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Consistency and Solubility Changes in Herring (*Clupea harengus*) Light Muscle Homogenates as a Function of pH

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Fish muscle proteins can be isolated from a variety of low-value raw materials by solubilization in either acid or base. If the consistency of the resulting solution is sufficiently low, it is possible to recover most of the solubilized proteins and remove most of the lipids by centrifugation. Lipid removal should greatly stabilize the isolated proteins. In a previous investigation into the use of herring for production of these protein isolates, it was observed that this species had particularly high consistency values when the proteins were solubilized. This study was undertaken to determine the consistencies obtained with herring light muscle tissue over the pH range covered by the two processes, from about pH 2.7 to 10.8. Protein solubility was compared to consistency of the resultant solutions. Maximum consistencies of the homogenates, ∼220 and ∼175 mPa·s, were obtained at pH values of approximately 3.5 and 10.5, respectively. Consistency began to increase approximately when solubilization began. Storage of homogenates at pH 2.7 decreased the consistency over a 10 min time period. The magnitude of the consistency peaks at both acid and alkaline pH values increased when using ice-stored as well as frozen-stored herring, especially in the acid range. Protein solubility at pH <4 and pH ≥10.8 slightly decreased after post-mortem storage of the herring muscle. It is suggested that the observed changes in consistency result from the expansion and solvation of protein aggregates which eventually dissociate into smaller units, perhaps even monomers.

KEYWORDS: Herring; *Clupea harengus*; muscle; pH; solubility; consistency; viscosity; proteins

INTRODUCTION

Today, ∼90% of the estimated 2.5 million tons of herring that are captured (*1*) are directed toward nonhuman consumption as fishmeal and fertilizers. Isolation of herring proteins for food production would be a more responsible way of using a nutritious and abundant raw material. However, the seasonality, small size, and lipid instability of herring have made it difficult to extract stable and functional proteins for use in human food products (*2*, *3*).

Recently, procedures have been developed and scaled up for recovering stable, functional proteins from low-value muscle sources such as herring (*4*−*10*). The muscle proteins are first solubilized; this may be done either at low pH (pH ≤ 3.5) or at high pH (pH ≥10.5). At this point, insoluble materials may be separated from the soluble myofibrillar, cytoskeletal, and sarcoplasmic proteins by a technique such as centrifugation, which can separate oils by flotation. Insoluble components heavier than the solution such as skin, bones, and even cellular membranes can be removed in the sediment. The soluble proteins are then precipitated by adjusting the pH and/or ionic strength and recovered by a second centrifugation. The recovered proteins retain their functionality, including their ability to form a gel.

When we used herring light muscle in the acid and alkaline processes, high consistency developed in the protein solubilization step (*11*). This caused a large gelatinous "extra sediment" to form in the first centrifugation. This sediment trapped ∼17% of the solubilized proteins and reduced maximum protein yields from 88 to 74% and from 83 to 68% in the acid and alkaline processes, respectively (*11*). Using acid isolation, the efficiency of total lipid removal was also reduced as the homogenate consistency increased (*11*). Herring light muscle produced higher

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homogenate consistencies and higher lipid levels in the final isolates than did mackerel light muscle (*5*, *9*, *11*).

At a constant protein concentration, the flow properties of a dilute biopolymer suspension largely depend on the conformation and aggregation of the biopolymer molecules (*12*). To a first approximation, the relationship between the viscosity of a muscle protein homogenate and the effective volume of protein molecules/aggregates can be described by the equation (*12*)

$$\frac{\eta}{\eta_1} = \left(1 - \frac{R_V c}{0.57\rho}\right)^{-1.52} \quad (1)$$

where η = homogenate viscosity, η_1 = viscosity of liquid surrounding the particles, c = protein concentration (in kg m^{-3}), ρ = protein density (in kg m^{-3}), and R_V = effective volume ratio of the protein molecules/aggregates in solution. R_V can be expressed as

$$R_V = V_E/V_A = \frac{4\pi r_g^{\,3}\rho N_A}{3M} \quad (2)$$

V_E is the effective volume of the protein molecule/aggregate in solution (i.e., the volume swept out during complete rotation) and V_A is the actual volume occupied by the protein molecules/aggregates, r_g is the radius of gyration of the molecules/aggregates, N_A is Avogadro's number, and M is the molecular weight.

Thus, the viscosity of a protein homogenate is thought to be directly related to the extent of swelling (i.e., increased R_V) and solubilization (i.e., decreased R_V) of protein aggregates and thereby also to changes in environmental conditions such as pH, salt, and raw material processing history. Hermansson and Åkesson (*13*) found that less soluble proteins, with high initial swelling, showed high concentration-dependent viscosity. In suspensions of promine-D, caseinate, and whey proteins, there was a positive correlation between viscosity and solubilization between pH 5.9 and 10.9 (*13*).

The objective of this study was to investigate changes in consistency and protein solubility in a herring light muscle homogenate over the pH range covered by the acid and alkali solubilization processes (*4*−*10*); ≤2.7 to ≥10.8. The pH-dependent consistency and solubility properties were also to be studied as a function of herring post-mortem age and the presence of added salt.

MATERIALS AND METHODS

Materials. Fresh herring was obtained from D&B Bait, Gloucester, MA, and transported on ice to the University of Massachusetts Marine Station (~15 min). The herring was caught in the spring of 2000 and in the fall of 2001. The post-mortem age generally ranged between 6 and 36 h. The herring was used following periods of ice storage (in a 4 °C refrigerator) or after frozen storage (−18 °C). White muscle was manually excised and ground through a 5 mm plate using a kitchen grinder (KitchenAid Inc., St. Joseph, MI).

Production of Acidified and Alkalized Protein Homogenates. Ground muscle (120 or 160 g) was homogenized for 1 min (speed 50) with 9 volumes of ice-cold distilled water using a Kinematica GmbH Polytron (Westbury, NY) connected to a Variable Autotransformer (Staco Energy Products Co., Dayton, OH). The proteins in the homogenate were solubilized by dropwise addition of 2 N HCl or 2 N NaOH until reaching pH values of 2.5−2.7 or 10.8−12.5.

Proteins and Protein Solubility. Total proteins were measured according to the method of Lowry et al. (*14*) as modified by Markwell et al. (*15*). Protein solubility (percent) was expressed according to the following formula: (protein concentration in supernatant after centrifugation at 18000*g* (4 °C for 15 min)/protein concentration in homogenate before centrifugation) × 100.

Electrophoresis. Proteins in the acidified and alkalized herring light muscle homogenates produced from fresh and ice-stored herring were separated according to the electrophoresis procedure described by Laemmli (*16*). Precast mini linear gels 4−20% (OWL Systems, Woburn, MA) were used to separate proteins on a vertical PAGE Mini device (Daiichi Scientific, Tokyo, Japan) with a constant current of 30 mA per gel. The protein samples were diluted 2-fold in a premade sample buffer (Sigma, St. Louis, MO) and heated for 1 min at 100 °C. Fifteen micrograms of proteins was added to each lane. Protein bands were fixed using a 1 h incubation in 12% trichloroacetic acid, followed by overnight staining using Pro-Blue (Owl Separation Systems, Portsmouth, NH). Scanning of the stained gels was accomplished using a Hoefer model GS 300 scanning densitometer (Hoefer Scientific, San Fransisco, CA) in the transmittance mode with a model 365W densitometer analysis software for protein quantification. A standard curve was constructed using high molecular weight SDS-PAGE standards (Sigma) on a linear gradient as described by Hames (*17*).

Consistency. Consistency was followed during solubilization and precipitation of proteins. Five hundred milliliters of the homogenates was placed into a 600 mL Pyrex beaker on ice. Consistency was measured in duplicate at 4−6 °C using a "HAT" Brookfield Syncrolectric viscometer (Stoughton, MA) equipped with a no. 2 spindle at 60 rpm. A manufacturer's chart allowed for the readings to be converted to mPa·s.

Measurements of pH. The pH was recorded with an Orion combination epoxy Ross Sure-Flow Electrode (Orion Research Inc., Beverly, MA) in conjunction with a pH-meter (Orion Research Inc., Boston, MA).

Statistics. At each pH, the homogenate consistency and solubility were measured twice, with the average value shown in the presented figures. Each experiment was repeated from two to nine times using different batches of herring. The figures shown illustrate typical data obtained. In determinations of the amounts of HCl and NaOH that had to be added for the homogenates to reach the end-pH values (2.7 and 10.8), nine replicates were done. To measure initial consistency at pH 7, 7 replicates were done. Standard deviations (SD) were here calculated using Excel 2000 (Microsoft Corp., Seattle, WA).

RESULTS

Character of the Herring Light Muscle Homogenate. The herring light muscle homogenate contained one part muscle and 9 volumes of distilled water, which resulted in a protein concentration of ~20 mg/mL (i.e., 2%) and a lipid concentration of 2.5 mg/mL (i.e., 0.25%). **Figure 1** shows the protein distribution in acidified and alkalized muscle homogenates made from fresh herring (lanes 2 and 4) and herring stored for 11 days on ice (lanes 3 and 5). In the homogenates from ice-stored herring, the SDS-PAGE procedure recovered relatively lower levels of polypeptides tentatively identified as myosin (~200 kDa), nebulin (600−800 kDa), and titin (>2000 kDa) (see black arrows in **Figure 1**, lanes 2 and 4).

Amounts of Acid and Base Needed for pH Adjustment of the Herring Light Muscle Homogenate. To adjust the herring light muscle homogenate from an initial pH of 7 to pH 2.7 and 10.8, HCl and NaOH to final concentrations of 43 and 19.4 mM, respectively, needed to be added. This increased the calculated ionic strengths of the homogenates from the initial ~0.017 at pH 7 to 0.061 ± 0.008 at pH 2.7 and to 0.037 ± 0.002 at pH 10.8 (n = 9, mean ± SD). The homogenate dilutions due to acid/base additions, 1.006 and 1.004 times at pH 2.5 and 11, respectively, were considered too small to have any impact on the observed changes in consistency. The amount of 2 N HCl needed to reach pH 2.7 (220 μL/g of muscle) was reduced by 25 and 30% when using fish stored on ice for 2 and 6 days, respectively (**Figure 2**). In the presence of 25 and 100 mM salt, ~25% more 2 N NaOH was needed to reach pH 11.

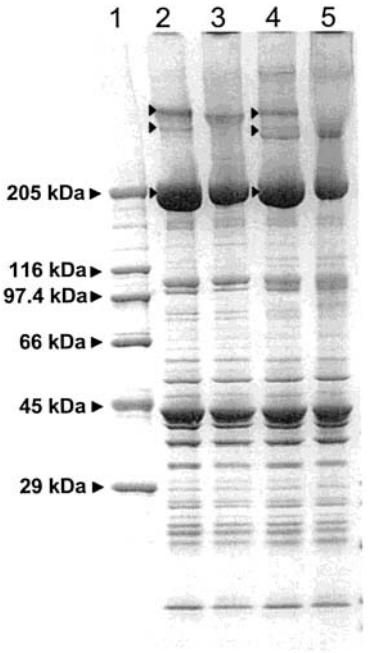

Figure 1. SDS-PAGE (4–20%, linear gradient) of acidified (pH 2.7) and alkalized (pH 10.8) homogenates made from fresh herring (lanes 2 and 4) and from herring stored for 11 days on ice (lanes 3 and 5). The herring was caught in the spring of 2000. Lane: 1, high (29–205 kDa) molecular weight SDS-PAGE standards; 2, proteins from acidified (pH 2.7) herring light muscle homogenate made from fresh herring; 3, proteins from acidified (pH 2.7) herring light muscle homogenate made from ice-stored herring; 4, proteins from alkalized (pH 10.8) herring light muscle homogenate made from fresh herring; 5, proteins from alkalized (pH 10.8) herring light muscle homogenate made from ice-stored herring. Proteins were added at 15 μg/lane.

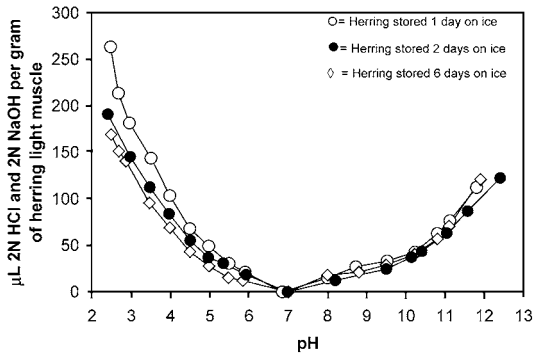

Figure 2. Amounts of 2 N HCl and 2 N NaOH needed to adjust a herring light muscle homogenate from pH 7 to pH 2.5 and from pH 7 to pH 11, respectively. The herring was caught in the fall of 2001 and had been stored on ice for 1, 2, or 6 days prior to excision of the white muscle.

Solubility of Proteins in the Herring Light Muscle Homogenate as a Function of pH. At the pH of the unadjusted herring light muscle homogenate (pH ~7), ~20% of the proteins in the homogenate were soluble. This is consistent with the level of sarcoplasmic proteins usually found in muscle tissue. Upon acidification and alkalization, the protein solubility increased until reaching 96% at pH ~2.5 and 93% at pH 11.8 (**Figure 3**); the most dramatic changes took place between pH 4 and 4.5 and between pH 9.5 and 11. At pH 4.5 and 9, high consistency started to develop in the homogenate (**Figure 3**). Proteins from herring aged for 6 days on ice were slightly less soluble at pH <4 and pH >10.8 as compared to proteins from fresh herring (**Figure 4**). After storage of herring for 18 days at −18 °C, the protein solubility at pH 2.7 was reduced from 87 to 76% and, at pH 10.8, from 83 to 73% (data not shown). Protein solubility was slightly reduced by 25 mM NaCl at pH 4.5 and 3.5, but not at pH 2.5, at which ~90% of the proteins were soluble in homogenates with and without 25 mM salt (**Figure 5**). With 100 mM salt, the protein solubility was greatly reduced at pH 3.5 and 2.5. On the alkaline side, no great changes in protein solubilities were seen after the addition of 25 or 100 mM salt (**Figure 5**).

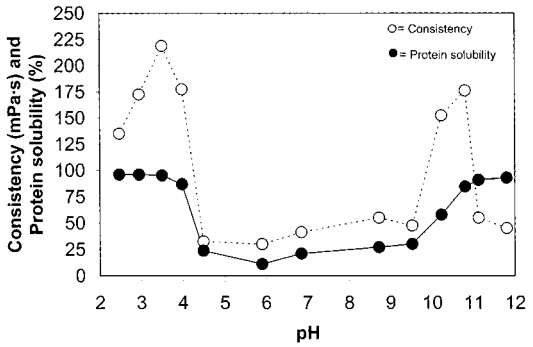

Figure 3. Protein solubility and consistency at 4–6 °C of a herring light muscle homogenate at selected pH values between 2.5 and 12. The herring was caught in the fall of 2001 and was used after <1 day on ice.

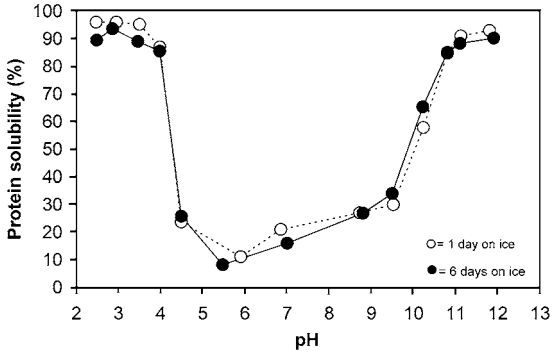

Figure 4. Protein solubility of a herring light muscle homogenate at selected pH values between 2.5 and 12. The herring was caught in the fall of 2001 and was used after 1 and 6 days on ice

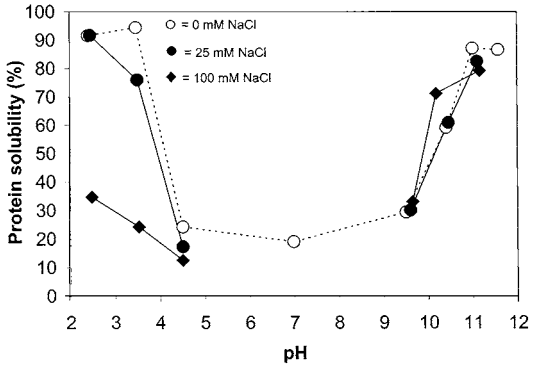

Figure 5. Protein solubility of a herring light muscle homogenate at selected pH values between 2.5 and 11. The herring was caught in the fall of 2001, and the homogenate was fortified with 0, 25, and 100 mM NaCl.

Consistency of the Herring Light Muscle Homogenate as a Function of pH. At pH 7, the consistency of the herring light muscle homogenate was 44 ± 10 mPa·s (n = 7). When the homogenate was acidified, the consistency increased sharply below pH 4.5, reached a maximum at pH 3.5, and decreased sharply when the pH was decreased below this value (**Figures**

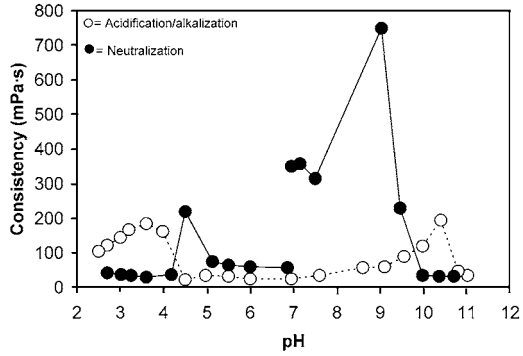

Figure 6. Consistency at 4–6 °C of a herring light muscle homogenate when it was adjusted from pH 7 to pH 2.5 and 11 and back from pH 2.5 and 11 to pH 7. The herring was caught in the fall of 2001 and was used after <1 day on ice.

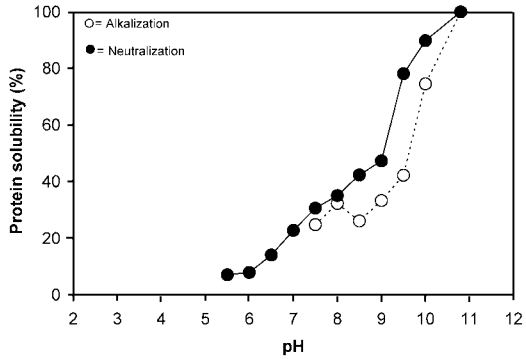

Figure 7. Protein solubility at 4 °C of a herring light muscle homogenate when it was adjusted from pH 7 to 11 and back from pH 11 to 5.5. The herring was caught in the fall of 2001.

3 and **6**). When the homogenate was alkalized, the consistency increased sharply above pH 9.5, reached a maximum value at pH 10.5, and decreased sharply when the pH was increased further (**Figures 3** and **6**). When the acidified and alkalized muscle homogenates were neutralized, large consistency peaks occurred at pH ∼4.5 and 9, especially at pH 9 (750 mPa·s) (**Figure 6**). To investigate how the latter was related to protein insolubilization, protein solubility was followed during neutralization of an alkalized muscle homogenate. **Figure 7** shows how the most extensive insolubilization was seen between pH 10 and 9.5, thus where the consistency started to increase. No increased consistency developed when sarcoplasmic protein fractions isolated from the herring muscle homogenate were acidified and alkalized by themselves.

Ice storage of the whole herring for up to 3 days prior to excision of the white muscle increased the size of the acid and alkaline consistency peaks in the homogenates prepared by 40 and 15%, respectively (**Figure 8a**). Using fish from a different season and year, 6 days of prestorage on ice increased the acid and alkaline consistency peaks by 5 and 40%, respectively (data not shown). After prestorage of the whole herring for 18 days at −18 °C, the acid and alkaline consistency peaks increased by 50 and 25%, respectively (**Figure 8b**).

With muscles having similar post-mortem ages, maximum consistency at pH 3.5 and 10.5 differed depending on seasons/batches (**Figures 3**, **6**, and **8**), more so on the acid side (∼125−225 mPa·s) than on the alkaline side (150−180 mPa·s). The magnitude of the sharp reduction of consistency at extreme pH values was also less consistent on the acid than the alkaline side. At pH 10.8, the consistency of the homogenate usually fell to values below 50 mPa·s, whereas at pH 2.5−2.7, the

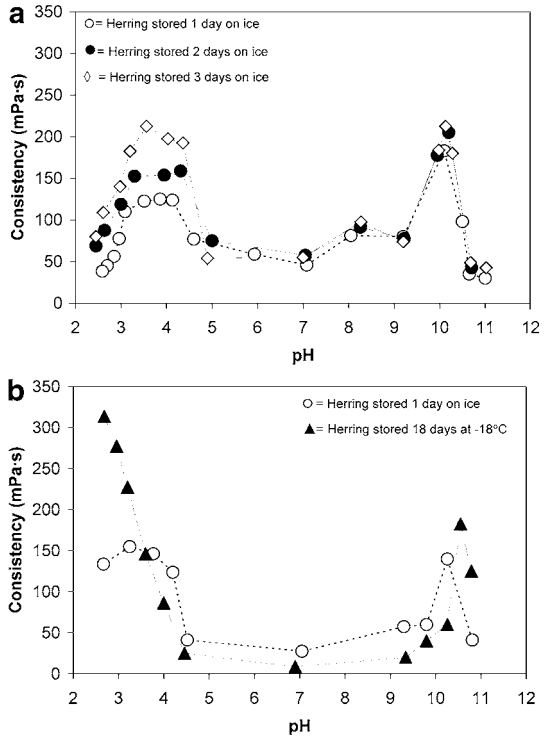

Figure 8. Consistency at 4–6 °C of a herring light muscle homogenate at selected pH values between pH 2.5 and 11: (a) homogenate made from herring that had been stored on ice for 1, 2, or 3 days prior to excision of the white muscle; (b) homogenate made from herring that had been stored on ice for 1 day or at –18 °C for 18 days prior to excision of the white muscle. The herring was caught in the spring of 2000.

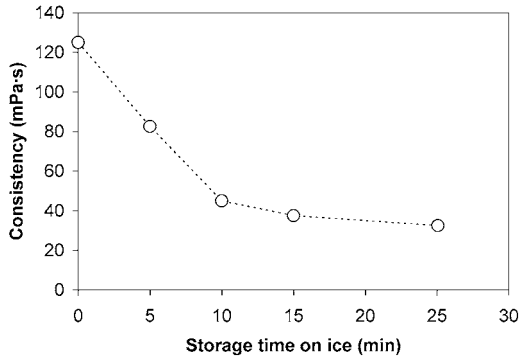

Figure 9. Changes in consistency at 4–6 °C during storage of an acidified (pH 2.7) herring light muscle homogenate on ice. The herring was caught in the fall of 2001.

homogenate was often still highly viscous (**Figures 3**, **6**, and **8**). When the latter occurred, the reduction in consistency was induced by storing the acidified homogenate for short periods of time on ice (**Figure 9**).

NaCl (25 and 100 mM) was added to fresh herring light muscle homogenates prior to acidification and alkalization to evaluate if this could be used to reduce the consistency (**Figure 10a**). On the acid side, 25 mM NaCl increased the magnitude of the consistency peak by 45%, whereas 100 mM NaCl decreased it by ∼65%. On the alkaline side, the maximum consistency was reduced by 25% at 25 mM NaCl and by 50% at 100 mM NaCl. To investigate at what concentration the effect of NaCl changed from increasing to reducing with regard to the acid consistency peak, NaCl at 10, 25, 50, 75, and 125 mM was added prior to acidification (**Figure 10b**). The magnitude of the peaks gradually increased up to 25 mM NaCl and then

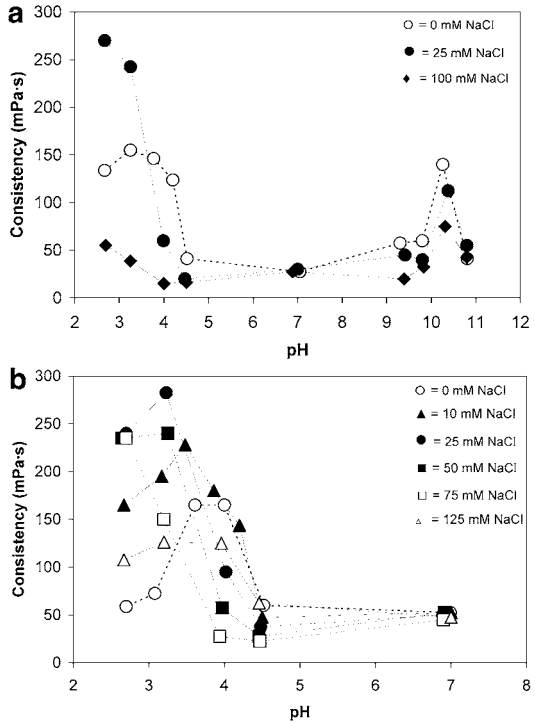

Figure 10. Consistency at 4–6 °C of a herring light muscle homogenate at selected pH values between pH 7 and 2.5 (a and b) and between pH 7 and 11 (a): (a) homogenates with 0, 25, and 100 mM NaCl added; (b) homogenates with 0, 10, 25, 50, 75, and 125 mM NaCl added. The herring was caught in the spring of 2000.

started declining. The magnitude of the acid consistency peak at 125 mM NaCl was lower than that without salt present. However, at more extreme pH values, consistency was greater at 125 mM salt than without salt. NaCl at 10−75 mM pushed the maximum consistency from pH 3.7 toward lower pH values.

DISCUSSION

The herring light muscle homogenate is a highly complex system consisting primarily of muscle proteins (∼2%) and fat (∼0.25%) dispersed in a continuous aqueous phase containing low molecular weight compounds. At the original pH, the proteins of this suspension exist both as individual proteins, for example, sarcoplasmic proteins, and as aggregates, for example, the myofibrillar proteins. Sarcoplasmic proteins constitute ∼20−30% of total proteins and have mostly spherical shapes (*18*). The myofibrillar proteins make up ∼70−75% and, on a molecular level, are mostly rod-shaped (e.g., myosin) or form rodlike filaments (e.g., actin). Moreover, at neutral pH, myofibrillar proteins are primarily bound in myofibril segments and myofibril bundles (*19*), which also have rodlike fibrous shapes.

As shown in eq 1, the viscosity of a protein homogenate is a function of the effective volume ratio, R_V, of the protein molecule or protein aggregate. R_V is the ratio between the "effective" volume (V_E) of the protein molecules/aggregates in solution and the actual volume (V_A) occupied by the protein molecules/aggregates (eq 2). Thus, R_V increases with increased V_E. The V_E can be described as the volume swept out by the molecule/aggregate during complete rotation and depends on, for example, the three-dimensional structure of the molecules/aggregates in solution (*12*). For molecules/aggregates forming compact globular structures (e.g., sarcoplasmic proteins), V_E is close to V_A, and therefore $R_V \sim 1$. Molecules/aggregates with extended structures in solution (e.g., rigid rods or random coils) have larger volume ratios than those that have compact structures. Expansion of a coil, for example, due to electrostatic repulsion, further increases its V_E and, thus, raises the viscosity (*20*).

The V_E of protein aggregates in a dilute solution is different from the volume occupied by the molecules making up the aggregate. For sarcoplasmic proteins and certain myofibrillar proteins (e.g., the proteins of the thin filaments), it is believed that aggregates would have a larger V_E than the individual molecules. This is because the molecules of the aggregate trap some of the continuous phase, giving them a higher effective volume (V_E) than actual volume (V_A).

On the basis of the above, it is suggested that two major factors explain the thickening−thinning profiles observed in the herring light muscle homogenate during pH adjustments away from neutrality: (i) an increase in the effective volume (V_E) of existing protein aggregates (e.g., myofibril segments/bundles) due to expansion/swelling and (ii) conversion of existing protein aggregates into smaller "pieces" or isolated protein monomers with lower V_E due to solubilization. It is suggested that the major driving forces causing expansion/swelling and solubilization during pH adjustment of the herring light muscle suspension are electrostatic repulsive forces induced by the addition/removal of hydrogen ions and/or salt ions into the system. Aggregation of proteins during the pH adjustments is thought to be induced by hydrophobic forces.

The suggested relationship between consistency and solubility is strengthened by **Figures 3**, **5**, and **10**. **Figure 3** shows that both solubility and consistency start changing at the same pH during acidification and alkalization of the homogenates. Consistency then declines dramatically when the solubilization ceases. **Figures 5** and **10** show how 100 mM sodium chloride reduced both the protein solubility and the consistency at acidic pH values.

The way the consistency responded to low levels of salt (10 and 25 mM at acidic pH, **Figure 10b**) gives some support to the hypothesis that solubilization and/or removal of certain cytoskeletal proteins by salt allows for more extensive swelling of the rest of the myofibrillar proteins (*19*, *21*, *22*). Up to 25 mM, consistency increased while at the same time the salt lowered the pH at which the maximum consistency developed. Above 25 mM, salt decreased the pH at which the consistency began and lowered the maximum consistency from what it was at 25 mM. It was previously found that a prewash of minced light muscles from mackerel (*19*) and herring (*21*) with 25 and 150 mM salt removed certain proteins that hold the structural elements together, for example, desmin and α-actinin. These prewashes also allowed for higher solubilization of the myofibrillar proteins after a subsequent reduction of the ionic strength. In this study, where the ionic strength was not altered after the salt addition, a tentative removal of "solubility-inhibiting peptides" by >25 mM salt was counteracted by the decreasing solubilization of myofibrillar proteins (**Figure 5**). It has previously been described how negative charges on proteins can be neutralized by salt cations favoring anion binding and "salting out" (*23*). With chicken dark muscle, the consistency at pH ∼5 increased up to 20 mM of added NaCl, whereas at ≥40 mM, it decreased (*10*). The data in **Figure 9** show that consistency decreased with time at acid pH. This finding might suggest that there is a time-dependent solubilization of some of the "solubility-inhibiting peptides". From the fact that most of the consistency changes were over in ∼10 min, it is unlikely that the effect from time reflects an enzymic breakdown.

When acidified and alkalized homogenates were neutralized (**Figure 6**), high consistency developed at pH ~4.5 and 9, thus ~1 pH unit higher on the acid side and ~1.5 pH units lower on the alkaline side compared to during the original pH adjustments. Although the change in the maximal consistency was relatively minimal on the acid side, it was nearly 4 times higher on the alkaline side. In addition, it remained very high even at pH 7. It was found that the largest decreases in the solubility during precipitation of herring light muscle proteins took place in the same pH ranges as the large consistency peak developed (**Figure 7**). **Figure 7** also illustrates that there was a hysteretic effect on solubility just as on consistency, although not as pronounced. Between pH 11 and 8, the proteins became insoluble at a lower pH on the way back to neutrality. Between pH 8 and 9, the difference between the alkalization and neutralization curves was ~1 pH unit, as compared to the difference in the location of the consistency peak, which was ~1.5 pH units. The lower pH required for equal solubilization on the way down may partially explain the change in position of the consistency peak, again establishing the correlation between the consistency and solubilization. That the consistency peak at pH 9 had a higher intensity compared to that at pH 4.5 could be due to any or all of the following reasons: (i) that there is on average more protein in the particles, that is, they are larger; (ii) that the particles have higher axial ratios; (iii) that the particles are more loosely structured; or (iv) that there is some type of a cross-connection between particles. It seems to be unlikely that changes in consistencies of the individual proteins have a primary role in the enormous consistency rise occurring during neutralization of alkalized homogenates. When Kristinsson (*24*) brought cod myosin (0.84 mg/mL in 20 mM Tris) from pH 7.5 to pH 2.5 or 11 and then back to pH 7.5, there was very little difference in the consistency of any of these modified myosins.

It is also unlikely that conformational changes of individual myofibrillar proteins were involved in the consistency changes observed during acidification and alkalization. The larger myofibrillar monomers, that is, myosin, titin, and nebulin, have axial ratios of ~125 (myosin) and ~1250 (titin and nebulin), which would allow maximum concentrations of ~6.3% myosin and 0.6% each of titin and nebulin for stable existence of the dissolved phase. The herring light muscle homogenate contained ~0.7% myosin, ~0.15% titin, and ~0.07% nebulin (*11*). At these concentrations, these proteins could probably not restrict each other from moving freely in the protein suspension. A contribution from conformational changes in the sarcoplasmic protein fraction is also unlikely as there were no consistency changes seen when the sarcoplasmic protein fraction alone was subjected to acidification or alkalization. Earlier studies of the viscosity of bovine serum albumin during acidification and alkalization supported this observation (*20*).

The consistency peak at pH 3.5 increased when ice-stored or frozen-stored muscle was used (**Figure 8**). A reason for this could be slight protein denaturation due to the freezing or to accumulation of lipid free radicals and free fatty acids during the storage. The latter can induce formation of S−S bridges and/or hydrophobic interactions, respectively, and, thus, cause cross-linking of monomers and aggregates to make them larger and persistent at more extreme pH values (*25*). This is supported by the slight reductions in protein solubility at extreme acidic and alkaline values after ice storage (**Figure 4**) and frozen storage of herring. The lower recoveries of titin, nebulin, and myosin in the SDS-PAGE analysis of herring homogenates from aged fish (**Figure 1**) also suggest there might have been cross-linking reactions. No hydrolysis products could be seen in the electrogram.

Several observations made in this study indicate that different underlying mechanisms are involved in the consistency development and solubilization during the acidification and alkalization processes. These observations include the different responses to salt (**Figures 5** and **10a**), to aging (**Figures 2** and **8**), and to neutralization (**Figure 6**). Furthermore, the homogenates made from fresh herring light muscle did not always exhibit the large reduction in consistency at pH <3.5 that they did at pH >10 (**Figures 3**, **6**, and **8**). In a cod myosin suspension, Kristinsson (*24*) found full unfolding of the myosin headgroup and partial unfolding of the rod part at pH 2.5. At pH 11, only the headgroup unfolded (*24*). It is possible that such molecular differences also affected the supramolecular structure. There is also the possibility that acid induced more conformational/structural protein changes of the herring muscle homogenate than did alkali because there are more ionizable groups having their pK_a values in the pH range of 2.5−7 than in the pH range of 7−11. Glutamic acid and aspartic acid, which have pK_a values at 4.2 and 3.8, respectively (*26*), make up ~25% of the total amino acids in herring muscle (*27*). Lysine, tyrosine, and cysteine, which have pK_a values at 9.5−10.5, 9.1−10.8, and 9.1−10.8, respectively (*26*), together only make up ~15% (*27*).

The presented results illustrate the importance of controlling protein solubility to control consistency changes during acid and alkaline solubilization of herring light muscle proteins. When acid and alkaline solubilizations were used to isolate herring light muscle proteins (*11*), low consistency facilitated the recovery of solubilized proteins and the removal of lipids by centrifugation (*11*). This study clearly showed the advantage of using a fresh raw material to reduce consistency, whereas the responses to salt were more complex. The latter was thought to arise in the fine balance that exists between swelling and insolubilization of proteins caused by low and high levels of salt, respectively. To reduce consistency by altering the pH used to solubilize the proteins is limited by two factors: on the one hand, the wish for solubilizing as much as possible of the protein (**Figure 3**) and, on the other, the increasing amounts of acid and base that are needed for adjusting the homogenate to extreme pH values (**Figure 2**). pH values of 2.7 and 10.8 were previously found to be a good balance between these features, which is further supported by the presented data.

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