ABSTRACT

Functional Stability of Antioxidant-washed, Cryoprotectant-treated Beef Heart Surimi During Frozen Storage

BAOWU WANG and YOULING L. XIONG

Beef heart surimi was prepared in the presence or absence of propyl gallate and blended with or without cryoprotectants (sorbitol, sucrose) prior to frozen storage at −15°C, −29°C, and −70°C up to 52 wk. Protein solubility, gelling characteristics, water-holding capacity, cooking yield, and emulsifying properties decreased during storage at −15°C and −29°C for control surimi (without cryoprotectants). Propyl gallate alone did not influence functionality changes. However, functional properties of surimi subjected to long-term cryogenic storage should be mixed with cryoprotectants and antioxidants to preserve functionality.

Key Words: beef heart, surimi, antioxidant, cryoprotectant, protein functionality

INTRODUCTION

The renewed interest in utilization of animal by-products as human food has led to considerable progress in research specifically aimed at improving the functionality and palatability of beef heart muscle. Wan et al. (1993) and Xiong et al. (1993), using a washing procedure similar to fish surimi manufacture with the incorporation of antioxidants in the washing solution (Kelleher et al., 1994), produced a surimi-like material from beef heart with inhibited lipid oxidation and improved gel-forming ability. In a previous study (Wang et al., 1997), beef heart surimi (BHS) prepared in the presence of propyl gallate and stored below −15°C was stable in chemical characteristics for up to 12 wk.

The most important functional properties of muscle food are gelation, water-binding, and emulsifying ability. Frozen storage, an essential step in the process of BHS production, can bring about detrimental changes in functionality of the surimi material. In fish surimi, cryoprotectants are usually added to protect the functionality of surimi protein (Lee, 1990). Park et al. (1996) showed that addition of cryoprotectants prior to freezing had no protective effect on gel-forming ability of surimi-like materials prepared from beef or pork skeletal muscles. Other researchers (Park et al., 1993) have reported that cryoprotectants had marked positive effects on protein solubility and gelation properties of minced beef. Hence, the question whether cryoprotectants can effectively preserve the functionality of muscle proteins from homiootherm is unresolved. Information is needed concerning the effects of freezing temperature and factors known to protect proteins from denaturation (e.g., antioxidants, cryoprotectants) on the functionality of BHS during frozen storage.

The objective of our study was to elucidate the effects of the common cryoprotectants sorbitol and sucrose and the antioxidant propyl gallate in protecting the functionality of BHS during long-term frozen storage.

MATERIALS & METHODS

Materials and preparation of beef heart surimi

Beef hearts (24–28 h postmortem) were obtained from a local meat packing plant. Preparation of BHS and storage conditions were described previously (Wang et al., 1997). Washed BHS blended with or without Cryo-protectants (4% sorbitol, 4% sucrose) were stored frozen at −15°C, −29°C, and −70°C for 1 day and 2, 4, 12, 24, and 52 wk before analysis.

Protein solubility

Loss of protein solubility, an indication of protein denaturation and aggregation, was monitored. Protein solubility was measured as described by Xiong and Brekke (1989). Protein concentration, determined by the Bradford method (Gornall et al., 1949), was adjusted to 5 mg/mL with 0.6M NaCl in 50 mM sodium phosphate buffer (pH 6.0). The protein suspension was set at 4°C for 18 h prior to centrifugation at 5,000 × g for 15 min. Protein solubility was expressed as the concentration of protein in the supernatant divided by the concentration of protein in the original suspension multiplied by 100.

Gel-forming ability

Dynamic rheological properties and gel strength of BHS were determined based on the methods of Wan et al. (1993). The pellet of BHS was diluted to a protein concentration of 50 mg/mL with 0.6M NaCl, 50 mM sodium phosphate buffer (pH 6.0). The sol was set at 4°C for 18 h to ensure maximal protein solubility prior to rheological measurements. A Model VOR rheometer (Bohlin Instruments, Inc., Cranbury, NJ) equipped with parallel plates (upper plate dia 3.0 cm) was used for dynamic rheological measurements during protein gelation. Protein gels were formed by heating the BHS sol from 20 to 73°C at 1°C/min, and the sample temperature during heating was verified with a thermocouple. The gelling samples were sheared at a fixed frequency (0.1 Hz) with a maximum strain of 0.02.

To determine gel strength, gels were prepared by heating 5 g of the above BHS sol in glass vials (15 mm i.d. × 65 mm l) from 20 to 75°C at 1°C/min in a water bath, followed by chilling in an ice slurry. After overnight setting at 4°C, gels, while still in the vials undisturbed, were equilibrated at room temperature (24°C) for 40 min and subsequently penetrated using a steel rod (12.5 mm dia with flat end) attached to the load cell (1 kg capacity) in a Model 4301 Instron universal testing machine (Instron Corp., Canton, MA). The crosshead speed was set at 20 mm/min. The force required to disrupt the gels (first peak) was used to represent gel strength.

Cooking yield and water-holding capacity

Cooking yield and water-holding capacity (WHC) were determined as described by Daum-Thumberg et al. (1992). About 2 g aliquots of protein sol were weighed into glass vials (15 mm dia × 40 mm l) and set at 4°C for 18h. The protein sol was cooked in a water bath at 1°C/min to 75°C. Cooked gels were chilled in an ice slurry and stored at 4°C for 18h. Gel weights were recorded after the vials were inverted and the cooked-out liquid was absorbed with a paper towel. Cooking yield was calculated as the weight of bloated gel divided by the weight of protein sol then multiplying by 100. Gels after the cooking yield determination were placed in thimbles folded with filter paper (2 pieces with a 5.5 cm dia at the outer layer and 1 piece with a 7.5 cm dia at the inner layer) and centrifuged at 30,000 × g (Sorwall RC-5B, Du Pont Instruments, Fairview, TN, Rotor SS34 16,000 rpm) for 15 min. After centrifugation, the weight of the gel was recorded and the mois-
tured remaining in the gel was determined by heating in a 100°C oven (AOAC, 1990). WHC was expressed as moisture content (g) after centrifugation divided by the protein content (g) in the gel.

**Emulsifying properties**

Emulsifying activity index (EAI) and emulsion stability (ES) were measured as described by Li-Chan et al. (1985). Pellets of BHS were prepared as a 1% protein suspension by mixing with 0.6 M NaCl in 50 mM sodium phosphate buffer (pH 6.0). After setting at 4°C for 18 h, the sol was mixed with corn oil at a (oil):4(sol) ratio (v/v). The mixture was emulsified using a micro (250 mL) Waring Blender (No. 707SB). Blending (2 min) was done using the high speed setting, and the temperature of the emulsion mixture was kept within 4-10°C during homogenization. The emulsion was allowed to set for 10 min. Subsequently, turbidity of the diluted emulsion was measured at 500 nm. EAI (unit: m²/g) was calculated according to Pearce and KinSELLA (1978) with the oil fraction corrected as:

\[
\text{EAI} = \frac{2 \times 2.303 \times C}{(1 - \phi) \times 10^7 \times A_{500} \times \text{dilution}}
\]

where \(C\) is protein concentration (g/mL) before emulsification, and \(\phi\) is oil volume fraction (v/v) of the emulsion. The measurement was repeated every 30 min until the absorbance reading at 500 nm decreased to half the initial value. The time elapsed (min) before \(A_{500}\) reached its half value was recorded as an indicator of ES.

**Statistical analysis**

Statistical analysis was performed as described previously (Wang et al., 1997). Specifically, the design of the study was a randomized complete block splitplot. The blocking factor was the replicate, i.e., the 3 repeated experiments run on different days (factor R). The whole plot consisted of the 12 treatment combinations, i.e., a 2×2×3 factorial treatment with factor A (propyl gallate, 2 levels), factor B (cryoprotectants, 2 levels) and factor C (freezing temperature, 3 levels). The subplot was the number of measurements at different time intervals (factor D). The overall F test was done using linear models of general AOV with Statistix 3.5 software package (Analytical Software, Inc., St. Paul, MN) for microcomputers. Significance was defined at \(P \leq 0.05\) unless otherwise specified. When the main effects were significant, two-way and three-way interactions were analyzed. Differences of means were analyzed using least significance difference (Snedecor and Cochran, 1989).

**RESULTS & DISCUSSION**

**Protein solubility**

Treatment of BHS with cryoprotectants before freezing stabilized protein solubility although there were some slight decreases after 12 wk storage at −15°C or −29°C (Fig. 1). Without cryoprotectants, protein solubility decreased substantially (\(P<0.05\)) after 12 wk at −15° or −29°C. The storage temperature of −7°C showed maximum protection of protein solubility for all surimi samples up to 24 wk. Buttkus (1970) had reported that the formation of insoluble, high-molecular-weight protein aggregates in myosin solutions increased as the temperature decreased to below the freezing point and reached a maximum near the eutectic point (−11°C) of the myosin-KCl-water solution. Solubility is an important functional characteristic related to protein hydration and is a prerequisite for many other functional attributes of muscle proteins in processed meats. Hence, factors such as structure of proteins, the pH and ionic strength of the medium, and various other intrinsic and extrinsic parameters could influence protein solubility. In our study, essentially all sarcoplasmic proteins were removed by repeated washing. Hence, solubility of BHS could be attributed to myofibrillar proteins only. Myofibrillar proteins are susceptible to freezing treatment and their solubility usually decreases during frozen storage (Xiong, 1997).

To minimize deleterious changes in surimi during frozen storage, including loss of solubility and denaturation of myosin, various hydrophilic compounds particularly polyols have been added to washed fish mince subjected to freezing treatment (Noguchi et al., 1975; Lee, 1990). It is generally hypothesized that these cryoprotectants afford their stabilizing effect through changing the water structure and modification of hydrophobic interactions that may be essential for protein structure and intermolecular association. The effectiveness of the two cryoprotectants we used (i.e., sucrose and sorbitol) in maintaining the solubility of BHS proteins presumably resulted from weakened protein-protein interactions by increasing the carbohydrate-mediated protein hydration.

Analysis of variance showed that cryoprotectants, frozen temperature, and storage time were major factors affecting protein solubility of BHS (\(P<0.01\), Table 1). There were also two-way interactions (\(P<0.01\) or \(P<0.05\)) among the three factors, showing that low freezing temperature or cryoprotectants could offset the detrimental effects of prolonged storage or elevated freezing temperatures (−15° or −29°C).

**Gel-forming abilities**

**Storage modulus (\(G^′\)).** In general, the effects of propyl gallate on rheological properties of the gelling BHS were not significant (\(P>0.05\)). However, cryoprotectants and freezing temperature had a marked impact on thermally-induced rheological changes above 50°C (Fig. 2). The overall rheological pattern, in terms of the \(G^′\) evolution over temperature, was similar among all BHS samples. Without cryoprotectants, \(G^′\) values of BHS after 52 wk storage at −15° or 29°C were low, suggesting that those surimi samples were unable to form an elastic gel network. However, all samples stored at −7°C produced similar rheograms to fresh surimi, including the magnitude of \(G^′\) (\(P>0.05\)), which was strong evidence that the low storage temperature was a potent protective factor for structure-forming proteins. When the peak \(G^′\) values (−53°C, Fig. 2) were plotted against storage time, it became apparent that the gel-forming ability of BHS gradually decreased over time especially for samples stored at −15°C and −29°C (Fig. 3). The protective effects (\(P<0.05\)) of cryoprotectants were evident after 12 wk storage at −15° or −29°C. When the final \(G^′\) values (at 73°C, Fig. 2) were plotted as a function of storage time, relationships similar to those for peak \(G^′\) between BHS samples, with or without the cryoprotectants or propyl gallate, were observed (Fig. 4). Hence, both the \(G^′\) values associated with the rheological transition (−53°C) and the \(G^′\) values at the end of heating (75°C) could be used to characterize BHS gelation.

Storage modulus is a good index for the gel-forming ability of food proteins. The higher the \(G^′\) value, the greater the gel-forming ability of the protein system, assuming that...
the protein gel is made up of a highly viscoelastic network (Ferry, 1980). Although antioxidant mixtures (propyl gallate, ascorbate, sodium tripolyphosphate) enhanced the gelation of fresh BHS (Wan et al., 1993; Xiong et al., 1993), our data indicated that propyl gallate alone was incapable of preventing losses in gelling ability of BHS. Several previous studies showed that ascorbate (Nishimura et al., 1992; Lee and Lanier, 1995) and tripolyphosphate (Trout and Schmidt, 1986; Srinivasan and Xiong, 1996) could enhance gelation of myofibrillar proteins independent of antioxidants. Hence, we expected that propyl gallate alone would not alter the gelation behavior of BHS.

Gelation of food proteins is a function of the nature of the proteins and processing conditions such as pH, ionic strength, binding agent and heating regimen. There was evidence that under certain specific conditions, oxidation could promote functional performance of muscle proteins such as fish surimi (Srinivasan and Hultin, 1997) and beef cardiaca myofibrils (Srinivasan and Xiong, 1996). Whether or not oxidation can lead to enhanced gelation, emulsification and other physicochemical properties of muscle proteins seems to be determined by the extent of oxidative modification as well as type of changes in proteins. It has been hypothesized that limited oxidative modifications in proteins would facilitate balanced protein-protein and protein-solvent interactions conducive to gelation. Excessive oxidation would suppress protein functionality due to the formation of insoluble protein aggregates and destruction of some functional side chains (Xiong, 1996). According to Lee and Lanier (1995), gelation of surimi proteins involves both covalent and noncovalent bonds. The major covalent bonds are disulfide and glutamyl-lysine covalent linkage, and the major noncovalent bonds are hydrophobic interactions, hydrogen bonds and ionic bonds. Formation of such intermolecular bonds could theoretically be driven by oxidative stresses, and hence, affect the gelation process of surimi. However, a general prevention of myofibrillar proteins from freeze-induced denaturation and aggregation, whether or not oxidation is involved, seems to be most critical for stabilization of the functionality of BHS during frozen storage. This can be substantiated. Addition of cryoprotectants were shown to promote oxidation of lipids and protein side chains but minimize protein conformational changes and denaturation. Also low freezing temperature (under which both conformational and oxidative changes in proteins are greatly inhibited) (Wang et al., 1997) effectively diminished losses in gel elasticity of BHS, especially during

![Fig. 2—Rheograms of fresh and frozen stored (52 wk) beef heart surimi prepared with (+AO) or without (-AO) antioxidant and blended with (+CP) or without (-CP) cryoprotectants.](image)

![Fig. 3—Peak storage modulus of beef heart surimi prepared with (+AO) or without (-AO) antioxidant and blended with (+CP) or without (-CP) cryoprotectants during frozen storage. The peak (53°C) storage modulus values were obtained from the rheograms shown in Fig. 2.](image)

### Table 1—Analysis of variance for solubility of surimi protein, and for storage moduli and gel strength of surimi gel

<table>
<thead>
<tr>
<th>Variable</th>
<th>Protein solubility</th>
<th>Peak G&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Final G&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Gel strength</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F ratio</td>
<td>P value</td>
<td>F ratio</td>
<td>P value</td>
</tr>
<tr>
<td>Propyl gallate (A)</td>
<td>&lt;0.1</td>
<td>NS*</td>
<td>1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Cryoprotectant (B)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>18</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Temperature (C)</td>
<td>&lt;0.01</td>
<td>5.7</td>
<td>&lt;0.01</td>
<td>10</td>
</tr>
<tr>
<td>Time (D)</td>
<td>&lt;0.01</td>
<td>48</td>
<td>&lt;0.01</td>
<td>57</td>
</tr>
<tr>
<td>A*B</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A*C</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A*D</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B*C</td>
<td>8.8</td>
<td>&lt;0.01</td>
<td>3.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B*D</td>
<td>9.2</td>
<td>&lt;0.01</td>
<td>8.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C*D</td>
<td>2.0</td>
<td>&lt;0.05</td>
<td>3.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A<em>B</em>D</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A<em>C</em>D</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B<em>C</em>D</td>
<td>1.1</td>
<td>NS</td>
<td>2.8</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*The error term for A, B, C, A*B, A*C, B*C was (a*b*c-1)*(r-1); the error term for D, A*D, B*D, C*D, A*C*D, B*C*D was a*b*c*(d-1)*(r-1).

Peaks G and final G refer to the storage moduli at 60 and 75°C, respectively (See Fig. 2).

NS: nonsignificant.

**— not analyzed because the main effects were nonsignificant.
extended frozen storage (Fig. 3 and 4).

Analysis of variance showed that cryoprotectants, frozen temperature, and storage time had major effects (P<0.01, Table 1) on gelation. In addition, two-way and three-way interactions of these parameters were also apparent (P<0.01 or P<0.05), suggesting that losses of gelling ability of BHS would be minimized through the concerted effects of these storage parameters. More specifically, cryoprotectants and low storage temperatures had synergistic actions in preserving gel-forming ability, while prolonged storage time and elevated storage temperature had synergistic detrimental effects.

Gel strength. Similar to changes in $G'$, the changes in gel strength as determined by the Instron penetration test were affected primarily by cryoprotectants, freezing temperature, and storage time (P<0.01, Table 1), and not by propyl gallate. Addition of cryoprotectants maintained the gel strength at the initial level (2.3-2.8N) for the first 12 wk (Fig. 5). Beyond that, all samples stored at −15°C or −29°C showed marked decreases in gel strength, especially for surimi containing no cryoprotectants. Samples stored at −70°C did not show much decrease in gel strength even after 52 wk storage. Analysis of variance revealed a parallel relationship between changes in gel strength and those in $G'$ (Table 1).

Cooking yield (CY) and water-holding capacity (WHC)

Cooking yield and WHC are indices of water-retaining properties of protein foods. In muscle protein gels, both properties are closely related to texture and matrix characteristics of the gel. In the absence of cryoprotectants, CY of surimi samples stored at −15°C or −29°C began to decline after 2 wk (Fig. 6), corresponding to a similar change in WHC (Fig. 7). The results suggested that the increased cooking loss of BHS caused by frozen storage was due to the inability of the BHS protein and its gel matrix to bind or immobilize water. As storage time was prolonged, the loss of CY and WHC continued to occur with most of the loss occurring between 24 and 52 wk. Addition of cryoprotectants protected CY and WHC up to 24 wk. At −70°C, CY and WHC did not show any changes for any of the treatments, indicating that the low storage temperature was effective in preserving water-retaining ability of BHS.

Analysis of variance showed that cryoprotectants, frozen temperature, and storage time affected CY and WHC (P<0.01, Table 2). Significant interactions (P<0.05 for CY; P<0.01 for WHC) also existed between cryoprotectants and storage time, demonstrating that cryoprotectants could counterbalance some losses of CY and WHC during extended storage up to 24 wk.

Emulsifying properties

Cryoprotectants had a major (P<0.01) impact on emulsifying properties of BHS. Without cryoprotectants, EAI decreased markedly for samples stored at −15°C or −29°C (Fig. 8), coinciding with loss in protein solubility (Fig. 1). EAI is a measure of interfacial area created per unit of protein. The decrease in EAI would imply that less soluble or partially denatured BHS proteins became less effective in dispersing lipids through the formation of a cohesive membrane surrounding the fat droplets. Surimi samples stored at −70°C did not show changes in EAI even after 52 wk storage. Incorporation of cryoprotectants also substantially inhibited the decrease of ES (Fig. 9). Samples that did not contain cryoprotectants (control) showed a rapid decline in ES after 2 wk storage at −15°C or −29°C. The presence of propyl gallate did not effect any appreciable changes in emulsifying activity or stability of the emulsion. This sharply differed from the results in fish surimi where oxidized myofibrillar proteins, except those which were abused by freeze-thaw

---

**Fig. 4**—Final storage modulus of beef heart surimi prepared with (+AO) or without (−AO) antioxidant and blended with (+CP) or without (−CP) cryoprotectants during frozen storage. The final (73°C) storage modulus values were obtained from the rheograms shown in Fig. 2.

**Fig. 5**—Changes in gel strength (Instron penetration force) of beef heart surimi prepared with (+AO) or without (−AO) antioxidant and blended with (+CP) or without (−CP) cryoprotectants during frozen storage.

**Fig. 6**—Changes in cooking yield of beef heart surimi prepared with (+AO) or without (−AO) antioxidant and blended with (+CP) or without (−CP) cryoprotectants during frozen storage.
Table 2—Analysis of variance for cooking yield (CY) and water-holding capacity (WHC) of surimi gels and for surimi emulsifying activity index (EAI) and emulsion stability (ES)

<table>
<thead>
<tr>
<th>Variable</th>
<th>CY F ratio</th>
<th>CY P value</th>
<th>WHC F ratio</th>
<th>WHC P value</th>
<th>EAI F ratio</th>
<th>EAI P value</th>
<th>ES F ratio</th>
<th>ES P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propyl gallate (A)</td>
<td>0.4</td>
<td>NS</td>
<td>&lt;0.1</td>
<td>NS</td>
<td>&lt;0.1</td>
<td>NS</td>
<td>0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Cryoprotectant (B)</td>
<td>34</td>
<td>&lt;0.01</td>
<td>35</td>
<td>&lt;0.01</td>
<td>21</td>
<td>&lt;0.01</td>
<td>39</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Temperature (C)</td>
<td>7.2</td>
<td>&lt;0.01</td>
<td>8.0</td>
<td>&lt;0.01</td>
<td>3.1</td>
<td>NS</td>
<td>11</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Time (D)</td>
<td>6.4</td>
<td>&lt;0.01</td>
<td>15</td>
<td>&lt;0.01</td>
<td>2.1</td>
<td>NS</td>
<td>3.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>A*B</td>
<td>—**</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A*C</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A*D</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B*C</td>
<td>2.4</td>
<td>NS</td>
<td>1.6</td>
<td>NS</td>
<td>—</td>
<td>—</td>
<td>2.5</td>
<td>NS</td>
</tr>
<tr>
<td>B*D</td>
<td>2.3</td>
<td>&lt;0.05</td>
<td>6.2</td>
<td>NS</td>
<td>—</td>
<td>—</td>
<td>1.1</td>
<td>NS</td>
</tr>
<tr>
<td>C*D</td>
<td>1.1</td>
<td>NS</td>
<td>2.5</td>
<td>&lt;0.01</td>
<td>—</td>
<td>—</td>
<td>3.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A<em>B</em>C</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A<em>C</em>D</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B<em>C</em>D</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*a The error term for A, B, C, A*B, A*C, B*C, A*B*C was (a*b*c-1)(r-1); the error term for D, A*D, B*D, C*D, A*C*D, B*C*D was a*b*c*(d-1)*(r-1).
*NS: nonsignificant.
**—not analyzed because the main effects were nonsignificant.

The ability to stabilize fat particles in the form of hydrodynamic emulsion is an important property of muscle proteins in comminuted meat products such as frankfurters and bologna. When the fat particles are coated with surface-active salt-soluble proteins, their stability is expected to increase sharply due to a reduction in interfacial tension (Gordon and Barbut, 1992). Li-Chan et al. (1985) reported that the emulsifying ability of muscle proteins depended on the balance of solubility and hydrophobicity. Proteins with high solubility and hydrophobicity had excellent emulsifying ability. In our study, the changes in emulsifying ability of beef heart surimi were directly related to changes in protein solubility. This was expected because the protein solubility was low and hence, the determining factor for emulsifying properties would be protein solubility based on evidence established by Li-Chan et al. (1985). Srikar and Reddy (1991) reported that a decline in protein solubility caused a decrease in emulsifying capacity of frozen stored fish mince which was confirmed by results from our present study.

Analysis of variance showed that cryoprotectants, frozen temperature, and storage time all affected ES (P<0.01 or P<0.05, Table 2). Interactions between temperature and time were also positive. The changes in ES were apparently more closely related to changes in other functional properties than they were to EAI. The EAI may be high at the isoelectric point (pI) of a protein due to the lack of electrostatic repulsive interactions of protein molecules (hence, increasing the protein loaded at the oil/water interface), but at pI, ES is usually low due to flocculation and coalescence of the “neutral” fat droplets. In our preliminary studies we noted that EAI was more sensitive to changes in processing factors (e.g., homogenization condition) than treatments, showed improved emulsifying ability and ES when compared to nonoxidized proteins (Srinivasan and Hultin, 1997). The discrepancy may be attributed to the different emulsifying conditions used. In our study, the myofibrillar proteins were solubilized in 0.6M NaCl and 50 mM phosphate buffer (pH 6.0), but in their study, emulsions were prepared apparently in the absence of salt and the pH of the protein/oil mixture was not controlled.

The ability to stabilize fat particles in the form of hydrodynamic emulsion is an important property of muscle proteins in comminuted meat products such as frankfurters and bologna. When the fat particles are coated with surface-active salt-soluble proteins, their stability is expected to increase sharply due to a reduction in interfacial tension (Gordon and Barbut, 1992). Li-Chan et al. (1985) reported that the emulsifying ability of muscle proteins depended on the balance of solubility and hydrophobicity. Proteins with high solubility and hydrophobicity had excellent emulsifying ability. In our study, the changes in emulsifying ability of beef heart surimi were directly related to changes in protein solubility. This was expected because the protein solubility was low and hence, the determining factor for emulsifying properties would be protein solubility based on evidence established by Li-Chan et al. (1985). Srikar and Reddy (1991) reported that a decline in protein solubility caused a decrease in emulsifying capacity of frozen stored fish mince which was confirmed by results from our present study.

Analysis of variance showed that cryoprotectants, frozen temperature, and storage time all affected ES (P<0.01 or P<0.05, Table 2). Interactions between temperature and time were also positive. The changes in ES were apparently more closely related to changes in other functional properties than they were to EAI. The EAI may be high at the isoelectric point (pI) of a protein due to the lack of electrostatic repulsive interactions of protein molecules (hence, increasing the protein loaded at the oil/water interface), but at pI, ES is usually low due to flocculation and coalescence of the “neutral” fat droplets. In our preliminary studies we noted that EAI was more sensitive to changes in processing factors (e.g., homogenization condition) than...
was ES. Samples with a relatively low protein solubility could still produce a high EAI value when a proper processing condition was applied, while ES, gel strength and WHC were always found to be less than values for samples with a high protein solubility.

**CONCLUSIONS**

**STORAGE TEMPERATURE WAS THE SINGLE MOST CRITICAL FACTOR GOVERNING THE FUNCTIONAL STABILITY OF BEEF HEART SURIMI DURING FREEZING PRESERVATION.** When storage temperature was as low as −70°C, loss in functionality of beef heart surimi was almost completely inhibited irrespective of cryoprotectants. The marked improvement in functional properties of cryoprotectant-treated surimi compared to its control at higher frozen temperatures, however, necessitated the incorporation of cryoprotectants into the surimi prior to frozen storage. Due to the prooxidative effect of the cryoprotectants, propyl gallate or other effective antioxidants should also be incorporated into surimi processing for its extended frozen oxidative stability.

**REFERENCES**


Ms received 6/7/97; revised 10/20/97; accepted 10/27/97.

Journal article no. 97-07-73 of the Kentucky Agricultural Ex-periment Station. This research was supported by a CSRS/USDA National Research Initiative (NRI) grant, under Agreement Grant No. 94-37350-0051.