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PII: S0308-8146(97)00064-2

Food Chemistry, Vol. 61, No. 3, pp. 339–343, 1998

0308-8146/98 $19.00 + 0.00

Emulsifying properties of an ultrafiltered protein from minced fish wash water

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(Received 20 September 1996; accepted 20 February 1997)

Centrifugation and ultrafiltration were used to concentrate soluble proteins in the water used to wash minced muscle of sardine caught at two different times of year. The proteins thus extracted were largely of less than 67 kDa molecular weight. Part of the resulting concentrate was frozen-stored and part freeze-dried. Irrespective of the season of capture, samples exhibited high emulsifying capacity and emulsion stability, values being higher in freeze-dried samples. Both properties were virtually unaffected by solutions, with NaCl concentrations ranging from 0 to 3%. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Washing is one of the most important steps in surimi manufacture, in that it improves gel-forming ability due to leaching of a considerable amount of fat and sarcoplasmic proteins (Suzuki, 1981; Wu *et al.*, 1991; Lin *et al.*, 1995). Washing also separates myofibrillar proteins. Although, according to classical protein chemistry, NaCl (0.3–0.6%M) is needed to solubilize myofibrillar protein, recent studies (Stefansson & Hultin, 1994; Lin *et al.*, 1995) indicate that significant amounts of myofibrillar proteins are solubilized in very low ionic strength (near zero) solutions. The protein lost in the waste-water by leaching accounts for 15–30% of the total protein of minced meat, and some of these soluble solids could be highly functional proteins.

The recovery of water-soluble proteins from surimi waste water would permit more efficient utilization of marine resources and help reduce the costs of surimi production (Okazaki, 1994), besides limiting the pollution problems associated with disposal, since chemical oxygen demand is reduced more than 10-fold when the proteins are removed from the medium (Lin *et al.*, 1995).

Ultrafiltration is the principal method used in commercial dairy whey recovery and has been studied by several scientists as a means of recovering proteins from surimi waste water (Nishioka & Shimizu, 1983; Green *et al.*, 1984; Swafford, 1987; Pedersen *et al.*, 1987, 1989; French & Pedersen, 1990). This procedure permits effective concentration, although the final products thus obtained are dark in colour and have a strong flavour (Lin *et al.*, 1995), which has led the above mentioned authors to suggest that their main potential may be as animal feed.

The aim of the present work was to recover soluble proteins from minced fish wash water by centrifugation, followed by ultrafiltration, and to examine their emulsifying properties with a view to using them in products for human consumption, where colour and smell are not a consideration. The study was based on two variables: season of capture of the fish (winter or spring) and mode of storing–processing the protein concentrate (freezing or freeze-drying).

MATERIALS AND METHODS

Sample preparation

The fish used in these experiments were sardine (*Sardina pilchardus*, Walbaum), caught in February (winter, W) and May (spring, S) off the Mediterranean coast. Individuals were headed, gutted and washed in ice and water, then skin and bones were removed with a Baader deboning machine (model 694) with 3 mm-diameter drum orifices. Mince was held for 10 min at 0–3°C in an aqueous solution of 0.5% bicarbonate, proportion 3:1 (solution:minced muscle), stirring constantly. This liquid was recovered by pressing and used for the present study. The solution was centrifuged at 11 000 *g* for 30 min to remove mince particles and insoluble particulates of high molecular weight. The supernatant was filtered through Whatman No. 4 paper to remove suspended fat particles; then the solution was concentrated by ultrafiltration. For this purpose, a tangential

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flow membrane filter of 30 kDa was placed in a Minisette hardware filter holder and a pump provided recirculating flow. Minisette and filter were supplied by Filtron Technology Corporation (Massachusetts 01532, USA) and the pump was a Millipore variable Speed Tubing Pump.

Preparation of lots

In all cases, the wash water was concentrated down to one-fifth of the initial volume. The concentrate was frozen and stored at −18°C pending analysis. At the same time, part of the concentrate from the water used to wash the fish caught in May was freeze-dried for subsequent analysis.

The effects of protein concentration and ionic strength on emulsifying capacity and emulsion stability were analysed in the frozen concentrates from the February and May catches and in the concentrated freeze-dried lot from the May catch.

Analyses performed

To characterize the samples, crude protein, moisture and ash were determined by A.O.A.C (1984). Crude fat was analysed according to the method described by Bligh and Dyer (1959). Results were averages of three determinations and expressed as $g\,kg^{-1}$. The proximate compositions of sardine muscle samples were: lot W (winter): crude protein, 22.4 ± 0.54; crude fat, 4.62 ± 1.44; moisture, 73.05 ± 0.84; ash, 1.52 ± 0.05; and lot S (spring): crude protein, 18.3 ± 0.26; crude fat, 6.64 ± 0.29; moisture, 69.78 ± 0.59; ash, 1.61 ± 0.02.

SDS–Polyacrylamide gel electrophoresis (SDS–PAGE)

Soluble proteins were analysed by SDS–PAGE in a Phastsystem horizontal apparatus (Pharmacia LKB Biotechnology, Uppsala, Sweden) using 12.5% polyacrylamide gels. The samples were treated according to Hames (1985) (2% SDS, 5% β-mercaptoethanol and 0.002% bromophenol blue); the protein concentration was adjusted to $1\,mg\,ml^{-1}$, and samples were heated for 5 min in a boiling water bath. Samples were then centrifuged at 12 000 rpm for 1 min. One-microlitre aliquots were applied in the gels.

Electrophoresis conditions were 4 mA/gel, 250 V and 3 W. The protein bands were stained with Coomassie Brilliant Blue and the bands analysed on a 3CX Image Analyzer (Bio Image and Visage, Millipore Corporation, Ann Arbor, MI, USA). The molecular masses of the main component proteins in the samples were estimated by comparing their mobility with that of a standard high-molecular mass protein mix (Pharmacia LKB Biotechnology, Uppsala, Sweden).

Emulsifying properties

Preparation of samples

Samples were prepared by homogenizing the concentrate water with distilled water to obtain the different target concentrations. To study the influence of ionic strength, different amounts of NaCl were added to concentrate water at $15\,mg\,ml^{-1}$.

Emulsifying capacity

This was determined by the method of Montero and Borderías (1991). In order to prepare an emulsion, 5 g of extract were added to 20 ml of H_2O and 4.2 g of oil and blended in an Osterizer homogenizer in which two electrodes had been placed so that they were in contact with the emulsion. During blending, oil was added continuously until the emulsion was unable to hold more and collapsed. Collapse of the emulsion, taken as the cessation of conductivity, was measured by a polymeter connected to the electrodes. The amount of oil added was quantified and emulsifying capacity was expressed as g oil per g homogenate or g oil per g protein. Four replications were performed for all determinations.

Emulsion stability

Emulsion stability was tested for the various samples by stopping the addition of oil prior to the point at which collapse of the emulsion was known to occur. In the emulsion preparation, 80% of the oil needed to collapse the emulsion for measurement of emulsifying capacity was therefore added to the water concentrate. Then, 25 g of this emulsion was centrifuged for 20 min at 6000 rpm (4000 *g*). The heavy supernatant was collected in capsules and dried to constant weight. The supernatant constituted the aqueous and fatty fractions not retained in the emulsion; these were expressed as % oil released (dry weight of supernatant $\times 100$/initial weight of sample) and % water released (weight of supernatant −dry weight of supernatant)$\times 100$/initial weight of sample). The precipitate (the emulsion remaining) was expressed as % emulsion stability (weight of precipitate $\times 100$/initial weight of emulsion). Four replications were performed for all determinations.

The effect of protein concentration (0, 5, 10, 15, 20 and $25\,mg\,ml^{-1}$) and the effect of NaCl addition (0, 0.5, 1, 2 and 3% NaCl) at a protein concentration of $15\,mg\,ml^{-1}$ were analysed.

Statistical analysis

Two-way analysis of variance was carried out for the different samples. The computer program used was Statgraphics (STSC Inc., Rockville, USA). The difference of means between pairs was resolved by means of confidence intervals using a LSD range test. The level of significance was set for $P \leq 0.05$.

RESULTS AND DISCUSSION

The amount of protein present in the concentrate water was 50 mg ml^{-1}. In each case, the protein concentration was adjusted to the desired level with water. Both lots were dark in colour and smelled strongly of fish, just as reported by Lin *et al.* (1995).

Most of the peaks in the electrophoretic profiles (Fig. 1) reflect proteins of less than 67 kDa molecular weight, with only insignificant quantities of higher-weight proteins, which would indicate that this wash water contained largely sarcoplasmic proteins and some myofibrillar proteins of low molecular weight. This finding agrees with Lin *et al.* (1995), who reported considerable quantities of myofibrillar proteins (myosin and actin) when the muscle was washed with water several times in succession, myosin and actin appearing from the second wash on, while sarcoplasmic proteins were largely found in the first wash. As Fig. 1 shows, the profiles were similar in centrifuged and concentrated wash water; this is because only molecules of less than 30 kDa were removed by the filter membrane used. Profiles were also similar, regardless of whether the concentrated water was frozen or freeze-dried.

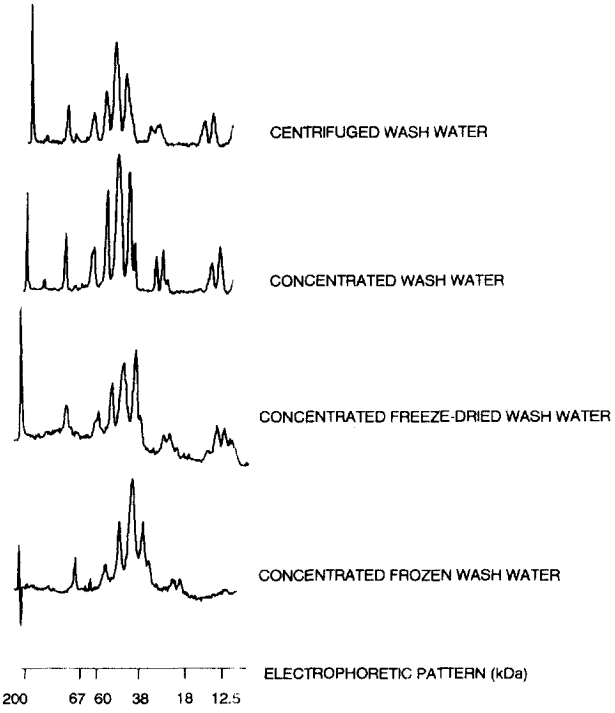

Fig. 1. Sodium dodecyl sulphate–polyacrylamide gel electrophoreis (SDS–Page 12.5%) of centrifuged minced wash water protein, concentrated minced wash water protein, concentrated freeze-dried minced wash water protein and concentrated frozen minced wash water protein from mincing process.

The emulsifying capacity of concentrate water diluted to different concentrations is shown in Fig. 2, expressed as g oil per g homogenate. The emulsifying capacity of frozen concentrate of wash water increased to a similar extent in either lot (F-W and F-S) in response to changes in protein concentration. Montero and Borderías (1991) reported such an increase in samples of collagenous material from trout (*Salmo irideus Gibb*) and hake (*Merluccius merluccius*), although the values were considerably higher. On the other hand, Jiménez-Colmenero and Borderías (1983) and Borderías *et al.* (1985) reported emulsifying capacity values within a similar range for a homogenate of muscle protein from a variety of species, namely bonito (*Sarda sarda bloch*), cod (*Gadus morhua*), horse mackerel (*Trachurus trachurus*), blue whiting (*Micromesistius potassou*) and also chicken and pork. Borderías *et al.* (1985) found somewhat lower values when examining the emulsifying capacity of sarcoplasmic proteins from muscle of these species.

At 1 mg ml^{-1}, only the freeze-dried lot (FD-S) would emulsify although, at higher concentrations, this lot exhibited rather less emulsifying capacity than did the frozen lots (F-S and F-W). Again, values were greater ($P \leq 0.05$) at the highest concentration. This could be related to protein aggregation, which would interact with the two phases, thus creating a barrier to coalescence, as frequently occurs in drying processes (Cheftel *et al.*, 1985). Kinsella (1976) suggested that although solubility is an important factor affecting the distribution of the proteins at the interface, other factors, such as the proportion of the hydrophobic groups, also play a role in determining the emulsifying capacity.

When emulsifying capacity was plotted per unit of protein (Fig. 3), it was found to decrease ($P \leq 0.05$) as

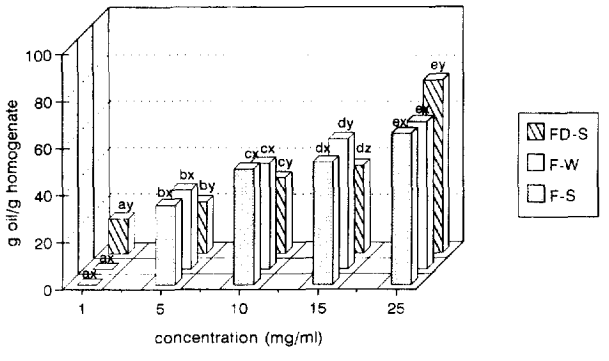

Fig. 2. Emulsifying capacity (g oil per g homogenate) of minced wash water protein at different concentrations.

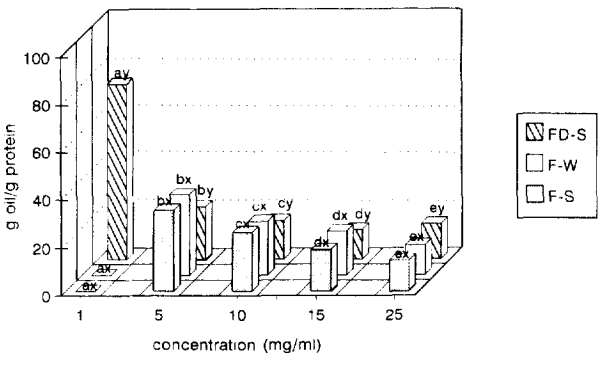

Fig. 3. Emulsifying capacity (g oil per g protein) of minced wash water at different concentrations.

protein concentration increased. The explanation of this is that, when protein concentration increases, steric reasons cause a shift in the polypeptide chains, resulting in a reduced tendency for the molecules to act as an interface in the emulsion (Kinsella, 1976). This phenomenon has been reported in fish myofibrillar proteins (Borderías *et al.*, 1985) and in soluble collagen (Montero & Borderías, 1991).

Emulsion stability was similar ($P \leq 0.05$) in the two frozen lots (F-S and F-W), which both exhibited a very sharp increase ($P \leq 0.05$) between 1 and 5 mg ml^{-1} (Fig. 4). At higher concentrations, between 5 and 25 mg ml^{-1}, emulsion stability was very much the same in all cases. In this connection, Cofrades *et al.* (1996) reported that protein concentration did not affect emulsion stability in actomyosin isolated from muscle of hake (*Merluccius merluccius*) at concentrations of 5–15 mg ml^{-1}. The part of the emulsion that did not remain stable was chiefly the aqueous phase, rather than the oleic phase (Fig. 4). The unstable part tended to decrease gradually ($P \leq 0.05$) as protein concentration increased, except in the freeze-dried lot (FD-S) at low concentrations (1–5 mg ml^{-1}), where there was considerable loss of the fatty portion and low emulsion stability. At 1 mg ml^{-1} the frozen lots did not actually form an emulsion.

The emulsifying capacity of the different lots scarcely varied ($P \leq 0.05$) when considered as a function of NaCl concentration in the concentrate water (Fig. 5). In the frozen lots, emulsifying capacity remained stable up to ionic strength values corresponding to the addition of 2% NaCl, at which point there was a slight decrease. Montero and Borderías (1991) found the same effect in collagenous material from the connective tissue and skin of trout, which suggests that 2% NaCl may mark the point of protein insolubilization or the onset of 'salting out'. However, Cheftel *et al.* (1985) found that, in meat emulsions, levels of 3–6% NaCl favoured emulsifying capacity as a result of protein solubilization ('salting in'). Ramatham *et al.* (1978) found, in groundnut protein, that increased NaCl concentration (between 0.6 and 3%) caused a slight decline in emulsifying capacity. Addition of NaCl reduces emulsifying capacity, probably because at certain concentrations the salt ions compete for water with proteins, thus increasing repulsion of proteins in the interface.

As NaCl concentration increased, emulsion stability dropped significantly and by the same amount in the frozen lots (F-W and F-S), and more sharply in the freeze-dried lot (Fig. 6). At the same time, there was a significant difference in the aqueous portion but not in the fatty (oleic) portion (Fig. 6), which was very low in all cases.

Following these studies, it is thought that the particular characteristics of this concentrate make it an ideal product for use in emulsion-type products for human consumption.

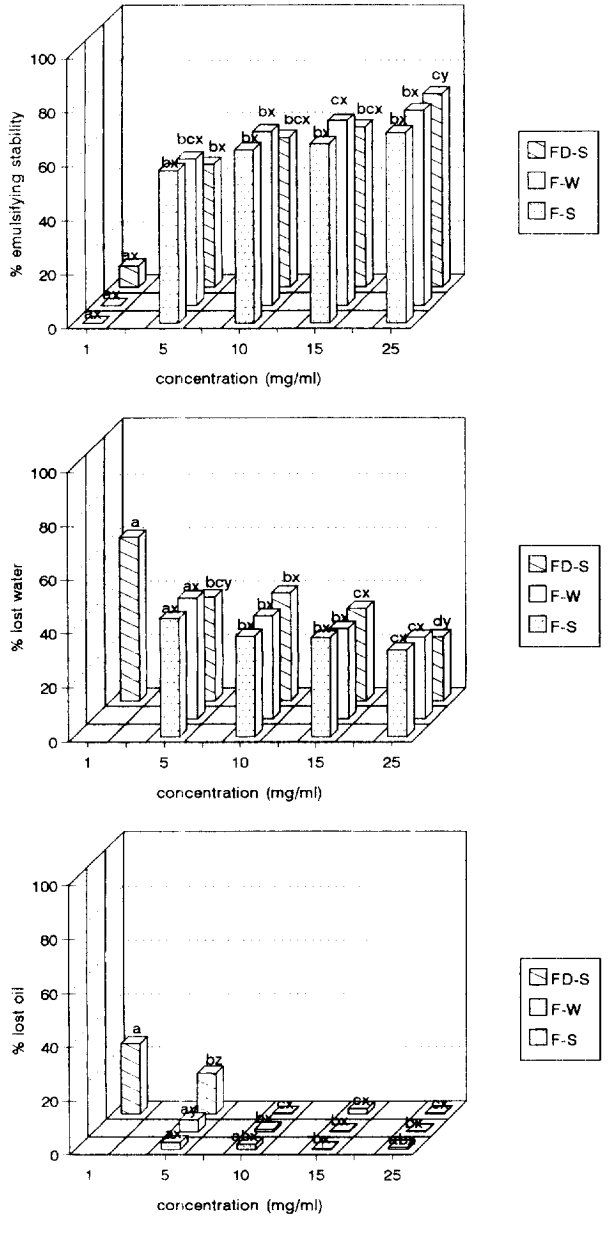

Fig. 4. Stability of emulsion (%), water lost (%) and oil lost (%) of minced wash water protein at different concentrations.

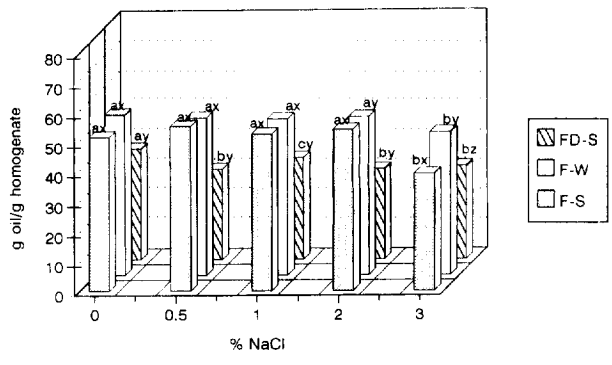

Fig. 5. Emulsifying capacity (g oil per g homogenate) of minced wash water protein at different proportions of NaCl.

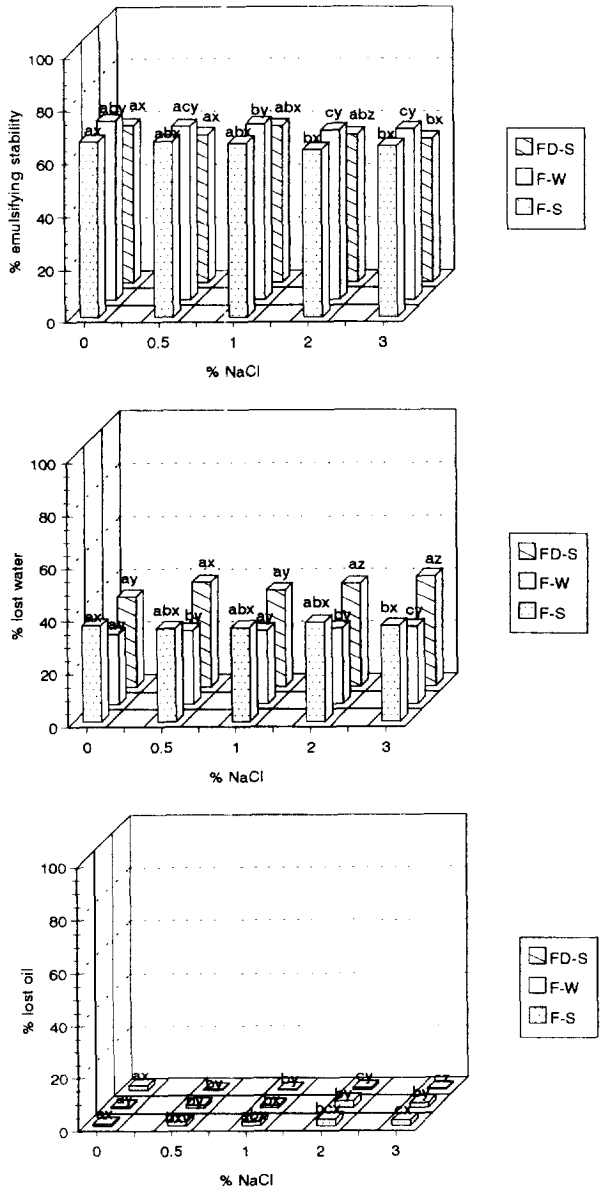

Fig. 6. Stability of emulsion (%), water lost (%) and oil lost (%) of minced wash water protein at different percentages of NaCl.

ACKNOWLEDGEMENT

This research was financed by the Comisión Interministerial de Ciencia y Tecnología under project ALI-910899-C03-01 (1991/1994).

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