Emulsifying and Gelation Properties during Freezing and Frozen Storage of Hake, Pork, and Chicken Actomyosins As Affected by Addition of Formaldehyde

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This paper describes an experiment designed to analyze the influence of freezing and frozen storage on aromatic hydrophobicity and emulsifying and thermal gelation properties of natural actomyosin (NAM) derived from myosystems with differing stability to low temperatures (chicken, pork, and hake). The effect of formaldehyde on these parameters was also studied. It was found that hydrophobicity, emulsifying activity index, and gel strength of the natural actomyosin were generally influenced by the species, the freezing and storage process, and the presence of formaldehyde. Chicken NAM proved the most stable to both formaldehyde and low temperatures. Changes in the characteristics of chicken actomyosin induced during freezing and frozen storage favored greater gel strength.

Keywords: *Actomyosin; frozen storage; functional properties; hydrophobicity; formaldehyde*

INTRODUCTION

The emulsifying and gel-forming properties of myofibrillar proteins (essentially myosin and actomyosin) are extremely important in terms both of the development of technological processes and of the final characteristics of products based on meat and fish muscle (Asghar et al., 1985; Nakai and Li-Chan, 1988; Acton and Dick, 1989).

Freezing and frozen storage cause certain alterations in the characteristics of meat and fish muscle proteins, which produce a decline in functional properties, including viscosity, emulsifying ability, and gel-forming capacity (Matsumoto, 1980; Jiménez Colmenero and Borderias, 1983; Morrissey et al., 1987). This loss of protein functionality reduces the product's suitability for subsequent use in processing (Awad et al., 1968; Miller et al., 1980). Such undesirable phenomena are caused essentially by denaturation and aggregation of myofibrillar proteins, chiefly actomyosin (Matsumoto, 1980; Tejada et al., 1996). In fact freezing/thawing has been found to increase the net surface hydrophobicity of proteins with or without loss of solubility (Hayakawa and Nakai, 1985; Li-Chan et al., 1985; Niwa et al., 1986a,b). However, the intensity and speed of such alterations is not the same in all actomyosin systems. Muscle proteins of fish and other marine animals are less stable to freezing than proteins from mammals and poultry (Matsumoto, 1980; Jiménez Colmenero and Borderías, 1983). The loss of protein functionality in some species during frozen storage has been partly attributed to the effect of formaldehyde (FA) (Gill et al., 1979; Poulter and Lawrie, 1979; Owusu-Ansah and Hultin, 1987; Careche and Tejada, 1990a) formed from demethylation of trimethylamine oxide, a compound present in most marine species. The mechanism through which FA affects the proteins is not fully understood, although work has been done on the nature of the aggregation process and the types of bonds involved in formaldehyde-forming species (Connell, 1975; Dingle et al., 1977; Gill et al., 1979; Owusu-Ansah and Hultin, 1987; Ang and Hultin, 1989; Ragarnsson and Regenstein, 1989; Del Mazo et al., 1994; Tejada et al., 1996; Careche and Li-Chan, 1997). In myosin isolated from cod (Ang and Hultin, 1989) and in hake natural actomyosin (NAM) (Del Mazo et al., 1994), FA accelerated the formation of high-molecular weight polymers. In cod myosin it has been reported (Careche and Li-Chan, 1997) that addition of FA produces changes in the secondary structure of the myosin, causes exposure of the hydrophobic aliphatic groups, and eventually leads to the appearance of covalent cross-links.

It is known that fish myosin and actomyosin have higher net surface hydrophobicity than mammalian or avian proteins (Li-Chan et al., 1985). Niwa et al. (1986a,b) attributed this to the more open and hence less stable structure of fish proteins. Considering these differences between meat and fish proteins, it would be most useful to analyze the effect of formaldehyde addition on actomyosin from different myosystems (both FA-forming and non-FA-forming), as this would help clarify aspects relating to the intrinsic functionality of muscle proteins and also the mechanisms by which FA acts.

This work pursued two objectives: first to analyze the gelling and emulsifying properties during freezing and frozen storage with reference to changes in net surface hydrophobicity and solubility of actomyosin from species having different functional stability at low temperatures and second to compare the changes in these parameters in the presence of a denaturing and aggregating agent like FA in order to arrive at a better understanding of the mechanics of FA action.

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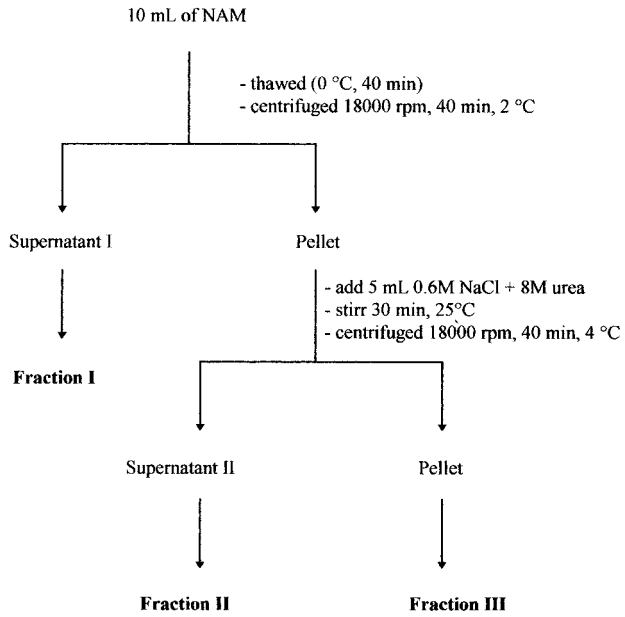

Figure 1. Flow diagram for preparation of the different protein fractions of NAM from chicken, pork, and hake NAM.

MATERIALS AND METHODS

Myosystems and Preparation of Actomyosin. The study was carried out on natural actomyosin (NAM) from hake (Merluccius merluccius L.), pork (longissimus dorsi) and chicken (mixture of pectoralis major and pectoralis minor muscles), analyzed in postrigor state. Chicken and pork muscles were purchased at a local market and had pH's of 5.6 and 5.8, respectively. Hake were caught in the Cantabric sea, gutted, and kept in ice until reaching the lab (within 48 h), the pH of the muscle was 6.7. NAM, isolated according to Kawashima et al. (1973), was extracted from 300 g of a homogenate of the above-mentioned muscles taken from at least three individuals of each species. All work was performed at 2−4 °C. Protein concentration was determined by microbiuret method (Itzhaki and Gill, 1964). The extracted natural actomyosin was adjusted to 10 mg/mL, 0.6 M NaCl, pH 7.0, with 50 mM tris-maleic buffer (pH 7.0), to study hydrophobicity and emulsifying activity index (EAI), and to 15.0 mg/mL, 0.15 M NaCl, and pH 6.0 (conditions achieved by dialysis), for the study of gel-forming properties. These conditions were chosen as optimum on the basis of previous findings (Cofrades et al., 1996a,b, 1997). Two different types of model system were made, one consisting solely of the protein extract (control lot) and the other containing the protein extract plus 200 ppm of formaldehyde (FA) added to the buffer solution used to adjust protein concentration. The conditions for addition of formaldehyde were established to prevent locally high concentrations of FA.

For each species aliquots of the control and FA-treated samples (10 mL placed in sealed plastic tubes for hydrophobicity and EAI and 10.0 g placed in plastic jars, 23-mm diameter and 25-mm height, for examination of gels) were frozen at −24 °C (± 2 °C) and stored at −12 °C (± 2 °C) for up to 17 weeks.

For analysis, samples were thawed in ice water (40 min) and the parameters listed below were determined. Two replicates were made for all analyses, and in each replicate all variables except gel strength were measured in duplicate.

Protein Solubility. Solubility of NAM in 0.6 M NaCl and 0.6 M NaCl + 8 M urea was performed as shown in Figure 1. Under these conditions three fractions were obtained: salt soluble protein (fraction I), urea-soluble protein (fraction II), and insoluble protein (fraction III); the latter was obtained by difference on the basis of the sum of fractions I and II. The protein content of fractions I and II was determined by using a microbiuret method according to Itzhaki and Gill (1964). Results are expressed as percent of soluble protein with respect to the total protein content.

Electrophoresis. SDS−PAGE electrophoresis of fresh actomyosin from the different species was performed with a vertical slab gel apparatus (Pharmacia LKB Biotechnology, Uppsala, Sweden), using 12.5% polyacrylamide gels according to Jiménez Colmenero et al. (1994). The samples were treated according to Hames (1985) (2% SDS, 5% β-mercaptoethanol, and 0.002% bromophenol blue) and then heated for 5 min in a boiling water bath. Samples were then centrifuged at 12 000 rpm for 1 min. Aliquots of 12.5 μL were applied to the gels, containing 1 mg/mL of the fresh NAM from each species.

Electrophoresis conditions were 20 mA/gel and 250 V. Protein bands were stained with Coomassie brillant blue and the bands analyzed on a 3CX image analyzer (Bio Image and Visage, Millipore Corp., Ann Arbor, MI). The molecular weight (MW) of the main proteins in the samples was estimated by comparing their mobility with that of a standard high-MW protein mix (Pharmacia LKB Biotechnology).

Aromatic Hydrophobicity (SoANS). Aromatic surface hydrophobicity was determined with 8-aniline-1-naphthalene sulfonic acid (ANS) according to Hayakawa and Nakai (1985). Twenty-microliters aliquots of a stock 8 mM solution were added to 4 mL of a protein solution with a concentration of 0.005−0.025 mg/mL. After they were mixed well by vortexing, the samples were incubated in a water bath at 25 °C. Fifteen minutes after adding the probe, the fluorescent intensity of each solution was measured, starting with the most diluted one. The excitation wavelength was 390 nm and the emission wavelength 470 nm. Measurements were performed on a Hitachi model F-2000 fluorometer (Japan). The fluorescent intensity of protein without ANS (protein blank) and a blank corresponding to the fluorescent intensity of the buffer + ANS were measured. The fluorescent intensity of each blank solution was subtracted from the fluorescent intensity of the protein with ANS. Measurement was continued until aggregation rendered it impossible. Surface hydrophobicity was expressed as the slope of the linear regression between the fluorescent intensity of the protein solutions and the protein concentration in arbitrary units.

Emulsifying Activity Index (EAI). This was determined on protein extracts adjusted to a concentration of 5.0 mg/mL, following the procedure of Pearce and Kinsella (1978) as modified by Li-Chan et al. (1984). Three milliliters of the NAM solution were homogenized (1800 rpm, 1 min) with 1 mL of olive oil in a microchamber connected to an Omnimixer. An aliquot was immediately taken from the bottom of the solution and diluted 200-fold with a solution of SDS (0.3%). This mixture was vortexed to obtain a uniform dispersion, from which absorbance was determined at 500 nm. Results were expressed in meters squared per gram.

Gel Strength (GS). A penetration test was used to determine gel strength. Gel preparation consisted of thermal treatment of jars (23-mm diameter and 25-mm height) at a heating rate of 1 °C/min up to 60 °C. Samples were heated in a water bath (Julabo Labortechnik GmbH, Seelbach, Germany) connected to a Julabo PRG1 temperature programmer. The gels were cooled in ice for 30 min, then immediately rheological analyses were conducted (2 ± 1 °C) using a stainless steel rod (diameter 5 mm) attached to a 100 N cell and connected to the head of an Instron model 4501 Universal texturometer (Instron Engineering Corp., Canton, MA). The rod penetrated 20 mm into the gels at a constant speed of 10 mm/min. The corresponding force−displacement curves were obtained and analyzed by using the Instron Series IX software and a Hewlett-Packard Vectra ES/12 computer. Gel strength was taken as work of penetration (in J × 10^{-6}), which was calculated as the area enclosed by the curve from the first moment of contact with the surface until the rupture point.

Statistical Analyses. Since it was not possible to complete the measurement of some of the parameters over the entire frozen storage period in two of the species, the same statistical treatment could not be applied to all the parameters. Thus, two sets of three-way analysis of variance were performed by using an F test, a first set to determine the effects of the freezing process, species, and addition of FA, and a second to determine the effects of frozen storage, species, and addition of FA in determination of the EAI and surface aromatic hydrophobicity. For the gel strength of chicken NAM, two sets of two-way ANOVAs were performed, one for the freezing

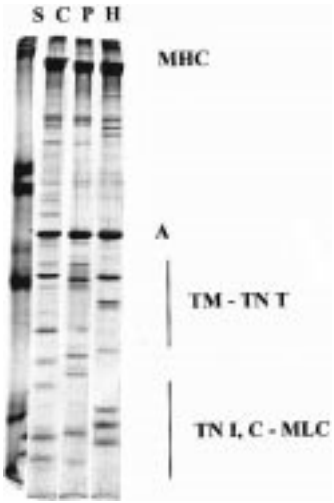

Figure 2. SDS−PAGE profiles of fresh natural actomyosin from chicken (C), pork (P), and hake (H). MHC, myosin heavy chain; A, actin; TM-TN T, tropomyosin and troponin T; TN I,C-MLC, troponins I and C and myosin light chains. (S) High molecular weight standards.

process and addition of FA and the other for frozen storage and addition of FA. In the case of pork NAM, two sets of analyses of variance were performed: one (one-way) to see the effect of the freezing and storage process on samples without FA and the other (two-way) to determine the effect of the storage process and addition of FA to pork NAM. The differences in means between pairs were resolved by LSD tests to obtain confidence intervals. Level of significance was set at $P < 0.05$. The statistical package used was Statgraphics statistical graphics system, version 5.0.

RESULTS AND DISCUSSION

The yield of NAM from pork, chicken, and hake was 7.2, 9.9, and 7.7 g/100 g of muscle, respectively. Electrophoretic profiles were quite similar for the three species, the majority bands being myosin heavy chain (MHC) and actin (Figure 2). A similar amount of MHC (expressed in arbitrary units, IOD) was found in pork and chicken NAM, and a slightly larger amount was found in hake (MHC IOD: 0.57−0.63 in chicken; 0.65−0.63 in pork; 0.75−0.78 in hake). The main differences in protein profiles were in the zone of tropomyosin and troponin-T and in the zone of troponin C and I and myosin light chain. Similar characteristics of NAM to that of these species have been reported by Jiménez Colmenero et al. (1994).

Protein Solubility. *Salt-Soluble Protein* (*0.6 M NaCl, Fraction I*). In the control samples, no differences ($P > 0.05$) were found in the levels of solubility exhibited by fresh (pre-freezing) NAM of chicken and hake (Table 1). Values were lower ($P < 0.05$) in pork NAM. Addition of FA to fresh NAM caused a decrease ($P < 0.05$) in salt-soluble protein in NAM of all the species considered. The decrease was greatest in hake NAM.

At the end of frozen storage, the amount of salt-soluble protein in the control samples differed according to species. While chicken NAM retained almost the same level ($P > 0.05$) as when fresh, the amount of protein in this fraction of hake NAM was 50% less (Table 1). The behavior of pork NAM was halfway between that of the other two. The fact that insolubilization of salt-soluble protein was greater in fish than in meat species agrees with the findings of Jiménez-Colmenero and Borderías (1983) on protein homogenates of chicken, pork, and blue whiting muscle stored for 8 months at −20 °C.

Table 1. Protein Solubility (%) in 0.6 M NaCl (Fraction I) and in 0.6 M NaCl + 8 M Urea (Fraction II) of Chicken, Pork, and Hake Natural Actomyosin (NAM) Fresh and after 17 Weeks of Frozen Storage (−12 °C), with and without Added Formaldehyde[a]

		fraction I		fraction II	
		fresh	17 weeks	fresh	17 weeks
chicken	control	$90.0^{a}{}_{1}$	$85.9^{a}{}_{1}$	$10.2^{abc}{}_{1}$	$16.7^{a}{}_{2}$
	FA	$72.9^{b}{}_{1}$	$29.7^{b}{}_{2}$	$8.24^{a}{}_{1}$	$54.7^{b}{}_{2}$
pork	control	$81.9^{c}{}_{1}$	$63.9^{c}{}_{2}$	$11.5^{c}{}_{1}$	$7.9^{c}{}_{2}$
	FA	$76.3^{b}{}_{1}$	$25.7^{b}{}_{2}$	$9.12^{ac}{}_{1}$	$8.8^{c}{}_{2}$
hake	control	$89.1^{a}{}_{1}$	$43.6^{d}{}_{2}$	$7.74^{a}{}_{1}$	$43.5^{d}{}_{2}$
	FA	$63.2^{d}{}_{1}$	$20.0^{e}{}_{2}$	$36.4^{d}{}_{1}$	$9.6^{c}{}_{2}$

[a] Control = samples without formaldehyde, FA = samples with added formaldehyde. Different letters in the same column and different numbers in the same row between each fraction indicate significant differences ($P < 0.05$).

Where FA was present, the protein content of fraction I was considerably lower by the end of storage ($P < 0.05$), particularly in hake. This loss of solubility was greater than in the controls (without FA) (Table 1). The insolubilizing effect of FA on frozen salt-soluble proteins is consistent with the findings of other authors (Del Mazo et al., 1994) in studies of fish protein in the presence of exogenous FA.

Urea-Soluble Protein (*0.6 M NaCl + 8 M Urea*) (*Fraction II*) *and Insoluble Protein* (*Fraction III*). In the control samples, urea-soluble protein content (fraction II) did not differ significantly in fresh NAM from chicken, pork, and hake. The low solubility of fraction II (7−10%) is consistent with the high content in fraction I (80−90%) (Table 1). Addition of FA to fresh NAM produced no changes ($P > 0.05$) with respect to the controls as regards urea-soluble proteins in NAM from chicken and pork (Table 1). However, addition of FA to hake NAM produced a pronounced increase ($P < 0.05$) in the protein content of fraction II (36.4%), corresponding to the decrease in salt soluble protein (Table 1). These results suggest that hydrogen bridges and hydrophobic interactions may play a role in aggregation of fresh NAM from hake, unlike chicken and pork in which addition of FA appeared to cause no appreciable changes.

In chicken NAM, by the end of the storage period the presence of FA had caused an increase ($P < 0.05$) in urea-soluble protein (Table 1), while in hake NAM this fraction decreased ($P < 0.05$), leaving a high percentage (around 70%) of insoluble proteins (fraction III). This would suggest that in the aggregation caused by FA during freezing and frozen storage there is greater involvement of covalent bonds in natural actomyosin from hake and pork.

Surface Aromatic Hydrophobicity. For samples before freezing, levels of SoANS were higher in hake NAM than in chicken or pork NAM (Figure 3), which is consistent with the findings of other authors (Li-Chan et al., 1985; Niwa et al., 1989). The freezing process affected hydrophobicity in different ways according to species. Whereas in hake NAM, SoANS increased significantly, in chicken and pork NAM it was not significantly affected by the freezing process (Figure 3).

During frozen storage of NAM lots without FA, the hydrophobicity of the hake protein increased signifi-

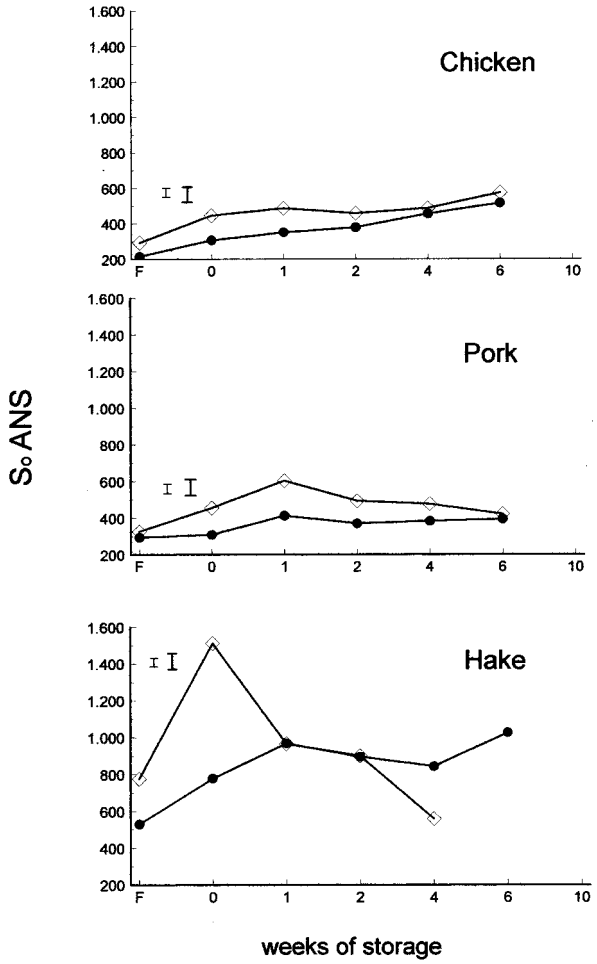

Figure 3. Changes in surface aromatic hydrophobicity (SoANS) (in arbitrary units), without (●) and with (◇) added FA, of chicken, pork, and hake natural actomyosin (NAM) during freezing (−24 °C) and frozen storage (−12 °C). F = AM before freezing. The vertical bars show the confidence interval (95%). The fine error bar shows the effect of FA and the freezing process, and the thick one shows the effect FA and frozen storage.

cantly in the first week (Figure 3). In chicken NAM, aromatic hydrophobicity increased gradually, the highest values occurring at the end of the study period. In pork NAM, unlike the other two species, although the freezing process had no effect on aromatic hydrophobic locations, hydrophobicity increased ($P < 0.05$) during the first week of frozen storage, with no significant changes thereafter ($P > 0.05$).

Whereas addition of FA to fresh NAM produced no significant changes ($P > 0.05$) in aromatic hydrophobicity of chicken or pork NAM (Figure 3), in hake NAM it caused a significant increase in SoANS. These results confirm that fish proteins are more sensitive to denaturing agents of this kind, as was reported by Buttkus (1971).

Freezing process of the model systems in the presence of FA caused a significant increase in the aromatic hydrophobicity of NAM in all three species, particularly hake (Figure 3). During frozen storage, considerable interspecies differences were detected as a result of FA action. The hydrophobicity of chicken and pork underwent only minor variations, so that after 6 weeks' storage SoANS values were similar to those of samples without FA, whereas in hake NAM hydrophobicity fell sharply (Figure 3). This phenomenon was accompanied by the formation of visually detectable aggregates which caused some interference in measurement of the parameter, and from the fourth week onward determination was impossible in hake NAM.

A number of authors have reported that both the freezing process (Hayakawa and Nakai, 1985; Li-Chan et al., 1985; Niwa et al., 1986a,b; Jiménez Colmenero et al., 1991) and frozen storage (Jiménez Colmenero et al., 1991) can produce an increase in the hydrophobicity of myofibrillar proteins. The change in surface hydrophobicity produced by the freezing process is smaller in myosin and actomyosin solutions from warm-blooded animals than in those from lean fish species; this has been attributed (Niwa et al., 1986a,b) to the fact that the proteins from the latter have a more open and, hence, less stable structure, so that they are more exposed to the action of denaturing agents.

Del Mazo et al. (1994), in NAM from hake stored at −18 °C, also observed an increase in surface aliphatic hydrophobicity upon freezing in the presence of FA and a subsequent decrease as in the present results. With isolated myosin, some authors have reported an increase in surface hydrophobicity in the presence of FA during frozen storage (Ang and Hultin, 1989), while others (Careche and Li-Chan, 1997) have found a decrease, as in the present experiment. SoANS results could be the consequence of a balance between proteins remaining in solution with more exposed hydrophobicity and aggregates with less exposed hydrophobicity (Careche and Li-Chan, 1997). Jiménez Colmenero et al. (1991) reported that surface hydrophobicity was lower in frozen stored pork myosin extracts with 58% solubility than in the soluble fraction of the extracts.

Emulsifying Activity Index. Initial levels of the EAI were similar in chicken and hake NAM, and these were significantly higher than in pork NAM (Figure 4). These results are consistent with the findings of Jiménez Colmenero and Borderías (1983) in salt-soluble proteins from various meat and fish species.

The freezing process did not significantly affect the EAI of the three species, either with or without FA ($P > 0.05$) (Figure 4). During frozen storage of the samples without FA, there were no changes in the EAI of chicken, hake, or pork NAM, but in the case of pork NAM, there was pronounced functional deterioration from the tenth week of storage (Figure 4). Some authors have found frozen storage to cause a decline in emulsifying ability (Jiménez Colmenero and Borderías, 1983; Careche and Tejada, 1990a,b; Srikar and Reddy, 1991), deterioration being greatest in fish proteins, followed by pork, and last chicken (Jiménez Colmenero and Borderías, 1983). Grabowska and Sikorski (1974) suggested that loss of emulsifying ability was due to denaturation induced by the freezing process and more specifically by a decrease in salt-soluble protein content. This explanation is consistent with findings at our own laboratory (Cofrades, 1994; Cofrades et al., 1996b) and with the results of Table 1 for chicken NAM. After 17 weeks of frozen storage (−12 °C) there were no changes ($P < 0.05$) in the salt-soluble protein content (fraction I), which maintained its initial values (Table 1). It would therefore seem logical for the EAI to remain virtually unchanged. On the other hand, by the end of 17 weeks' frozen storage salt soluble protein of pork NAM had decreased to 63.9% (Table 1), so that the fall

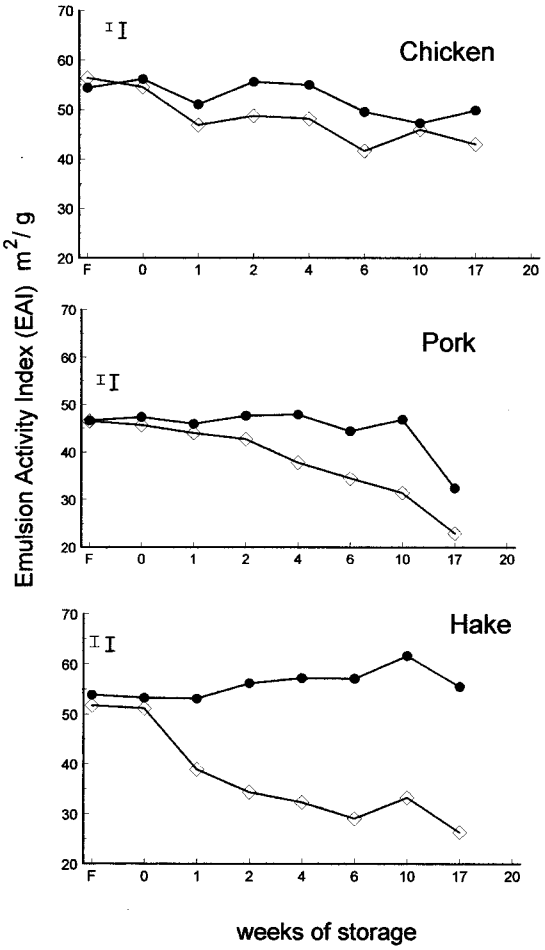

Figure 4. Changes in emulsifying activity index (EAI) (m^2/g), without (●) and with (◇) added FA, of chicken, pork, and hake natural actomyosin (NAM) during freezing (−24 °C) and frozen storage (−12 °C). F = AM before freezing. The vertical bars show the confidence interval (95%). The fine error bar shows the effect of FA and the freezing process, and the thick one shows the effect of FA and frozen storage.

in the EAI by the end of storage (Figure 4) may be attributable to a moderate decrease in salt-soluble protein.

The case of hake NAM was different in that although there was no decrease in the EAI during frozen storage (Figure 4), by the end of 17 weeks at −12 °C the content in salt-soluble protein had fallen to 43.6% (Table 1). This behavior would seem to suggest that other factors besides protein solubility also affected this functional property. In this connection, it has been noted that hydrophobicity and solubility together play an important role in the emulsifying properties of proteins extracted with salt from beef muscle, so that greater surface exposure of the hydrophobic locations could favor emulsifying properties (Nakai and Li-Chan, 1988). This suggestion is consistent with the results of protein solubility (Table 1) and surface hydrophobicity (Figure 3). At the end of frozen storage a major portion of the hake protein was solubilized in 0.6 M NaCl + 8 M urea (Table 1), which suggests that under these conditions proteins, although aggregated, have a high potential of hydrophobic interactions. The fact that the sum of fraction I and fraction II gave rise to similar levels of protein solubility (around 90%) in both fresh and frozen hake NAM (Table 1) might help explain the fact that emulsifying properties were comparable in hake NAM when fresh and after 17 weeks' storage (Figure 4). The increased surface hydrophobicity observed in hake NAM at the end of storage reflects the importance of hydrophobicity of salt-insoluble proteins for emulsifying capacity (Gaska and Regenstein, 1982).

Frozen storage of NAM in the presence of FA caused a decrease in the EAI in all three species (Figure 4). This was most pronounced in hake NAM, where by the end of the first week of storage the EAI had fallen to well below the levels recorded for chicken and pork NAM. These results could be connected with loss of solubility in chicken, pork, and hake NAM in fraction I and fraction II in the presence of FA (Table 1) plus a fall in net surface hydrophobicity in the case of hake NAM. Thus, the sum of salt-soluble and urea-soluble protein by the end of storage was 84.4, 34.5, and 29.7% for NAM of chicken, pork, and hake, respectively. This would suggest that covalent bonding was chiefly responsible for the protein aggregation that caused loss of solubility and hence of emulsifying activity. This suggestion is further supported by studies on formaldehyde action during frozen storage of cod myosin (Careche and Li-Chan, 1997) and hake actomyosin (Del Mazo, 1997).

Gel Strength. The behavior of NAM with respect to gel-forming properties also differed according to species. In fresh samples without FA, gel strength was similar for chicken and hake NAM, while that of pork NAM was significantly lower than in either (225, 245, and 190 J × 10^{-6}, respectively). Gels made from pork NAM exhibited loss of GS ($P < 0.05$) as a result of the freezing process (Figure 5). Addition of FA to fresh samples produced no changes ($P > 0.05$) in the gel-forming ability of either species, while the freezing process of the samples with FA produced a decrease of this parameter in chicken NAM. The case of hake NAM was different in that samples lost gel-forming ability as a result not only of the freezing process but also of addition of FA to the fresh protein; this parameter was therefore not presented either graphically or statistically.

In the samples without FA, while GS in pork NAM remained unaltered over 10 weeks' storage, in chicken NAM GS began to increase after 4 weeks, reaching a maximum at the end of 6 weeks' storage (Figure 5). With respect to the presence of FA, there was no clear trend in the evolution of gel-forming ability of pork NAM with FA during the first 4 weeks of storage; after the fourth week, the pork NAM lost all gel-forming ability (Figure 5). Chicken NAM with FA, on the other hand, retained gel-forming ability to the end of storage, although the gels were significantly weaker than the control samples.

The reason for the different behavior of the gel-forming properties of hake as opposed to chicken and pork actomyosin as a result of both the freezing process and addition of FA may be connected with the fact that fish proteins are less stable than mammal or poultry proteins (Matsumoto, 1980; Jiménez Colmenero and Borderías, 1983). This behavior is consistent with the fact that salt-soluble protein (0.6 M NaCl) decreases more in hake than in chicken or pork NAM in the early stages of frozen storage (Table 1). Under such conditions the protein aggregation is such that there is no possibility of an orderly protein−protein interaction which might favor formation of a gel structure.

One important aspect is the fact that gels made from chicken NAM which had been stored for 4 weeks at −12

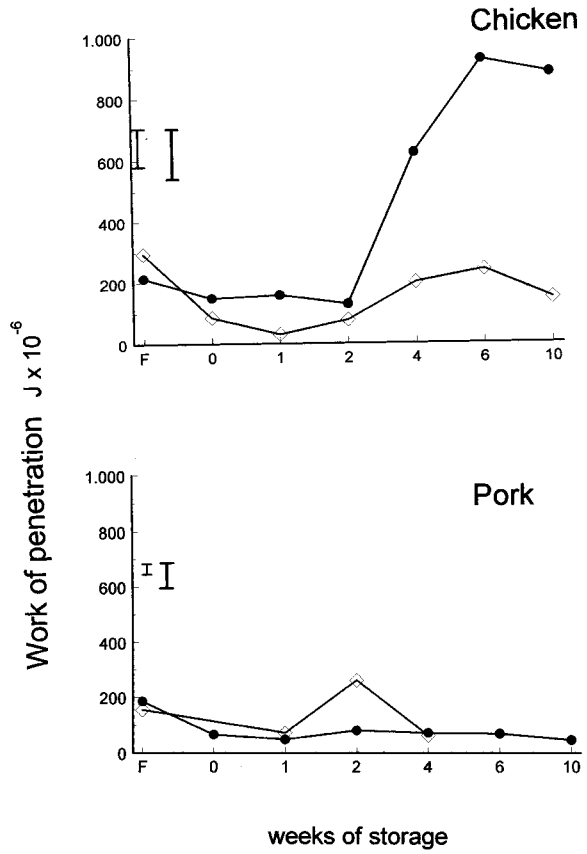

Figure 5. Changes in gel strength, measured as work of penetration ($J \times 10^{-6}$), without (◇) and with (●) added FA, of chicken and pork natural actomyosin (NAM) during freezing (−24 °C) and frozen storage (−12 °C). F = AM before freezing. The vertical bars show the confidence interval (95%). In the case of chicken NAM the fine error bar refers to the effect of FA and the freezing process and the thick bar to the effect of FA and the time during frozen storage. In the case of pork NAM the fine error bar refers to samples without FA and the thick bar to samples with FA.

°C exhibited much higher GS than gels made from fresh NAM (Figure 5). It is well-known that certain protein denaturation is a prerequisite for formation of the kind of ordered structure that is involved in gelation. This implies initial unfolding of the native protein, thus exposing the hydrophobic groups, which interact to form a three-dimensional network (Itoh et al., 1979; Niwa et al., 1989). It has been suggested that it is hydrophobic interactions which predominate in this kind of aggregation (Liu et al., 1982). For their part Li-Chan et al. (1985) reported that controlled denaturation without aggregation or coagulation augmented exposure of the hydrophobic groups, generally resulting in an improvement in some functional properties such as gel-forming ability, so that stronger gels are formed. Niwa et al. (1989) and Niwa (1992) found that treatment with urea of myofibrillar proteins from different species, including chicken, favored greater exposure of the hydrophobic groups, resulting in the formation of gels with similar characteristics to gels made from species which provoked setting. A similar effect to that produced by urea treatment could have been produced in chicken NAM by frozen storage. The gradual and moderate increase in aromatic hydrophobicity during this process (higher than twice of fresh protein) (Figure 3) could resemble controlled denaturation like that occurring in fish proteins at low temperatures (setting), so that better-quality gels are made than when fresh protein is used. Where FA is added, however, the denaturation is so rapid that there is no time to achieve the structural characteristics needed for formation of a good gel.

In summary, this experiment shows that the presence of FA appears to induce various modifications in NAM, which differ according to species (Figures 3−5). This suggests that there are intrinsic factors, characteristic of each species, which modulate the vulnerability of the actomyosin to attack from formaldehyde. This finding is consistent with the results of a study on protein solubility, in different agents, of NAM from chicken, pork, and hake which had been stored under similar conditions to those described in the present article (Cofrades, 1994) and also with studies on the effect of FA on actomyosin from fish species which produce FA at different rates (Tejada et al., 1996; Torrejon, 1996).

The results from chicken NAM could have interesting technological implications in that controlled processes might be used to induce denaturation by freezing in order to improve the functional behavior of the chicken proteins for the purpose of gel formation. However, a more exhaustive study is still needed to confirm the findings of the present experiment.

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