ELSEVIER

Food Chemistry 78 (2002) 149–156

Food Chemistry

www.elsevier.com/locate/foodchem

The effect of frozen storage on the functional properties of the muscle of volador (*Illex coindetii*)

C. Ruiz-Capillas*, A. Moral, J. Morales, P. Montero

Instituto del Frío, (C.S.I.C.) Ciudad Universitaria, 28040 Madrid, Spain

Received 7 August 2001; received in revised form 21 November 2001; accepted 21 November 2001

Abstract

Functional properties of muscle proteins of volador (*Illex coindetii*) were evaluated during frozen storage and classified according to gender and anatomical part of the animal. Solubility of protein in 5% NaCl, in all lots, showed a significant increase in the initial months and then a decrease. This solubility was generally greater in the mantles than in the arms. The viscosity was initially very high and fell rapidly, and there were no significant differences between the lots. This initial viscosity was greater in the arm lots than in the mantle ones. Likewise, extracts of the muscle of the arms also had the greatest initial emulsifying capacity values ($P \leqslant 0.05$). Soluble collagen, in an acid medium-exhibited a similar trend in all lots, throughout frozen storage. At the early stages of storage, soluble collagen remained stable or increased slightly, and then tended to become insoluble. The lowest solubilities were for the muscles of the arms. Myofibrillar protein and collagen solubilities, as well as emulsifying capacities were effective for detecting molecular changes in the proteins during frozen storage and results showed that, the volador mantles were more suited to frozen storage than the arms.

Keywords: Volador; Muscle; Viscosity; Emulsifying capacity; Myofibrillar; Collagen; Solubility; Frozen storage

1. Introduction

Since the 1960s the cephalopod industry, with refrigerator ships, has been important in the waters off the north-east coast of Africa (Lozano-Soldevilla, 1992). Among the wide variety of species of cephalopods caught, volador (*Illex coindetii*) is the one of greatest commercial and economic importance in Spain. When cephalopods are stored frozen, a series of significant changes occur in the flesh: mainly myofibrillar protein denaturation and aggregation and decreased protein functionality, which result in reduced water-binding capacity and changes in texture (Matsumoto, 1980; Srikar & Reddy, 1991). These changes are extremely important for the subsequent technological treatment of this species and they are associated with its spoilage because they determine the length of its storage time. Although protein changes during frozen storage have been mainly attributed to myofibrillar proteins, there are studies that indicate that changes also occur in the sarcoplasmic proteins (LeBlanc & LeBlanc, 1992) and in the connective tissue proteins, and in the latter there is collagen aggregation and cross-linking (Borderías & Montero, 1985; Montero & Borderías, 1990a, 1990b). All the changes occurring in proteins during frozen storage, as a result of the aforementioned factors, appear as changes in the functional properties of proteins. Thus, a number of authors observe that both the solubility and apparent viscosity, and even the emulsifying capacity are closely related to the changes that occur in muscle proteins and, depending on the extent of these changes, one or other technique will be more appropriate for detecting them (Borderías, Jiménez-Colmenero & Tejada, 1985; Jiménez-Colmenero, Tejada & Borderías, 1988; Matsumoto, 1980; Montero & Borderías, 1990a, 1990b). However, these kinds of studies have been done in fish: mainly, hake, sardine and trout, and no references have been found on functional properties or changes in cephalopod proteins during either iced or frozen storage.

On the other hand, only few studies have been performed on the physiological and anatomical characteristics of cephalopods and their relation to possible

* Corresponding author. Tel.: +34-9-1449-2300; fax: +34-9-1549-3627.

E-mail address: claudia@if.csic.es (C. Ruiz-Capillas).

0308-8146/02/$ - see front matter
PII: S0308-8146(01)00392-2

technological applications. Thus, in this study, we consider possible changes in the muscle attributable to sex, since Mangold (1987) in a study on the physiology of cephalopods indicated that the short time in which females reach sexual maturation can produce considerable changes in muscle proteins. We have also considered the anatomical location of the muscle: the mantle or the arms, with a distinct physiological function in the live animal, which can have a distinct effect on the storage of each muscle. Finally, the storage of cephalopod containing viscera is very common and, since the viscera can alter the muscle proteins during frozen storage and later when the muscle is defrosted, it is another factor that has been considered.

The present work therefore sets out to identify the behaviour of volador muscle during frozen storage by considering the changes in some of the functional properties of its proteins: protein soluble in 5% NaCl, apparent viscosity, emulsifying capacity and collagen solubility in acetic acid. The study was done on mantle and arm specimens of males and females gutted and ungutted.

2. Materials and methods

2.1. Materials

The species of cephalopods used for this study was volador (*I. coindetii*, Vérany, 1839): a batch of 200 kg. This species was caught by dragnet from the Galician platform. The cephalopods were placed in boxes with ice and when they reached port, they were taken to the Instituto del Frío in an isothermal lorry, where they arrived a day later. At the laboratory, the batch was divided into male (M) and female (H). The average length and weight of the males (M) were 156 mm and 107.3 g, respectively, and the females (H) 157 mm and 111.3 g, respectively. Each lot was further divided, one had gutted specimens (e), flushed with sea water and the other had ungutted specimens (w). Then the lots were divided again according to the anatomical region into mantles and arms. In the lot of gutted specimens, the male and female arms were mixed to form just one lot (e-arm), since, for this species, the anatomical region with the greatest commercial interest is the mantle. There were eventually seven lots (M-w-mantle, M-w-arm, H-w-mantle, H-w-arm, M-e-mantle, H-e-mantle, e-arm). About 600–1000 g of at least 10 specimens from each lot were packed in polythene, and they were frozen in a cupboard with horizontal plates (Sabroe brand Aarhus model. Denmark) at −35 °C until the temperature was −20 °C in the thermal centre and storage was in a forced-convection chamber at −20 °C. The frozen storage study was done over 16 months.

2.2. Sample preparation

One day before the analysis, the lot that was going to be analysed was transferred to a refrigeration chamber at 0/2 °C to be defrosted.

2.3. Protein solubility in 5% NaCl

Soluble protein was determined using a modification of the technique of Ironside and Love (1958). This consisted basically in homogenizing two grammes of minced muscle with 40 ml NaCl (5% pH 7–7.5), for 1 min in an Ultra-Turrax macerator. This was followed by centrifuging at 10,000×***g*** for 30 min. The process was repeated twice more. The supernatants were mixed to make up the 5% NaCl-soluble fraction. Following separation of the soluble and insoluble proteins, the amount of nitrogen was determined by the Kjeldahl method, following the procedure of the AOAC (1995). The entire process was conducted at 0–4 °C. Results were averages of at least three replicates.

2.4. Isolation of collagen

The connective tissue was isolated according to the method of Borderías and Montero (1985), involving removal of noncollagenous material by extensive extraction with 0.14M NaCl and 0.05M proteases inhibitors [1 mM phenylmethanesulfonyl fluoride (PMSF), 1 mM *p*-chloromercuribenzoate, and 10 mM ethylenediaminetetraacetic acid (EDTA) and stirring during 24 h, repeated at least three times.

2.5. Solubilization of collagen

The solubility of the isolated connective tissue in acetic acid was determined by the method of Borderías and Montero (1985), with some modifications. Basically, it consisted of homogenizing 2 g of connective tissue with 0.5M acetic acid in an Ultra-turrax macerator (ratio 1:25 w/v) for 1 min, containing 1 mM phenylmethanesulfonyl fluoride (PMSF), 1 mM *p*-chloromercuribenzoate, and 10 mM ethylene-diaminetetraacetic acid (EDTA). The mixture was continuously stirred for 24 h at 2–4 °C and then centrifuged at 35,000 ***g*** for 1 h. The process was carried out three times, and the supernatants were combined. These supernatants contained the acid-soluble collagen. The results, the averages of at least three replicates, were expressed as percent collagen solubilized in acid, which was calculated by determining the hydroxyproline in the soluble fraction, and the insoluble fractions, according to the method of Leach (1960). The entire process was carried out at 0–4 °C.

2.6. Apparent viscosity

The apparent viscosity was determined with the technique devised by Borderías et al., (1985) and modified by Moral, Ruiz-Capillas, Morales, and López (1994). In this technique, 50 g of the sample was homogenised with 200 ml distilled water, adjusting the pH to 5.7–5.8 before and after homogenisation, and then the homogenate was centrifuged. The precipitate obtained was suspended in 5% NaCl, pH 6.8–6.9, homogenised and filtered through a gauze. The filtrate was left at 0–4 °C for 45–60 min, and then the viscosity was measured; three readings were taken at 3-min intervals, with spindle number 3, and speed of 12 rpm, using a Brookfield-LV viscometer (Brookfield Engineering NABS, Stoughton, Mas, USA). All of the process was done in the cold (0–4 °C). The values obtained were expressed in centipoises (cP) and the results were the averages of at least three replicates.

2.7. Emulsifying capacity

Emulsifying capacity was determined, with the technique of Tejada, Borderías, and Jiménez-Colmenero (1987), on the extract obtained, to determine apparent viscosity. Twenty grammes of this extract was homogenised with 80 ml of distilled water and 16.8 g of oil in an Osterizer homogeniser, which had an automatic system for adding the oil. When the emulsion breaks, there is an increase in its resistance which is measured by an ohmmeter and this stops further oil being added. The results were expressed in grammes of emulsified oil for 20 grammes of extract. The results were the averages of at least three replicates

2.8. Statistical analysis of results

Two-way analysis of variance (ANOVA) was carried out to determine differences. The mean differences between pairs were resolved by confidence intervals, using a least significance range test at ($P \leqslant 0.05$). The computer program used was Statgraphics (STATC Inc., Rockville, MD).

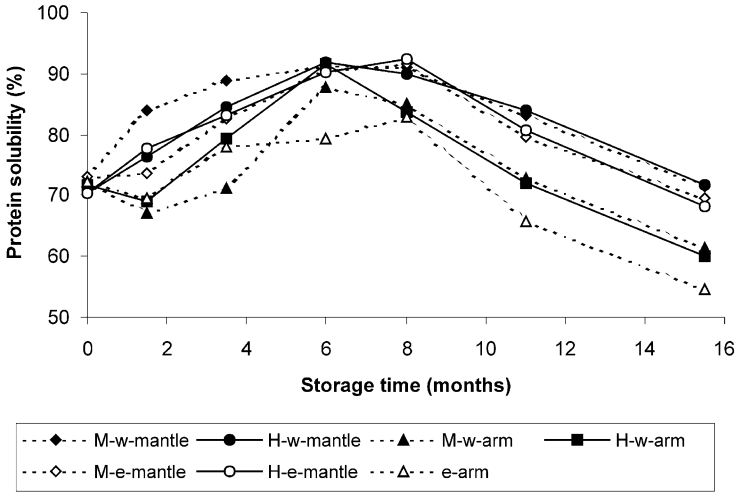
3. Results and discussion

3.1. Protein soluble in 5% NaCl

Initial protein solubility in 5% NaCl (Fig. 1) was similar in all the lots and it had values higher than 60% throughout frozen storage. All the lots exhibited a significant increase in solubility in the initial months, and then protein solubility fell, as is characteristic of protein aggregation. An initial increase was also observed on frozen storage of sardine muscle (Montero, Gómez-Guillén, & Borderías, 1996). This could be due to the fact that, at the early stages of storage, there is, as a result of the formation of ice crystals, a redistribution of hydrogen bridges and hydrophobic interactions, leading to denaturation, which is seen as increased solubility; this denaturation precedes progressive aggregation (Matsumoto, 1980). This behaviour could also result from the intense activity of cephalopod muscle enzymes which, in squid and octopus, is three and seven times greater than in fish muscle (Hurtado, Borderías & Montero, 1999). This enzyme activity could partly be responsible for keeping the protein solubility values high due to its effect on the protein-extracting processes (Iguchi, Tsuchiya & Matsumoto, 1981; Ke, Smith-Lall & Pond, 1981; Kolakowska, Kwiatrowska, Lachowicz, Gajowiecki, & Bortnowska, 1992). Moral, Tejada and Borderías (1983) also obtained protein solubility figures of 61% or higher for squid muscle (*Loligo vulgaris*) after 13.5 months of frozen storage and they showed that protein solubility was beginning to fall slightly after 4.5–6 months of frozen storage. Iguchi et al. (1981) did not notice any great changes in the evolution of soluble protein in the mantle of pota (*Ommastrephes sloani pacificus*) during frozen storage. Yet Paredi and Crupkin (1997) did observe a decrease in actomyosin, and also in volador (*I. argentinus*) throughout frozen storage. However, in fish, an evolution in protein solubility similar to that determined in volador has not been observed (Jiménez-Colmenero & Borderías, 1983; Montero & Borderias, 1990a). As regards the evolution in mantles, the lots that were gutted (open symbols) showed very similar behaviour, and there were no significant differences between males and females. However, in ungutted mantles during the early stages of storage, the male mantles were more soluble than the female ones and, at the sixth month, the behaviour was similar ($P \leqslant 0.05$). Probably, this high degree of solubility in ungutted males (between 80 and 90%) could have been affected by the breaking of the viscera during storage and the transfer of some enzymes to the muscle. The evolution in protein solubility in the arms (Fig. 1) generally exhibited lower values than in the mantle. Significant differences were detected in the ungutted volador lots in the first analyisis where the female arms were more soluble. At the eighth month there were no differences ($P \leqslant 0.05$) between these lots and there was a progressive decrease. Ungutted arm solubility showed the lowest values throughout storage.

3.2. Apparent viscosity (η_{app})

Apparent viscosity of the proteins obtained from the male and female volador mantle lots, homogenised with 5% NaCl, did not show any significant differences between the gutted and ungutted lots, throughout frozen storage (Fig. 2). This initial viscosity, in turn, was



	Storage time (months)						
Lots	0	1.5	3.5	6	8	11	15.5
M-w-mantle	1/a	1/b	1/c	1,2/c	1/c	1/b	1/a
H-w-mantle	1/a	2,4/b	2/c	1/d	1/d	2/c	2/a
M-w-arm	1/a	3/b	3/a	2/c	2/c	3/a	3/d
H-w-arm	1/a	3/a	4/b	1/c	2/d	3/a	3/e
M-e-mantle	1/ae	2/a	4/b	3/c	1/d	4/bc	1/e
H-e-mantle	1/a	4/b	2/c	1,2/d	1/d	1,4/b,c	1/a
e-arm	1/a	3/a	4/b	3/b	2/c	5/d	4/e

M, H, w, eviscerated: male, female, whole. Different letters in the same row indicate significant differences between the storage months ($p \leq 0.05$). Different numbers in the same column indicate significant differences between the different treatments ($p \leq 0.05$).

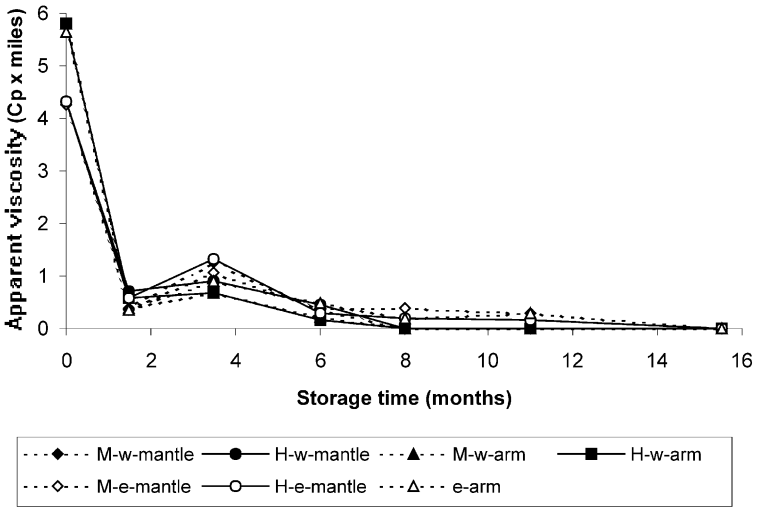
Fig. 1. Soluble protein (%) in muscle of volador during frozen storage.

significantly different between the arm and mantle lots, and it was very high in the arm lots. In all cases, viscosity was initially very high, but it was very unstable and fell rapidly. After a slight increase ($P \leqslant 0.05$) between the second and fourth month, it decreased completely. These small increases in viscosity have previously been detected by authors for this type of fish, and possibly could be attributed to the initial protein aggregation with few cross-linkings, which gives rise to large particles that continue to possess a high water-binding capacity (Borderías, Jiménez-Colmenero & Tejada, 1985); particle size may have a greater influence on viscosity at this stage. This drastic reduction in apparent viscosity, because of freezing, has also been observed by different authors in protein extracts of hake, chicken and pork muscle (Noguchi & Matsumoto, 1971; Umemoto, Kana, & Ixata, 1971).

Moreover, freezing and frozen storage could cause changes in myofibrillar protein conformation in the saline extract, which would cause a decrease in the viscosity values because of the reduced protein axial relation, and there could also be a greater dissociation of actomyosin into myosin and actin which would give low viscosity figures, as occurs in chicken and hake actomyosin (Cofrades, Careche, Carballo, & Jiménez-Colmenero, 1996).

3.3. Emulsifying capacity

The evolution in the emulsifying capacity in myofibrillar proteins during frozen storage was very different between the lots (Fig. 3), which basically suggests that this technique is more appropriate for detecting changes in the muscle proteins of this species. As occurred with apparent viscosity, the arm muscle had the greatest initial values ($P \leqslant 0.05$). All the arm lots, either gutted or ungutted, male or female, showed a gradual decrease ($P \leqslant 0.05$) throughout storage, losing about 25% of their functionality. However, evolution in the emulsifying capacity was different between the gutted and ungutted mantle lots. The gutted lots exhibited a decrease (like the arm muscle), although the loss in functionality after a year was less (around 17%). This seems to indicate that, in these lots, there was protein aggregation that reduced the emulsifying capacity. Jiménez-Colmenero and Borderías (1983) indicated that the emulsifying



	Storage time (months)					
Lots	***0***	***1.5***	***3.5***	***6***	***8***	***11***
M-w-mantle	1/a	1/b	1/c	1,2/b	---	---
H-w-mantle	1/a	2/bc	2,3/b	1/c	---	---
M-w-arm	2/a	1/bc	3/b	1,2/c	---	---
H-w-arm	2/a	1,2/b	3/b	2/c	---	---
M-e-mantle	1/a	1/b	1,2/c	1,2/b	1/b	1/b
H-e-mantle	1/a	1,2/b	1/c	1,2/bd	1/d	1/d
e-arm	2/a	1/b	2,3/c	1/b	1/b	1/b

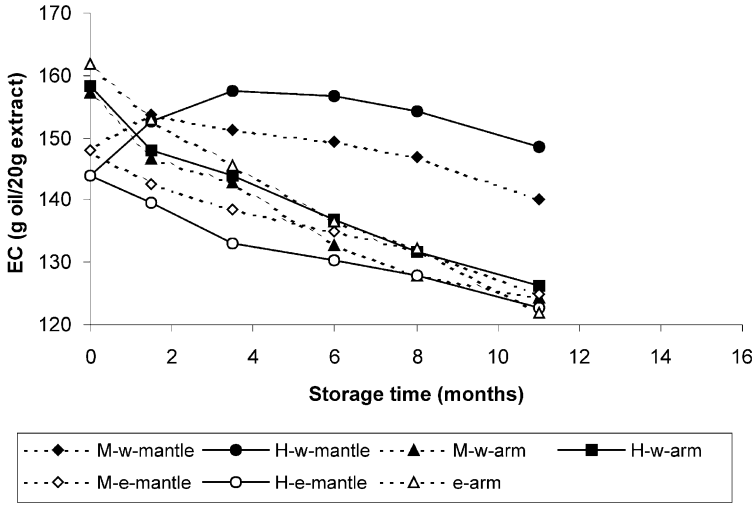
M, H, w, eviscerated: male, female, whole. Different letters in the same row indicate significant differences between the storage months ($p \leq 0.05$). Different numbers in the same column indicate significant differences between the different treatments ($p \leq 0.05$).

Fig. 2. Apparent viscosity (η_{app}) (cP × 1000) in extract of volador during frozen storage.

capacity in fish depends on protein solubility and it is more appropriate than solubility as a source of information on structural changes that take place in the protein molecules. However, the ungutted mantle lots showed a slight increase, only significant in some instances, as indicated in the tabulated part of Fig. 3. The fact that, in these lots, protein aggregation during storage was slower, may be due to the enzyme action by the visceral mass components that have penetrated the muscle. The emulsifying capacity figures found in this work were lower than those obtained by other authors for different species of fish, chicken and pork, which were from 200–230 g emulsified oil/20 g extract (Tejada et al., 1987). This may be due to the fact that the emulsifying capacity control was done on an extract obtained using the viscosity technique, where a specimen is flushed first with water to eliminate the non-protein nitrogenous fraction, low molecular weight peptides and water-soluble proteins, and the homogenate is also filtered to separate the protein aggregates and stroma proteins which affect the emulsifying capacity. The absence of these proteins although they are very few, means that the values given are lower, since there are studies that show that sarcoplasmic proteins intervene in the emulsifying capacity (Borderías & Montero, 1985; Tsai, Cassens, & Briskey, 1972). However, the evolution in volador emulsifying capacity is similar to that found in fish and chicken by other authors, who have observed that the evolution in emulsifying capacity decreased slightly throughout frozen storage (Jiménez-Colmenero et al., 1988; Tejada et al., 1987).

4. Collagen solubility in acid

Soluble collagen in an acid medium exhibited a similar trend in all the lots throughout storage (Fig. 4). In the early periods of storage, it remained stable or increased slightly, and then it tended to become insoluble, although this behaviour was different for each lot. Thus the lots that were more insoluble right from the start, around 10–15%, were the arm muscle ones. Furthermore, there were some differences ($P \leqslant 0.05$); the gutted male arm collagen was the most insoluble in the early stages of storage. In the gutted mantles there was a minor initial difference; however this was



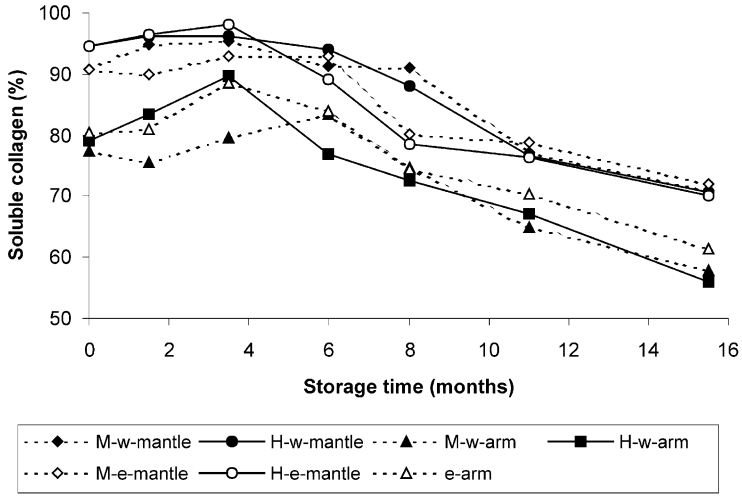
	Storage time (months)					
Lots	***0***	***1.5***	***3.5***	***6***	***8***	***11***
M-w-mantle	1/a	3/a	3,4/a	2/a	2/a	2/a
H-w-mantle	1/a	3/b	4/b	2/b	2/b	2/b
M-w-arm	2/a	3/a	2/c	1/d	1/d	1/d
H-w-arm	2/a	1,3/b	2,3/bc	1/c	1/c	1/c
M-e-mantle	1/a	1,2/ab	1,2/bc	1/c	1/cd	1/d
H-e-mantle	1/a	2/ab	1/bc	1/c	1/cd	1/d
e-arm	2/a	3/b	2,3/c	1/d	1/d	1/e

M, H, w, eviscerated: male, female, whole. Different letters in the same row indicate significant differences between the storage months ($p \leq 0.05$). Different numbers in the same column indicate significant differences between the different treatments ($p \leq 0.05$).

Fig. 3. Emulsifying capacity (EC) in extract of volador during frozen storage.

significant ($P \leqslant 0.05$) between the male (with less soluble collagen) and the female, and remained like this until the sixth month. However, in the ungutted lots these initial differences were not observed during frozen storage. Throughout storage there were hardly any differences between the gutted and ungutted lots, and this could be due to fact that the influence of the visceral proteases is not very great since the collagen can only be attacked by a small group of enzymes (collagenase and pepsin). Generally, it also seems that the degree of insolubility in the mantle collagen is lower (around 18%) with respect to the arms, where it is around 25%, on average.

No studies have been found on the behaviour of cephalopod collagen solubility in an acetic acid medium (0.5M) during frozen storage and there are hardly any studies on other kinds of fish like hake and trout (Montero & Borderías, 1990a, 1990b). The collagen insolubility values in acetic acid (0.5M) obtained in hake and trout were initially similar to those found for volador in this study. Yet the increase in insolubility was greater in the former than in volador. Moreover, the solubility was more apparent in hake than in trout, and this could be due to the progressive presence of formaldehyde in hake stored frozen, which would promote a greater degree of aggregation in the collagen molecules. The decrease in collagen solubility during frozen storage could be due to the formation of stable acid and keto-imine heat links (Bailey, 1974). In cephalopods at the beginning, most of the collagen links are labile to acids, i.e. they are non-cross-linking molecules and fibres joined by aldimin links that are not very stable in low pH conditions, although they are resistant to the action of saline solutions (Miller & Rhodes, 1982). It has been shown that the predominant link in cephalopod collagen (*Loligo vulgaris* and *Sepia officinalis*) is the aldimine dehydro-hydroxy-lysine-norleucine (dHLN) one. In cephalopod collagen, there are between 8 and 10 times more reducible links than in mammal in collagen, which would explain the high collagen solubility of this species in acid (Sikorski, 1984).



	Storage time (months)						
Lots	***0***	***1.5***	***3.5***	***6***	***8***	***11***	***15.5***
M-w-mantle	1/a	2/b	1,2/b	1,2/a	3/a	1/c	1/d
H-w-mantle	2/a	2/a	2/a	1/a	3/b	1/c	1/d
M-w-arm	3/a	4/a	4/b	3/c	2/a	3/d	3/e
H-w-arm	3/a	3/b	3/c	4/a	2/d	3/e	3/f
M-e-mantle	1/a	1/ab	1/b	1/b	1/c	1/c	1/d
H-e-mantle	2/a	2/bc	2/c	2/d	1/e	1/e	1/f
e-arm	3/a	3/ac	3/b	3/c	2/d	2/e	2/f

M, H, w, eviscerated: male, female, whole. Different letters in the same row indicate significant differences between the storage months ($p \leq 0.05$). Different numbers in the same column indicate significant differences between the different treatments ($p \leq 0.05$).

Fig. 4. Soluble collagen (%) in 0.5 m acetic acid of volador muscle during frozen stage.

5. Conclusions

From the functional properties studied (soluble protein, viscosity, emulsifying capacity and collagen solubility in acid) it appears that the volador mantles are better suited to frozen storage than the arms, and no significant differences were detected between males and females. The mantles stored with viscera, often had greater myofibrillar protein solubility and emulsifying capacity, which indicates a lower degree of protein aggregation. Myofibrillar protein and collagen solubilities, as well as the emulsifying capacity, were effective for detecting molecular changes in the proteins during frozen storage. On the other hand, apparent viscosity was unsuitable for studying changes in this species.

Acknowledgements

This research was financed by the EU under project TS3*-CT92–0109 and by Comisión Interministerial Científica y Técnica under project ALI93–1146-CO2–01-CE.

References

AOAC. (1995). *Official methods of analysis*. 16th ed. Arlington, VA: Association of Official Analytical Chemistry.

Bailey, A. J. (1974). Collagen: general introduction. In E. D. T. Atkins, & A. Keller (Eds.), *Structure of fibrous biopolymers* (pp. 115). Londres: Butterworth.

Borderías, A. J., & Montero, P. (1985). Changes in fish muscle collagen during frozen storage. Storage lives of chilled and frozen fish and fish products. In: International Institute of Refrigeration, Ed. *Storages lives of fresh and frozen fish and fish products* (pp. 85–92). Aberdeen, UK: Refrigeration Science and Technology, pp 85–92.

Borderías, A. J., Jiménez-Colmenero, F., & Tejada, M. (1985). Viscosity and emulsifying ability of fish and chicken muscle protein. *Journal of Food Technology*, *20*, 31–42.

Cofrades, S., Careche, M., Carballo, J., & Jiménez-Colmenero, F. (1996). Freezing and frozen stored of actomyosin from different species. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, *203*, 316–319.

Hurtado, J. L., Borderías, J., Montero, P., & An, H. (1999). Characterization of proteolitic activity in octopus (*Octopus vulgaris*) arm muscle. *Journal of Food Biochemistry*, *23*, 469–483.

Iguchi, S. M. M., Tsuchiya, T., & Matsumoto, J. J. (1981). Studies on the freeze denaturation of squid actomyosin. *Nippon Suisan Gakkaishi*, *47*, 1499–1506.

Ironside, J. I. M., & Love, R. M. (1958). Studies on protein denaturation

in frozen fish. I. Biological factors influencing the amounts of soluble and insoluble protein present in the muscle of North Sea Cod. *Journal of the Science of Food and Agriculture*, *9*, 597–617.

Jiménez-Colmenero, F., & Borderías, A. J. (1983). A study of the effects of frozen storage of certain functional properties of meat and fish protein. *Journal of Food Technology*, *18*, 731–737.

Jiménez-Colmenero, F., Tejada, M., & Borderías, A. J. (1988). Effect of seasonal variations on protein functional properties of fish during frozen storage. *Journal of Food Biochemistry*, *12*, 159–170.

Ke, P. J., Smith-Lall, B., & Pond, G. R. (1981). Quality enhancement of Canadian Atlantic squid (*Illex illecebrosus*) by various low temperature operations. In: International Institute of Refrigeration, Ed. *Advances in the refrigerated treatment of fish* (pp. 513–516). Aberdeen, UK: Refrigeration Science and Technology.

Kolakowska, A., Kwiatrowska, L., Lachowicz, K., Gajowiecki, L., & Bortnowska, G. (1992). Effect of fishing season on frozen storage quality of baltic herring. In G. Bligh (Ed.), *Seafood science and technology* (pp. 269–277). London: Fishing News Book.

Leach, A. A. (1960). Notes on a modification of the Newman and Logan method for the determination of hydroxyproline. *Biochemistry Journal*, *74*, 70–71.

LeBlanc, E. L., & LeBlanc, R. J. (1992). Determination of hydrophobicity and reactive groups in proteins of cod (*Gadus morhua*) muscle during frozen storage. *Food Chemestry*, *43*, 3–11.

Lozano-Soldevilla, F. (1992). Aspectos bromatológicos del volador (*Illex coindetii*) en aguas del Banco Sahariano. *Alimentaria, Septiembre*, 51–53.

Mangold, K. (1987). Reproduction. In P. R. Boyle (Ed.), *Cephalopods Life Cycles. Vol II. Comparative reviews* (pp. 157–200). London: Academic Press.

Matsumoto, J. J. (1980). Chemical deterioration of muscle proteins during frozen storage. In J. R. Whitaker, & M. Fujimaki (Eds.), *Chemical deterioration of muscle proteins* (pp. 95–124). Washington, DC: ACS Symposium Series 123; American Chemical Society.

Miller, E. J., & Rhodes, D. K. (1982). Preparation and characterization of the different types of collagen. In L. W. Cunningham, & L. W. Frederiksen (Eds.), *Methods in enzymology. Vol 82. Structural and Contractile Proteins* (pp. 33–64). London: Academic Press.

Montero, P., & Borderías, A. J. (1990a). Behaviour of myofibrillar proteins and collagen in hake (*Merluccius merluccius L*) muscle during frozen storage and its effect on texture. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, *190*, 112–117.

Montero, P., & Borderías, A. J. (1990b). Comportamiento de las proteínas miofibrilares y del colágeno del músculo de trucha (Salmo irideus Gibb) durante su conservación en estado congelado y su implicación en la textura. *Revista de Agroquímica y Tecnologia de los Alimentos*, *30*, 377–386.

Montero, P., Gómez-Guillén, M. C., & Borderias, J. (1996). Influence of subspecies, season and stabilization procedures in gel-forming ability of frozen minced muscle of sardine (*Sardina pilchardus*). *Food Science and Technology International*, *2*, 111–122.

Moral, A., Ruiz-Capillas, C., Morales, J. & López, M.E. (1994). A contribution to determining apparent viscosity in cephalopods. In: 24th *Western European Fish Technologists Association (WEFTA) meeting*. Nantes, (France).

Moral, A., Tejada, M., & Borderías, A. J. (1983). Frozen storage behaviour of squid (*Loligo vulgaris*). *International Journal of Refrigeration*, *6*, 54–57.

Noguchi, S., & Matsumoto, J. J. (1971). Studies on the control of denaturation of fish muscle proteins during frozen storage 2. Preventive effect of amino-acids and related compounds. *Nippon Suisan Gakkaishi*, *37*, 1115.

Paredi, M. E., & Crupkin, M. (1997). Biochemical properties of actomyosin from frozen stored mantles of squid (*Illex argentinus*) at different sexual maturation stages. *Journal of Agriculture and Food Chemestry*, *45*, 1629–1632.

Sikorski, Z. E. (1984). The role of collagen in the quality and processing of fish. *Critical Reviews in Food Science and Nutrition*, *20*, 301–334.

Srikar, L. N., & Reddy, G. V. S. (1991). Protein solubility and emulsifying capacity in frozen stored fish mince. *Journal of the Science of Food and Agriculture*, *55*, 447–453.

Tejada, M., Borderías, A.J., Jiménez-Colmenero, F. (1987). Myofibrillar and sarcoplasmic proteins: their role in alterations in certain functional properties of muscle during frozen storage. In: *Proceedings of the IUFoST international symposium chemical changes during food processing*. Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Valencia (Spain).

Tsai, R., Cassens, R. G., & Briskey, E. J. (1972). Emulsifying properties of purified muscle proteins. *Journal of Food Science*, *37*, 286–288.

Umemoto, S., Kana, K., & Ixata, K. (1971). Studies on quality of frozen stored alaskapollack surimi. 2. Changes inextractable protein of surimi during cold storage. *Nippon Suisan Gakkaishi*, *37*, 1100.