Recovery of Proteins as a Spun Product from Sardine Viscera and Heads

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To recover proteins as a spun product from sardine viscera and heads for the use of food ingredient, factors affecting the extraction of proteins were investigated. Factors examined were extraction time, temperature, NaCl concentration, and effect of pH. The most appropriate extraction was made by water at 22°C for 30 min at pH 10.5 (final value). In order to concentrate proteins, the extracted proteins were precipitated out by adjusting the pH to 5.0, and the precipitates were dissolved in NaOH solution for the preparation of spinning dope. The coagulation solution containing 20% NaCl or 20% CaCl₂ in 1 N acetic acid gave 40-59% recovery of proteins in spun products. On the other hand, a spun product prepared by using 1 N acetic acid in ethanol brought about 70% protein recovery and its lipid content was fairly low.

Recently there has been an increasing interest in the use of underutilized fish species, such as sardines and mackerels, which are caught near the coasts of Japan in large quantities. However, more than 50% of these fish species are used for non-human outlets, the most important of which is for animal feed as fish meal.¹⁾ Although a large amount of fish industry wastes such as viscera and head portions themselves are unsuitable for human consumption, their protein quality is substantially equivalent to that of fish muscle proteins.²⁻⁴⁾ Therefore, it would be important to recover proteins from fish wastes with acceptable properties as a food ingredient, because there has been a shortage of proteins with high biological value in the world. While fish protein concentrate (FPC) has been developed for this purpose, it usually lacks the functional properties of water-holding and gel-forming abilities. Consequently, it has limited application for food use. Spinning of vegetable proteins such as soybean proteins has been intensively studied,^{5,6)} but there is a scarcity of information available concerning the spinning of fish proteins.¹⁾

The purpose of this study was to develop the recovery technique of proteins from sardine viscera and heads as a spun product.

Materials and Methods

Sample

Sardines *Sardinops melanosticta* were used

throughout this study. The average body weight and length of the sample fish were 107 g and 22 cm, respectively. Head portions were cut off at the edge of operculum. The average weight of the viscera was 14 g, corresponding 13% of the total body weight, while that of the head was 20 g, which comprised approximately 19% of the total body weight. The proximate compositions of viscera and heads are given in Table 1.

Extraction and Recovery of Proteins from Viscera and Heads

Viscera and heads were minced by a Waring blender for 2 min and 5 g of minced sample was stirred with 50 ml of the extraction solution. Sodium chloride solutions (0 M-1.0 M) were used for the extraction and their pH values were adjusted to 1-12. The final pH value of the extract was measured. The extraction was carried out at 15°, 22°, 30°, and 40°C for up to 90 min.

After the extraction with NaCl solutions, they were centrifuged at 7,000×g for 15 min at 4°C and the precipitates were discarded. Total nitrogen and TCA soluble nitrogen contents in the supernatants were determined by the Kjeldahl method. The supernatants were then filtered through a glass wool to remove solidified fats, and pH values of the filtrates were adjusted to 5.0 with 1.0 M HCl or 1.0 M NaOH in order to precipitate proteins. After centrifugation at 14,000×g for 20 min at 4°C, the precipitates were collected (simply called protein isolate hereafter). Total nitrogen contents of protein isolates obtained

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were determined by the Kjeldahl method.

Spinning of Protein Isolates

The protein isolates obtained as described above were used for the preparation of the spinning dopes. Sodium hydroxide solution was added to the protein isolate to have NaOH/protein ratio (weight base) of 0.02–0.15, and the protein concentration of the resulting spinning dope was adjusted to 40–80 mg/g.

After the spinning dopes were thoroughly mixed, they were placed into a syringe with a stainless spinneret (diameter: 0.8 mm). The spinning dope was extruded into a coagulation solution. As a coagulation solution, 5–20% NaCl in 1 N acetic acid, 20% CaCl_2 in 1 N acetic acid, and 1 N acetic acid in ethanol were used in this study. The spun fibers were removed from the coagulation solution and left to drain of excess coagulation solution. After air-dried at room temperature overnight, solid, protein, lipid and ash contents of the spun products were analyzed.

Results and Discussion

Extractability of Proteins

Fig. 1 shows the effect of NaCl concentration on the extractability of proteins from viscera and heads at pH 2, 7.5, and 10.5 (final value). In the case of viscera, protein extractability remarkably decreased with increasing NaCl concentration at pH 2, while the effect of NaCl concentration was not so significant at pH 7.5 and 10.5. It was

shown that the extraction by water was most suitable for viscera. On the other hand, the protein extractability of head portions was lower over the range of NaCl concentrations and pH values tested in this study than that of viscera. The higher recovery of proteins from heads was obtained in the lower concentration of NaCl except at pH 7.5, where 0.4 M NaCl brought about a slightly higher extraction. The difference between the total N and protein N was larger in viscera than in heads, indicating the occurrence of protein hydrolysis by digestive enzymes present in viscera.

Fig. 2 illustrates the effect of extraction temperature and time on the protein extraction by water from sardine viscera. The extraction was carried out at 15°, 22°, 30°, and 40°C at pH 2, 7.5, and 10.5 (final value). At almost all conditions the total N extractability reached 80% within 30 min of extraction, whereas the protein N extractability decreased with longer extraction time, especially at higher temperatures at pH 7.5 and 10.5. It is apparent from these results that this decrease was concomitant with an autolysis of viscera proteins by the digestive enzymes. The higher recovery of the protein N was attained by the extraction at 22°C for 30 min at pH 10.5.

The influence of extraction temperature and time on the protein extraction from sardine heads is given in Fig. 3. The extraction of proteins from heads was carried out either by water at pH 2 and 10.5 or by 0.4 M NaCl at pH 7.5. Both the total N and protein N extractabilities were,

Fig. 1. Effect of NaCl concentration on the extractability of proteins from sardine viscera and heads. The extraction was carried out at 22°C for 30 min. pH indicated is the final value of the extract.

—○—: Total N,
—●—: Protein N.

Fig. 2. Effect of extraction temperature and time on the extractability of proteins from sardine viscera. Extraction was carried out by water and pH indicated is the final value of the extract. —○—: Total N, —●—: Protein N.

Fig. 3. Effect of extraction temperature and time on the extractability of proteins from sardine heads. Extraction was carried out by water except at pH 7.5 by 0.4 M NaCl, and pH indicated is the final value of the extract. —○—: Total N, —●—: Protein N.

on the whole, lower in heads than those in viscera at any extraction conditions. Moreover, the difference between the total N and protein N extractability in the head portion was quite small, suggesting that the influence of digestive enzymes on the protein extraction was negligible.

Fig. 4 presents the effect of pH on the extraction of proteins from sardine viscera and heads at 22°C. In the case of viscera, as much as 90% of the total N and 60% of the protein N were extractable at the alkali range regardless of the extraction solution used. Sodium chloride greatly

Fig. 4. Effect of pH on the extractability of proteins from sardine viscera and heads. The extraction was carried out at 22°C for 30 min.

—○—: Total N, —●—: Protein N.

Fig. 5. Effects of NaOH/protein ratio, protein concentration, NaCl concentration in the coagulation solution on the recovery of proteins in spun products.

inhibited the extraction of proteins at lower pH values. As the pH was altered toward the isoelectric point of around 5.0, the extractability of the protein N decreased to reach a minimal value of 4–15%. Similar findings have been reported by several investigators.^{1,7,8)} An analogous result was obtained in sardine heads, but the protein extractability was smaller at any pH values than that in viscera.

Though a high recovery of proteins from sardine viscera and heads was obtained by the extraction

with weak alkali solution as indicated above, the extracts themselves were not concentrated enough for spinning. It was, therefore, necessary to introduce a concentration step prior to the spinning process to give spun products of adequate strength.¹⁾ The simplest and most effective procedure for this purpose was to precipitate proteins by adjusting the pH of the extracts to the isoelectric point, pH 5.0 (Fig. 4). The precipitates thus obtained were dissolved in dilute NaOH solution to prepare the spinning dope.

Spinning of Proteins

Fig. 5 shows the effects of NaOH/protein ratio, protein concentration of the spinning dope, and the NaCl concentration in the coagulation solution on the recovery of proteins in spun products. The recovery of proteins decreased with increasing NaOH/protein ratio and the ratio of around 0.03 was most suitable. The protein recovery in spun products was higher from the viscera dope than that from heads dope. The recovery approached a plateau at the dope protein concentration of 45 mg/g for viscera and 55 mg/g for heads. It is apparent from Fig. 5 that the recovery of proteins increased with increasing NaCl concentration in 1 N acetic acid solution. The effect of NaCl concentration on the protein recovery was more significant for viscera dope compared to that for heads dope. The spinning conditions employed for viscera and heads dopes were as follows; NaOH/protein ratio: 0.028 and 0.035,

protein concentration: 48 and 55 mg/g, coagulation solution: 20% NaCl in 1 N acetic acid, respectively. The proximate compositions of the spun products thus prepared were determined and the recovery of each component was calculated (spun product I in Table 1). About 50% and 40% of proteins were recovered from viscera and heads, respectively. Ash content of the product remarkably increased due to the use of NaCl in the coagulation solution.

The spun product II was prepared by substituting CaCl_2 for NaCl in the coagulation solution. Recovery of each component was almost equivalent to that in the spun product I. In order to remove lipids as much as possible during the spinning process, 1 N acetic acid in ethanol was used as the coagulation solution (spun product III). The lipid content of the spun product III was significantly reduced whereas the protein content increased. Using ethanol in the coagulation solution appears to be appropriate for the preparation of spun products from sardine viscera and heads as far as the economical feasibility is not considered. It is revealed from this study that extraction, concentration and coagulation are the important steps for the preparation of spun products from sardine viscera and heads.

Table 1. Proximate compositions of spun products from sardine viscera and heads (%)

	Solid	Protein	Lipid	Ash
Viscera				
Raw material	63.8 (100)* ¹	7.6 (100)	55.5 (100)	0.8 (100)
Spun product I* ²	73.3 (13.6)	33.1 (51.8)	22.7 (4.8)	14.9 (221)
Spun product II* ³	84.7 (13.7)	43.2 (58.9)	21.5 (4.0)	14.1 (183)
Spun product III* ⁴	87.1 (10.2)	74.1 (73.0)	1.6 (0.2)	0.6 (5.6)
Heads				
Raw material	32.4 (100)	10.6 (100)	13.4 (100)	6.8 (100)
Spun product I* ²	76.4 (19.5)	50.1 (39.3)	7.9 (4.7)	18.1 (22.0)
Spun product II* ³	80.7 (22.4)	51.1 (43.4)	9.2 (6.2)	19.6 (25.9)
Spun product III* ⁴	89.1 (23.3)	85.7 (68.7)	1.2 (0.7)	2.2 (2.8)

*¹ Values in parentheses are % recovery based upon raw material.

*² 20% NaCl in 1 N acetic acid was used as a coagulation solution.

*³ 20% CaCl_2 in 1 N acetic acid was used as a coagulation solution.

*⁴ 1 N acetic acid in ethanol was used as a coagulation solution.

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