Inhibition of Protein and Lipid Oxidation in Beef Heart Surimi-like Material by Antioxidants and Combinations of pH, NaCl, and Buffer Type in the Washing Media†

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Protein and lipid oxidation and their inhibition by various washing media were monitored during the preparation of beef heart surimi-like material. Lipid oxidation was measured as increases in thiobarbituric acid (TBA)-reactive substances and conjugated dienes, and protein oxidation was determined as formation of protein carbonyls. Water-soluble antioxidant tripolyphosphate (0.2%) and lipid-soluble antioxidants propyl gallate (0.02%) and α-tocopherol (0.2%), added to the washing solution, were effective inhibitors of both lipid and protein oxidation. Ascorbate (0.2%) also inhibited lipid oxidation but caused an increase in protein carbonyls. Phosphate buffer (25 mM NaH_2PO_4/Na_2HPO_4), high final pH (7.0) of surimi pellet, and the presence of salt (0.1 M NaCl) all inhibited both protein and lipid oxidation during storage. Several processing options are thus available for the preparation of shelf-stable surimi-like material from beef hearts for possible utilization in formulated meat products.

Keywords: *Beef heart; surimi; protein; lipid; oxidation*

INTRODUCTION

Interest in utilizing edible meat byproducts as value-added products has increased in recent years. Processed meats provide variety to consumers with ever-changing needs influenced by the new information on diet and health. The use of beef heart muscle as an ingredient in restructured meat products has been limited by low protein functionality and less desirable flavor compared with skeletal muscle. However, many of the undesirable components (e.g., fat particles, pigments, and odorants) can be removed through mincing and washing (i.e., surimi preparation), thus allowing marketing the muscle mince as a premium functional ingredient. Success in utilization of washed minced beef heart largely depends on the stability of the product and the muscle protein functionality. McKeith *et al.* (1988) demonstrated that surimi-like material prepared from beef hearts had improved textural properties when compared with fish surimi. Kenney *et al.* (1992) showed that incorporation of washed beef cardiac muscle into restructured beef enhanced sensory and instrumental texture traits. Nevertheless, the incorporation led to increased lipid oxidation in the beef rolls.

Lipid peroxidation is a well-known phenomenon in stored muscle foods. The susceptibility of beef heart surimi to oxidative deterioration may be related to the residual heme compounds, iron, and polyunsaturated fatty acids (e.g., arachidonic acid), which are initially high in unwashed cardiac muscle (USDA, 1990). Heme iron (Greene, 1969; Govindarajan *et al.*, 1977; Kanner and Harel, 1985), nonheme iron (Johns *et al.*, 1989; Kanner *et al.*, 1991), and enzymic systems (Lin and Hultin, 1976; Kanner and Kinsella, 1983; Rhee *et al.*, 1983) have been implicated as major catalysts of lipid peroxidation in muscle foods. Muscle washing would substantially remove water- and low-salt-soluble substances, fat particles, and heme compounds (Toyoda *et al.*, 1992). However, preparation of surimi involves extensive cellular disruption caused by mincing of tissue. Oxygen is mixed with lipid and different prooxidants during washing, so lipid radicals can be generated. Mincing of tissue may also mimic the conditions of the "reperfusion syndrome" in which the reintroduction of molecular oxygen into the tissue that has undergone anaerobiosis results in the generation of active oxygen species (McCord, 1987). Thus, both proteins and lipids are susceptible to oxidative deterioration during surimi processing.

Several recent studies have shown that inhibition of lipid oxidation during myofibril preparation improves protein functionality of bovine cardiac muscle (Wan *et al.*, 1993; Xiong *et al.*, 1993) and reduces off-flavor associated with oxidation in Atlantic mackerel surimi (Kelleher *et al.*, 1992). Proteins and lipids contribute to much of the desirable textural and flavor attributes of muscle foods. Therefore, it would be important to minimize changes in both proteins and lipids during processing. The first objective of this study was to determine the optimal condition for minimizing lipid and protein oxidation during the preparation of surimi-like material from the edible portion of beef hearts. This was accomplished with different antioxidants and various combinations of pH, salt, and buffer type in the washing solution. The second objective was to assess the storage stability of washed muscle mince at 2 °C.

MATERIALS AND METHODS

Materials. Fresh beef hearts (24−28 h postmortem) were obtained from cattle (age 16−20 months) slaughtered at a local meat packing company. Beef hearts were individually vacuum packaged in polyethylene bags and frozen in a blast freezer (−29 °C). All heart samples were stored at −29 °C

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and used within 4 months. Coomassie protein assay reagent (no. 23200) was obtained from Pierce Chemical Company (Rockford, IL). Piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) disodium salt (PIPES) was purchased from Sigma Chemical Company (St. Louis, MO). All other chemicals used were of at least analytical grade.

Preparation of Surimi Pellet. All experiments were replicated with at least three different hearts using ground and washed muscle samples freshly prepared on different days. All samples for each experiment originated from the same hearts. A sample size of 100 g of ground muscle was used for each parameter studied in the same experiment. Beef hearts were thawed at 2 °C for 24 h. All sample preparations were carried out in a 5 °C walk-in cooler, unless otherwise specified. After the removal of caps, vessels, and external fat tissue, the lean muscle was diced into ~2-cm cubes, and ground through a 4.7-cm diameter orifice with a Kitchen Aid (Kitchen Aid Inc., St. Joseph, MI) mincer. The minced muscle was washed twice in 10 volumes (v/w) of washing solution (specified later) by stirring with a spatula for 1 min. The mixture was allowed to stand for 10 min, and was then strained on a nylon screen mesh (0.25 cm^2). A third and final wash was done in five volumes (v/w based on original weight of mince) of 25 mM phosphate buffer (NaH_2PO_4/Na_2HPO_4), pH 6.0 (unless otherwise specified), by blending in a Waring blender for 1 min at high speed. The pH of the homogenate was adjusted, if necessary, to 6.0 with 0.1 N HCl or 0.1 N NaOH, and the homogenate was centrifuged at 2000*g* for 15 min. The supernatant was discarded, and the pellet was stored on ice for specific periods (0, 1, 3, 5, and 7 days) to monitor oxidative changes in proteins and lipids.

Washing Solutions. When water-soluble antioxidants (ascorbate, tripolyphosphate) were used, they were incorporated into washing solutions at 0.2% (w/v) in all three washes. The washing solutions contained 25 mM phosphate buffer, pH 7.0 for the first two washes. The final wash was done in the same buffer at pH 6.0. Although phosphate is not a strong buffering agent at pH 6.0, it maintains ~10% of its maximal buffering capacity at this pH. Phosphate was also chosen because it is naturally present in muscle. Two control washings were run at the same time: one had phosphate buffer in all three washes but no antioxidants and was denoted "buffer" washing, and the other control had distilled deionized water in the first two washes and 25 mM phosphate buffer, pH 6.0 in the final wash and was denoted "water" washing.

In the experiments determining the effect of lipid-soluble antioxidants, the use of phosphate buffer, pH 7.0 in the first two washes was omitted because of the inhibitory effect of phosphate on lipid peroxidation (Figure 1). Hence, this experimental design would minimize interference by phosphate and facilitate definition of the role of lipid-soluble antioxidants. Lipid-soluble antioxidants (propyl gallate, α-tocopherol/vitamin E) were dissolved in 5 mL of 50% ethanol before being added to 100 g of minced muscle. The final antioxidant concentration was 0.02% of muscle tissue, unless otherwise stated. After thoroughly mixing for 5 min, the mince was washed twice in 10 volumes (v/w) of distilled deionized water. The final wash was done in 5 volumes (v/w) of 25 mM phosphate buffer, pH 6.0. A control in which the minced muscle was mixed with 50% ethanol only (5 mL/100 g of tissue), was prepared in the similar way.

When pH (7.0, 6.0, or 5.5) and nature of buffer (phosphate, PIPES) were varied in washing solutions, all three washes were done in the same buffer of the same pH. The pK_a of PIPES at 25 °C is 6.8 and its useful pH range is 5.8 to 7.8. PIPES was chosen as an alternate for phosphate because of its similarity in useful pH range and pK_a values. In addition, PIPES has no known effects on chelating metal ions. For studying the role of salt, two washes in distilled deionized water and a final wash in 25 mM phosphate buffer (pH 6.0), with and without 0.1 M NaCl, were done.

Preparation of Sample for Chemical Assays. A 2 to 3-g sample of surimi pellet was suspended in 6.0 mL of distilled deionized water and mixed well to form a fine smooth slurry with a protein concentration of 30−40 mg/mL. Protein concentration in the slurry was determined by the biuret method after diluting a 0.1-mL aliquot to 1.0 mL with distilled deionized water and reacting it with 4.0 mL of biuret reagent (Torten and Whitaker, 1964).

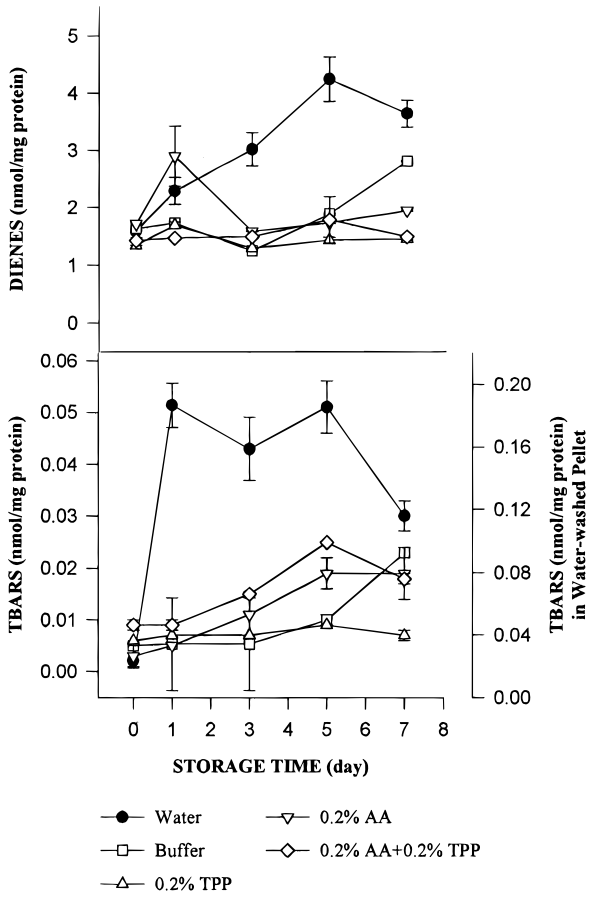

Figure 1. Changes in TBARS and conjugated dienes in beef heart surimi pellet during storage (0 °C). Surimi pellet was prepared in the presence of water-soluble antioxidants alone or in combination (tripolyphosphate, TPP; ascorbic acid, AA) added to all three washes. Two control washes, denoted buffer and water, were done in 25 mM phosphate buffer and distilled deionized water, respectively. Data for water-washed control pellet are presented on a different *Y*-axis scale and represents an average of four experiments (replications) assayed in duplicate. All other data represent an average of two experiments assayed in duplicate.

Carbonyl Content of Proteins. Protein carbonyls were assayed as hydrazone derivatives by reacting proteins with 2,4-dinitrophenylhydrazine (DNPH) as described by Levine *et al.* (1990), with some modifications (Srinivasan and Hultin, 1995). A 50-μL aliquot of sample, prepared as just described, was reacted with 2.0 mL of 10 mM DNPH in 2.0 N HCl for 1 h at room temperature; another 50-μL of sample with 2.0 mL of 2.0 N HCl served as control. The protein content of the final mixture dissolved in 6.0 M guandine, 20 mM phosphate (pH 2.3) was determined by reacting 20 μL of sample with 1.0 mL of Coomassie protein assay reagent and measuring absorbance at 595 nm after 10 min (Bradford, 1976). The difference in absorbance at 370 nm between HCl-treated and DNPH-treated samples was taken as a measure of reacted carbonyl groups, using a molar extinction coefficient of 22 400 M^{-1} cm^{-1}.

Thiobarbituric Acid-Reactive Substances (TBARS). The extent of lipid oxidation in the washed mince was determined by the TBARS measurement based on the method of MacDonald and Hultin (1987) using a molar extinction coefficient of 15 600 M^{-1} cm^{-1}. A 2.0-mL aliquot of a solution of 7.5% trichloroacetic acid (TCA), 0.1% propyl gallate (PG), and 0.1% EDTA was added to 1.0 mL of sample while mixing. The mixture was centrifuged, and the supernatant was used for reaction with thiobarbituric acid (TBA).

Conjugated Dienes. Lipid oxidation was also monitored by the formation of conjugated dienes which was measured

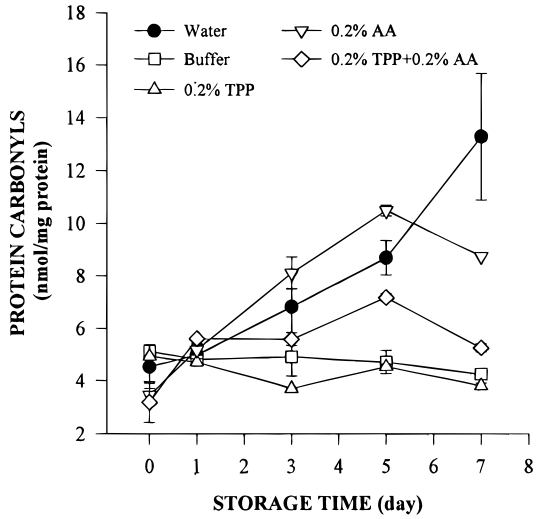

Figure 2. Effect of storage (0 °C) time on protein carbonyls in beef heart surimi pellet prepared in the presence of water-soluble antioxidants in the washing media. Symbols are the same as in Figure 1.

as increases in the absorbance at 233 nm (Esterbauer *et al.*, 1982) of a hexane, isopropanol extract. A 0.5-mL sample was extracted with 5.0 mL of combined hexane and isopropanol (3:2) for 1 min on a vortex and then centrifuged at 2000 × *g* for 5 min. A blank with distilled deionized water was similarly treated. The difference in absorbance at 233 nm was used for the determination of conjugated diene, using a molar extinction coefficient of 25 200 M^{-1} cm^{-1} (Buege and Aust, 1978).

Statistical Analysis. Data were analyzed by the general linear models procedure of the Statistix 3.5 software package (Analytical Software, Inc., St. Paul, MN) for microcomputers. Analysis of variance (ANOVA) was done to determine the significance of main effects (treatment; storage time). Significant ($p < 0.05$) differences between means were identified by the least significant difference procedure (Snedecor and Cochran, 1989).

RESULTS

Water-Soluble Antioxidants. The effect of water-soluble antioxidants incorporated into the washing solution on lipid peroxidation in the final surimi-like material (henceforth referred to as "surimi" without an inference to fish-based product) is shown in Figure 1. The extent of lipid oxidation as measured by TBARS or dienes was the same in all samples on the day of surimi preparation (analyzed within 2 h). However, during storage, there was a rapid increase in TBARS in the water-washed surimi sample after 1 day. The TBARS tapered off and started to decrease by day 7. Conjugated dienes also increased in water-washed samples during storage, reaching a maximum on day 5. Incorporation of tripolyphosphate almost completely inhibited both TBARS and diene formation in the surimi pellet throughout the storage period. Buffer-washed surimi pellet also showed remarkable stability during the initial stages of storage and showed a small increase in both dienes and TBARS after 7 days. Ascorbate-containing surimi pellets showed small increases in TBARS content towards the end of the storage period.

There was nearly a linear increase in protein carbonyls in the water-washed pellet with storage time (Figure 2). Washing in phosphate buffer, on the other hand, nearly completely inhibited formation of additional protein carbonyls in the surimi pellet during the storage period. A near total inhibition of protein carbonyls was also observed in tripolyphosphate-washed surimi pellet. However, a significant increase in protein carbonyls was observed in surimi pellets prepared in the presence of ascorbate alone or in combination with tripolyphosphate. Increase in protein carbonyls in the presence of ascorbate alone was much higher than in the presence of combined ascorbate and tripolyphosphate.

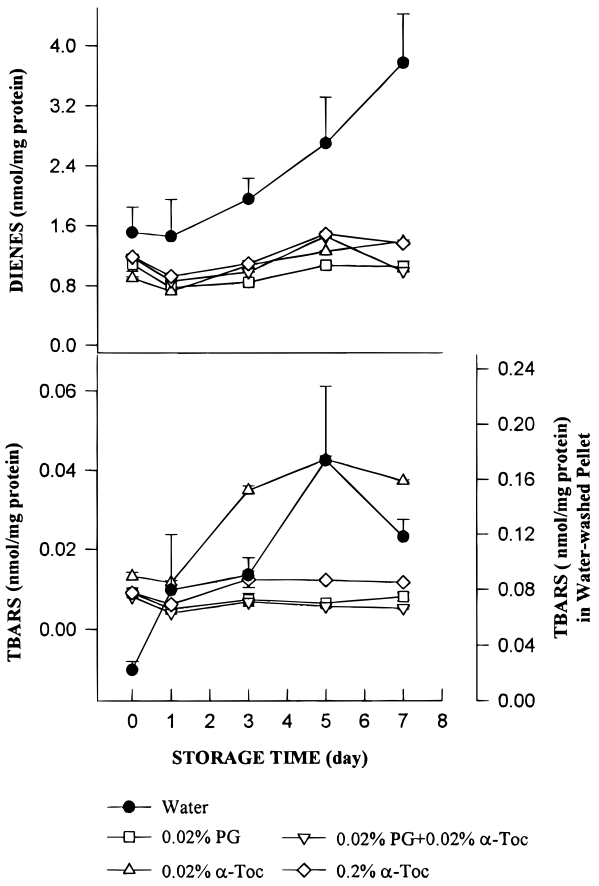

Figure 3. Changes in TBARS and conjugated dienes in beef heart surimi pellet during storage (0 °C). Surimi pellet was prepared in the presence of lipid-soluble antioxidants (propyl gallate, PG; α-tocopherol, α-Toc) added alone or in combination to minced muscle before washing. Data for water-washed pellet are presented on a different *Y*-axis scale.

Lipid-Soluble Antioxidants. As demonstrated in Figure 3, a nearly complete inhibition of lipid peroxidation, as measured by TBARS or dienes, occurred in surimi pellet containing propyl gallate by itself or in combination with α-tocopherol both during the preparation of the surimi pellet as well as throughout the storage period. However, α-tocopherol by itself, when used at a 0.02% level, was only partially inhibitory. Nevertheless, increasing the concentration of α-tocopherol to 0.2% resulted in nearly a total inhibition of lipid peroxidation.

The pattern of inhibition of protein carbonyl formation in the presence of lipid-soluble antioxidants was similar to the inhibition of lipid oxidation (Figure 4). When propyl gallate alone or in combination with α-tocopherol was incorporated into the surimi pellet, a nearly total inhibition of protein carbonyls occurred, during both the preparation and storage period. When α-tocopherol was added at a 0.02% level, protein carbonyls increased during storage. Increasing the concentration of α-tocopherol to 0.2% prevented the formation of new protein carbonyls in the surimi pellet (Figure 4), as was the case for the inhibition of TBARS formation (Figure 3).

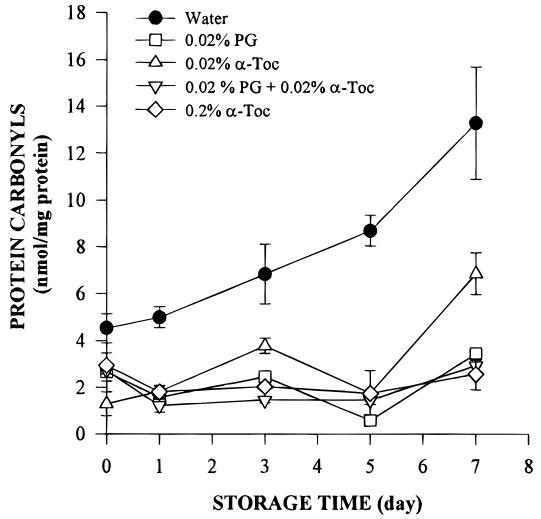

Figure 4. Effect of storage (0 °C) time on protein carbonyls in beef heart surimi pellet prepared in the presence of lipid-soluble antioxidants in the washing media (propyl gallate, PG; α-tocopherol, α-Toc).

Buffer and pH. In the preparation of surimi-like products, pH and the nature of the buffering agents of the washing media play important roles not only with regard to the stability of the product but also from a technological point of view. In the traditional surimi-making process, water, instead of a buffered solution, is usually used. However, in the present study with bovine cardiac muscle, we found that washing in distilled deionized water twice before washing in 25 mM phosphate buffer at pH 6.0 rendered the sample highly susceptible to oxidative deterioration (Figures 1 and 2). When 25 mM phosphate buffer at pH 7.0 was used for the first two washes, and the same buffer at pH 6.0 was used for a third wash, the sample was remarkably stable against oxidative changes (Figures 1 and 2). We tested further to see the interaction of the pH of the buffer and the type of the buffer on lipid and protein oxidation in the surimi pellet. All three washes were carried out in the same buffer with the same pH. As the pH of the phosphate buffer was decreased from 7.0 to 5.5, the extent of lipid peroxidation, as measured by TBARS and dienes formation, increased during storage (Figure 5). At pH 7.0, there was very little lipid oxidation in phosphate buffer. When PIPES was used as the buffering medium at pH 7.0, the inhibitory effect was not as much as it was for the phosphate buffer of a similar molar concentration. This result suggested that in addition to the inhibitory effect of higher pH on lipid peroxidation, phosphate contributed to the inhibitory action. At pH 7.0, protein carbonyl content of the surimi proteins was essentially constant ($p > 0.05$), irrespective of the nature of buffer used during surimi preparation or the storage (Table 1). However, the protein carbonyl content of the surimi proteins prepared at pH 6.0 and 5.5 were higher ($p < 0.05$) than the samples prepared at pH 7.0, both on day 0 and after 7 days of storage. An increase in protein carbonyls during storage was observed only when the pH of the buffer used for the preparation of surimi was 5.5 ($p < 0.05$).

Salt (NaCl). Salt is an important ingredient not only in the preparation of surimi-like products for dewatering purposes but also in the final product for gelation of muscle proteins. In the surimi industry, 0.15% salt (~26 mM) is typically used for dewatering surimi pellet that is normally not homogenized. In the present study, muscle was homogenized and washed in 0.1 M salt in

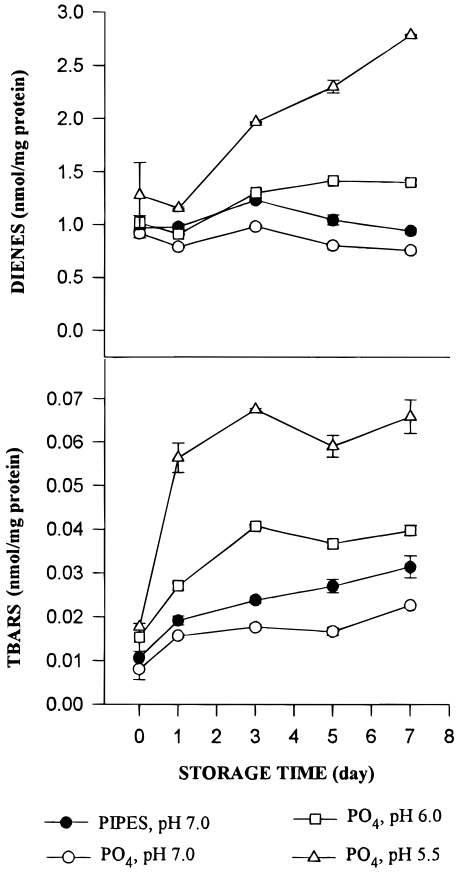

Figure 5. Changes in TBARS and conjugated dienes in beef heart surimi during storage (0 °C) as a function of pH and nature of buffer in the washing media. The concentration of phosphate buffer and PIPES used was 25 mM. All three washes were done in the same buffer of the same pH.

Table 1. Changes in Protein Carbonyl Content in Beef Heart Surimi during Storage (0 °C) as a Function of pH and Nature of Buffer Used[a]

	protein carbonyls (nmol/mg of protein)	
washing medium	day 0	day 7
PIPES, pH 7.0	3.30 ± 0.21^b	3.98 ± 0.03^c
PO_4, pH 7.0	3.43 ± 0.22^b	3.68 ± 0.15^c
PO_4, pH 6.0	4.59 ± 0.09^a	5.09 ± 0.03^b
PO_4, pH 5.5	**4.54 ± 0.10^a**	**7.10 ± 0.58^a**

[a] Mean ± SD values with the same superscript letter in the same column are not significantly different ($p > 0.05$); bold faced data in the same row are significantly different from each other ($p < 0.05$).

the final wash step to obtain purer myofibril pellets. The PIPES buffer, instead of a phosphate buffer was used for this study because phosphate *per se* may contribute to inhibition of lipid peroxidation as seen earlier (Figure 5). There was no significant difference ($p > 0.05$) at pH 7.0 in lipid peroxidation between the salt-washed sample and the sample washed in the absence of salt, as indicated by TBARS and dienes (Figure 6). However, at pH 6.0, fresh samples of both salt-washed and non-salt-washed pellets had a higher content of TBARS than those at pH 7.0. No significant differences ($p > 0.05$) in diene contents were observed among all fresh samples. During subsequent storage on ice, samples washed without salt had a much higher TBARS and diene content than samples washed with salt, suggesting that salt at a 0.1 M level was inhibitory of lipid peroxidation in beef heart surimi pellet. There were no significant differences in protein carbonyl content of

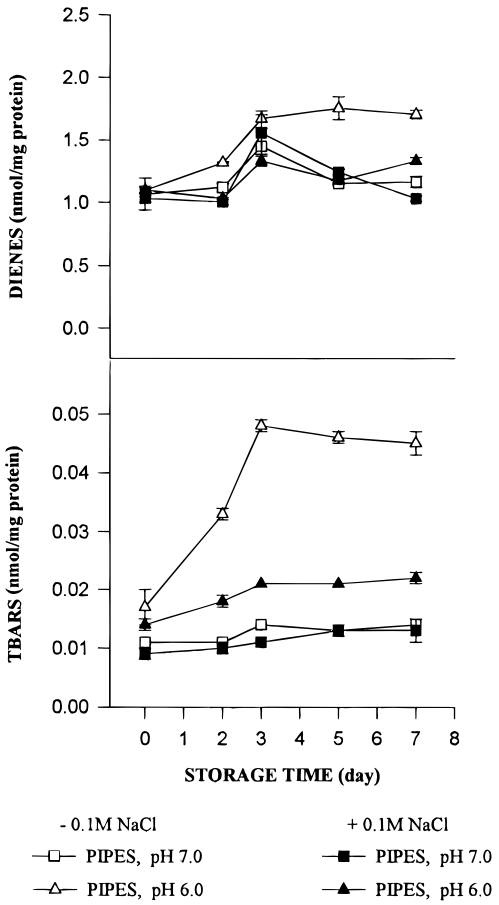

Figure 6. Effect of storage (0 °C) time on TBARS and conjugated dienes in beef heart surimi dewatered in the presence and absence of NaCl (0.1 M). PIPES buffers (25 mM) at pH 7.0 and 6.0 were used for all three washes. The final wash was done in the presence (+) or absence (−) of salt.

surimi proteins prepared in the presence and absence of salt (data not shown).

DISCUSSION

Heme pigments in the presence of hydrogen peroxide were suggested to form an active catalyst of "ferryl" species capable of initiating lipid peroxidation (Kanner and Harel, 1985). Hydrogen peroxide has also been shown to be capable of causing the release of iron from heme compounds, and the released iron was responsible for catalyzing lipid peroxidation (Rhee *et al.*, 1987). Whichever the pathway, the formation of hydrogen peroxide *in situ* may then become a prerequisite for heme pigments to be catalytically active for lipid peroxidation. Oxidation of oxymyoglobin to metmyoglobin was reported to result in the formation of superoxide that dismutates to hydrogen peroxide (Misra and Fridovich, 1972). Thus, any factor that favors oxidation of oxymyoglobin to metmyoglobin would result in the activation of heme compound to catalyze lipid peroxidation. Caughley and Watkins (1985) reported that high concentrations of H^+ (low pH) and, to a much lesser extent, certain anions like Cl^-, favored this reaction. Our results showed an increased amount of lipid peroxidation, as measured by TBARS and diene formation, with a decrease in pH, suggesting a possibly major involvement of heme-catalyzed oxidation. The washing process (a total dilution of 677×) removed most of the water- and low-salt-soluble constituents, including nonheme iron of the muscle tissue (Toyoda *et al.*, 1992), so residual heme iron could very well be the major catalyst of lipid peroxidation in this system. Nevertheless, a possible role for enzymic lipid peroxidation in this beef heart surimi system cannot be ruled out, even though washing may have reduced the concentration of NADH/NADPH to a large extent. The k_m value for NADH for the enzymic system in flounder microsomal fraction was reported to be only 1 μM (McDonald and Hultin, 1987). Because the enzymic system has activity over a broad range of pH values, it has a possible role as an initiator of pigment oxidation, as suggested by Rhee *et al.* (1984). Kenney *et al.* (1992) reported that muscle washing (without antioxidants) resulted in improved textural traits and decreased lipid oxidation in restructured beef rolls, compared with its unwashed counterpart, more so for cardiac muscle than for skeletal muscle. They attributed the difference in the behavior of heart and skeletal muscle to the removal of water-soluble proteins, in particular a 66 kDa protein in heart muscle. It is possible that extensive muscle washing substantially removes prooxidants such as those associated with mitochondria, as well as antioxidants, such as low molecular weight reducing substances and enzymes present in the heart muscle. Preliminary investigations have shown the residual hematin content in the surimi to be rather high (0.187 μmol/g) after three washes.

Lipid peroxidation and oxidation of oxymyoglobin to metmyoglobin are two closely linked phenomena in muscle systems. Hultin (1980) indicated that free radicals derived from lipid hydroperoxides were involved in the oxidation of meat pigment and that *in vitro* lipid peroxidation may precede pigment oxidation. Protection of minced muscle tissue against lipid oxidation by tripolyphosphate and ascorbate, as in the present study, was also shown by several researchers in various muscle types (Kelleher *et al.*, 1992; Wan *et al.*, 1993). Ascorbate can interfere with the protein carbonyl assay because it is capable of reacting with DNPH in a similar way as a carbonyl moiety. However, in the protein carbonyl assay, proteins were precipitated after the protein mixture was reacted with DNPH and the protein pellet was thoroughly washed in an ethanol: ethyl acetate (1:1) mixture. Thus, any unreacted DNPH or DNPH bound to lipid or other components, such as ascorbate or oxidized ascorbate, would mostly be removed. Accordingly, any increase in protein carbonyls as measured by this assay would be accounted for by carbonyl groups that are either part of the protein derivatives or exogenous but covalently linked to the protein. Increases in the protein carbonyls observed in ascorbate-containing sample (Figure 2) could have resulted from the combination of oxidized ascorbate covalently bound to protein and protein carbonyl derivatives formed with mediation by ascorbate/oxidized ascorbate.

Of particular interest was the observation that the relatively low concentration of orthophosphate (25 mM) used in the washing solution also conferred protection against oxidation to the surimi pellet. Inorganic phosphate was a potent inhibitor of fish muscle microsomal lipid peroxidation system *in vitro*, possibly by the interaction of phosphate with Fe^{3+} to form an insoluble complex (McDonald and Hultin, 1987). It may also be possible that washing two times in 25 mM phosphate buffer at pH 7.0 instead of distilled deionized water may have selectively removed some factors or cofactors that would catalyze lipid peroxidation or pigment oxidation in the system.

The effectiveness of propyl gallate, a potent free radical scavenger, in inhibiting lipid peroxidation in minced meat products has been well documented (Greene, 1969; Kelleher *et al*., 1992; Wan *et al*., 1993; Xiong *et al*., 1993). Therefore, it is not surprising to see the inhibitory effect of propyl gallate in the surimi pellet, even though bovine cardiac muscle is particularly rich in polyunsaturated fatty acid, heme compounds, and mitochondria. α-Tocopherol was also effective against lipid peroxidation but only at a 0.2% level compared with 0.02% for propyl gallate, indicating that the latter was of a stronger antioxidant activity. The difference in activity between the two antioxidants may also be due to a possible variation in distribution of these antioxidants in the aqueous, nonaqueous, and water/lipid interphase of the surimi pellet. Formation of protein carbonyls in the surimi pellet was also inhibited by propyl gallate and α-tocopherol, suggesting that protein oxidation was probably coupled with lipid oxidation in this system. The lipid content of the bovine cardiac muscle used for surimi preparation (i.e., after trimming the valves and adipose tissue) varied from 1.9 to 2.5% (average of 11% on dry weight basis). These lipids are primarily intramuscular and membranal in nature and are difficult to remove. The lipid content in the final surimi pellet on a 90% moisture basis varied from 1.3 to 1.9% (average of 16% on dry basis). Thus, the potential for protein oxidation via oxidized lipids is high in the present study. It must be noted that a quantification of protein oxidation by the measurement of carbonyl content alone would be an underestimation. A number of other oxidations, such as oxidation of histidine (Farber and Levine, 1986; Uchida and Kawakishi, 1993) and the loss of thiol groups (Takenaka *et al*., 1991), are possible and, in these cases, a carbonyl derivative is not formed.

Several studies on the effect of NaCl on lipid peroxidation have shown that NaCl acted as an antioxidant (Chang and Watts, 1950; Nambudiry, 1980) whereas many more recent reports have indicated that NaCl functioned as a prooxidant (Rhee *et al*., 1983; Salih *et al*., 1989; Kanner *et al*., 1991; Osinchak, *et al*., 1992). The latter studies suggested that NaCl enhances the activity of Fe^{3+} (Kanner *et al*., 1991) or that Cl^- derived from NaCl may improve solubility of Fe^{3+} and, hence, stimulate lipid peroxidation (Osinchak *et al*., 1992). We found that a salt concentration of 0.1 M in final washing solution conspicuously curtailed lipid peroxidation at pH 6.0 in the surimi pellet. This result suggested that Na^+ interfered with the ability of H^+ to catalyze redox reactions of myoglobin or that washing in 0.1 M NaCl may have removed some factors or cofactors that are capable of catalyzing lipid or pigment oxidation in beef heart muscle. If, in fact, the catalyst primarily responsible for lipid peroxidation is enzymic, then salt would be expected to exert an inhibition on the catalytic activity of the enzyme, as reported by McDonald and Hultin (1987). The exact role of salt in relation to variations in pH of muscle *in situ* on lipid oxidation in muscle foods is still not clearly understood.

In conclusion, inhibition of protein and lipid oxidation in surimi pellet from bovine cardiac muscle could be achieved by a variety of means, including a proper selection of pH of the washing media and type of the buffer, addition of salt, and incorporation of water- and lipid-soluble antioxidants, or any combinations thereof. This information is of potential use to food processors for designing processing parameters in the preparation of surimi-like material from under-utilized meat byproducts, such as beef hearts, depending on their end use. At the same time, the improved processing conditions would result in a stable finished product. Further research is needed to ascertain how surimi-like material prepared under different wash conditions would behave as a functional ingredient in cooked muscle foods, such as restructured meat rolls or loaves.

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