acid to pH 5.3 (isoelectric precipitation) followed by treatment with 100 ppm sodium hypochlorite reduced the bacterial load of the tap water permeate by a factor of 4, as shown inTable 2. This treatment, however, reduced

Table 1. Quality of protein solutions determined before and after ultrafiltration

Solution	Bacterial load (cfu/mL)	Protein content (g/L)	Soluble solids (g/L)
Tap water extract	7×10^7	2.09	22.0
Tap water permeate	2×10^8	0.15	9.0
Protein concentrate	5×10^7	8.41	44.0
Reused water extract	4×10^7	2.99	29.0
Reused water permeate	8×10^7	0.26	10.0

Table 2. Quality of ultrafiltration permeates treated with 1N HCl and 100 ppm sodium hypochlorite

Permeate	Bacterial load (cfu/mL)	Protein content (g/L)	Soluble solids (g/L)
Tap water	5×10^7 (2×10^8)*	0.16 (0.16)*	5.0 (9.0)*
Reused water	4×10^7 (8×10^7)*	0.26 (0.26)*	13.0 (10.0)*

*Indicates untreated permeates.

the bacterial load of the reused water permeate by only half. Table 2 also shows that isoelectric precipitation did not precipitate the proteins in the permeates. The isoelectric point used was that of myofibrilar and sarcoplasmic proteins. The results in Table 2 show that the small proteins (or protein fragments), which are less than 10 kD did not precipitate at this pH.

The soluble solids content of the treated tap water permeate was lower than that of the untreated permeate (Table 2). It was reduced by about 44%. However, this was not true for the treated reused water permeate. Isoelectic precipitation and treatment with sodium hypochlorite actually increased the soluble solids content of the treated reused water permeate.

The treated permeates were clear in appearance and did not have any odor that could be associated with deterioration protein solutions, even after four weeks of storage, compared to the protein extracts and untreated permeates. The protein extracts showed signs of deterioration (odor and cloudiness) after about a week of storage at 10°C while the untreated permeates showed signs of spoilage after about 10 day of storage at 10°C. This difference may have been due to the difference in their protein content. Signs of deterioration were more easily detected in the protein extracts since they had more proteins.

Conclusions

Ultrafiltration was used to concentrate a protein solution obtained by extraction from mechanically deboned turkey residue. The process was effective without significant clogging of the membrane by lipids during 3.5 hours of operation. The permeates from the ultrafiltration process were higher in bacterial load than the protein extracts. This was probably due to better metabolism of the small proteins (less than 10 kD), present in small amounts, by the bacteria. The protein and soluble solids content of the permeates were low compared to those of the original protein extracts. The use of an ultrafiltration permeates as a solvent in protein extraction caused only modest increases in the protein and soluble solids content of the resulting protein extract and permeate.

Isoelectric precipitation did not reduce the protein content of the permeate. Treatment of the permeates with sodium hypochlorite caused significant decreases in the bacterial loads of the permeates. The overall treatment of the permeate caused a reduction in the soluble solids content of the tap water permeate but caused a small increase in the soluble solids content of the reused were permeate. The treated permeates were clear in appearance and had no odor even after 4 weeks of holding at 10°C. With a few modifications, the treated permeates should be safe for disposal because of their low bacterial count, protein and soluble solids contents (hence BOD).

References

AOAC. 1984. *Official Methods of Analysis*, 14th ed. Association of Official Analytical Chemists, Washington, D.C.

APHA. 1965. *Standard Methods for the Examination of water and Wastewater*, 12th ed. American Public Health Association, N.Y.

Chi, S.P. and T.C. Chen. 1994. Selected chemical and physical characteristics of chicken broth from broiler deboning by-products. *J. Food Proc. Preserv.* **17**: 407-420

Fonkwe, L.G. and R.K. Singh. 1994a. Protein recovery from mechanically deboned turkey residue. *Trans. of the ASAE.* **37**: 527-534

Fonkwe, L.G. and R.K. Singh. 1994b. Extraction of salt and alkali soluble proteins from mechanically deboned turkey residue: Effect of some variables using response surface methodology. *J. Food Proc. Preserv.* **18**: 47-60

Fonkwe, L.G. and R.K. Singh. 1996a. Characterization of alkali-extracted protein prepared from deboned turkey residue. *J. Food Proc. Preserv.* **20**: 359-378

Fonkwe, L.G. and R.K. Singh. 1996b. Protein recovery from mechanically deboned turkey residue by enzymic hydrolysis. *Proc. Biochem.* **31**: 605-616

Fonkwe, L.G. and R.K. Singh. 1997. Production and characterization of gelatinous protein extracts from turkey deboner residue. *Proc. Biochem.* **32**: 309-318

Kijowski, J. and A. Niewiarowicz. 1985. A method of protein extraction from chicken bone residue and the chemical and electrophoretic characteristics of the extract. *J. Food Technol.* **20**: 43-49

McCurdy, S.M., P. Jelen, P. Fedec and D.F. Wood. 1986. Laboratory and pilot scale recovery of protein from mechanically separated chicken residue. *J. Food Sci.* **51**: 742-747, 753

Opiacha, J.O. 1989. Properties of dehydrated protein extracts from poultry bone residue. Ph.D. Thesis. The

Pennsylvania State University, State College, PA.

Opiacha, J.O., M.G. Mast, and J.H. MacNeil. 1991. In-vitro protein digestibility of dehydrated protein extract from poultry bone residue. *J. Food Sci.* **56**: 1751-1752

Ozimek, G., P. Jelen, L. Ozimek, W. Sauer and S.M. McCurdy. A comparison of mechanically separated and alkali extracted chicken protein for functional and nutritional properties. *J. Food Sci.* **51**: 748-753

Wallace, M.J.D. and G.W. Froning. 1979. Protein quality determination of bone residue from mechanically deboned chicken meat. *Poultry Sci.* **58**: 333-336

Smith, P.K., R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson and D.C. Klenk. 1985. Measurement of protein using bicinichoninic acid. *Anal. Biochem.* **150**: 76-85

Young, L.L. 1976. Composition and properties of an animal protein isolate prepared from bone residue. *J. Food Sci.* **41**: 606-608