Oxidation of Purified Bovine Myoglobin: Effects of pH, Sodium Chloride, Sodium Tripolyphosphate, and Binders

Chiao-Min Chen,† Dale L. Huffman,* W. Russell Egbert,‡ and Robert C. Smith

Department of Animal and Dairy Sciences, Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama 36849-5415

The effect of pH, sodium chloride (NaCl), sodium tripolyphosphate (STP), and binders on oxidation of purified bovine myoglobin was investigated in an in vitro incubation system. The rate of metmyoglobin formation decreased ($P < 0.05$) in a linear manner either as pH values increased from 4.5 to 6.5 or as STP concentrations increased from 0.1 to 0.5%. NaCl at either 1 or 2% increased ($P < 0.05$) the rate of metmyoglobin formation; STP decreased the rate of oxidation of myoglobin in the presence of either 1 or 2% NaCl.

INTRODUCTION

Color and appearance are prime factors that consumers use to judge the initial acceptability of meat products. Many nonmeat ingredients, such as NaCl, sodium tripolyphosphate (STP), and binders, used in manufacture of restructured products can alter the rate of discoloration that occurs during processing and/or storage. NaCl increases the rate of metmyoglobin formation in meat products, and STP reduces the rate of metmyoglobin formation (Huffman et al., 1981; Rhee et al., 1983; Ande, 1985; Chu et al., 1987; Trout, 1990). Use of binders such as crude myosin extract, calcium alginate, and surimi in the manufacture of restructured steaks may be advantageous since NaCl can be lowered or eliminated without detrimental effects to the textural properties of the product (Chen and Trout, 1991a). With increased interest in reducing dietary sodium intake, the use of binders may become an effective method to reduce sodium levels of restructured products.

The rate of restructured steak discoloration is slowed by addition of calcium alginate (Trout and Schmidt, 1990; Means and Schmidt, 1986). Since the oxidation rate of myoglobin is reduced as pH is increased (Chu et al., 1987; Trout, 1990), pH adjustment also has potential for reducing discoloration. The objective of this research was to determine the effect of pH, NaCl, STP, and various binders on the oxidation of purified bovine myoglobin in an in vitro incubation system.

MATERIALS AND METHODS

Additives and Reagents. Additives and reagents used were reagent grade NaCl, calcium carbonate, ammonium hydroxide, and phosphoric acid (Fisher Scientific Co., Pittsburgh, PA), food grade STP and carrageenan (Viscarin 389 SD; FMC Corp., Philadelphia, PA), sodium alginate (Manugel DMB, Kelco, Clark, NJ), whey protein concentrate (Alacen 882, New Zealand Milk Products, Inc., Petaluma, CA), isolated soy protein (Protein Technologies International, St. Louis, MO), wheat gluten (Supergluten 75, Ogilvie Mills, Inc., Minnetonka, MN), and surimi (Alaska Fisheries Association Kodiak, AK). Extracted beef crude myosin was prepared according to the method of Turner et al. (1979).

Myoglobin Preparation. USDA Choice bovine muscle (semimembranosus) was obtained 24 h post-mortem from John Morrell and Co., Montgomery, AL, and transported in a refrigerated chest at approximately 1–2 °C to the laboratory at Auburn University. Myoglobin was prepared from muscle according to the method of Wittenberg and Wittenberg (1981). Purified myoglobin stock solutions (10–20 mL) were dialyzed against 0.04 M phosphate buffer (1000 mL, pH 5.5) overnight before use. The dialysate was changed three times. All procedures were carried out at low temperature (0–5 °C). Concentration of myoglobin was determined from absorbance at 525 nm, using a molar extinction coefficient of 7.6 mM^{-1} cm^{-1} on the basis of molecular mass of 17 500 Da (Bowen, 1949).

Experiments. Six separate studies were conducted to determine the effects of pH, NaCl, and STP or binder on myoglobin oxidation.

pH Profile Study. Oxidation of purified myoglobin was examined at five pH values. Myoglobin solutions were adjusted to a pH of 4.5, 5.0, 5.5, 6.0, or 6.5 using either 1 M ammonium hydroxide or 1 M phosphoric acid.

NaCl and STP Profile Study. Eighteen myoglobin solutions were prepared in a factorial arrangement using three NaCl levels (0, 1, and 2%) and six STP levels (0, 0.1, 0.2, 0.3, 0.4, and 0.5%).

NaCl/STP Ratio Study. Nine different ratios of NaCl and STP (from 0 to 4.0 in 0.5 intervals) at a constant STP level of 0.5% were evaluated. Two myoglobin solutions were also prepared as controls, one with no additives and the second containing 0.25% NaCl with no added STP.

NaCl and STP Concentration Study. Concentration effects were studied by preparing five myoglobin solutions with a NaCl/STP ratio of 3.5. The concentrations of NaCl/STP (in percent) were as follows: 0.35/0.1, 0.70/0.2, 1.05/0.3, 1.40/0.4, and 1.75/0.5. Myoglobin solution with no additives served as a control.

Binder Profile Study. Myoglobin solutions were prepared with each of the following binders: calcium alginate, crude myosin extract, whey protein concentrate, wheat gluten, isolated soy protein, surimi, or carrageenan. A control treatment with no additive was also included.

Binder, NaCl, and STP Study. Myoglobin solutions were prepared with calcium alginate, crude myosin, surimi, or carrageenan with and without NaCl and STP.

Procedures. Treatment solutions were prepared by adding reagents, ingredients, or binders in a test tube with phosphate buffer (0.04 M phosphate buffer, pH 5.5). A known volume of purified myoglobin stock solution was added and mixed with each treatment solution. Myoglobin concentration of solutions was standardized to a concentration of 0.020–0.025 mmol/L (Krzywicki, 1982). In the binder studies, myoglobin solutions were centrifuged prior to determination of absorbance. Centrifugation was conducted at 1000 rpm for 2 min at each time interval using a Jalco centrifuge (Model 48, The Jalco Motor Co., Union City, IN). Prepared myoglobin solutions were incubated at 25 °C for 24 h, and metmyoglobin concentrations were determined at regular intervals (0, 1, 2, 3, 4, 5, 6, 9, 12, and 24 h). Absorbance was measured at 473, 525, 572, and 730 nm using a Perkin-Elmer (Norwalk, CT) Lambda 4A spectrophotometer.

† Present address: North Side Packing Co., Arnold, PA 15068.

‡ Present address: ADM Corp., Decatur, IL 62525.

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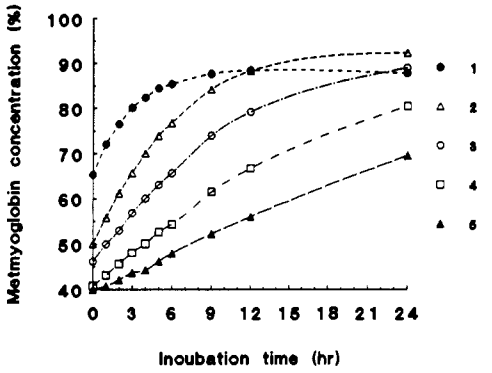

Figure 1. Effect of pH on metmyoglobin formation. Treatment: 1, 4.5; 2, 5.0; 3, 5.5; 4, 6.0; 5, 6.5 (SEM = 3.8% metmyoglobin).

Percent metmyoglobin was calculated according to the method described by Krzywicki (1979) and Chen and Trout (1991b).

Statistical Analyses. Data from each study were analyzed using a split-plot design with three replications. The whole plot conformed to a completely randomized design. The salt and STP profile study was a 3 × 6 factorial arrangement of treatments in a split-plot design. Analysis of variance procedures with the appropriate models to test the effect of treatment and incubation time were computed according to SAS (1982) procedures. Fisher's least significant difference test was used to determine differences between treatment means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

pH Profile Study. When the pH of myoglobin solutions was adjusted to values from 4.5 to 6.5, the concentration of metmyoglobin in samples at pH 4.5 was greater initially ($P < 0.05$) than that for all other treatments (Figure 1). Metmyoglobin concentration increased ($P < 0.05$) at all pH values over the incubation period. pH had a linear ($P < 0.05$) effect on rate of metmyoglobin formation; solutions with high pH had lower metmyoglobin concentration than solutions with low pH. Chang and Traylor (1975) reported that stability of the heme–globin linkage decreased when pH was lowered. This destabilization could lead to greater exposure of heme iron to components of the medium and lead to an increase in oxidation rate.

Salt and STP Profile Study. The effect of various levels of STP (from 0 to 0.5%) in combination with 0, 1, and 2% NaCl on oxidation of purified myoglobin was evaluated (Figure 2). The rate of myoglobin oxidation decreased as STP levels increased regardless of NaCl concentration (0, 1, or 2%). This protective effect increased as STP levels increased ($P < 0.05$) from 0.1 to 0.4% in 0.1% increments. Myoglobin solutions (equivalent NaCl content) containing 0.4 and 0.5% STP had similar ($P > 0.05$) rates of metmyoglobin formation.

In general, metmyoglobin concentrations were higher ($P < 0.05$) in myoglobin solutions containing 2% NaCl than in those containing 1% NaCl at equivalent STP levels and over time periods. Myoglobin solutions containing 0.1% STP with either 1 or 2% NaCl had metmyoglobin concentrations similar to myoglobin solutions without NaCl over the entire incubation period.

Myoglobin solutions containing either 1 or 2% NaCl had greater ($P < 0.05$) rates of metmyoglobin formation than myoglobin solutions without NaCl. There were no differences ($P > 0.05$) in the rate of metmyoglobin formation between myoglobin solutions containing 1 or 2% NaCl. These results agree with previous findings that NaCl has a pronounced pro-oxidant effect on myoglobin (Wallace et al., 1982; Asghar et al., 1990). Certain anions

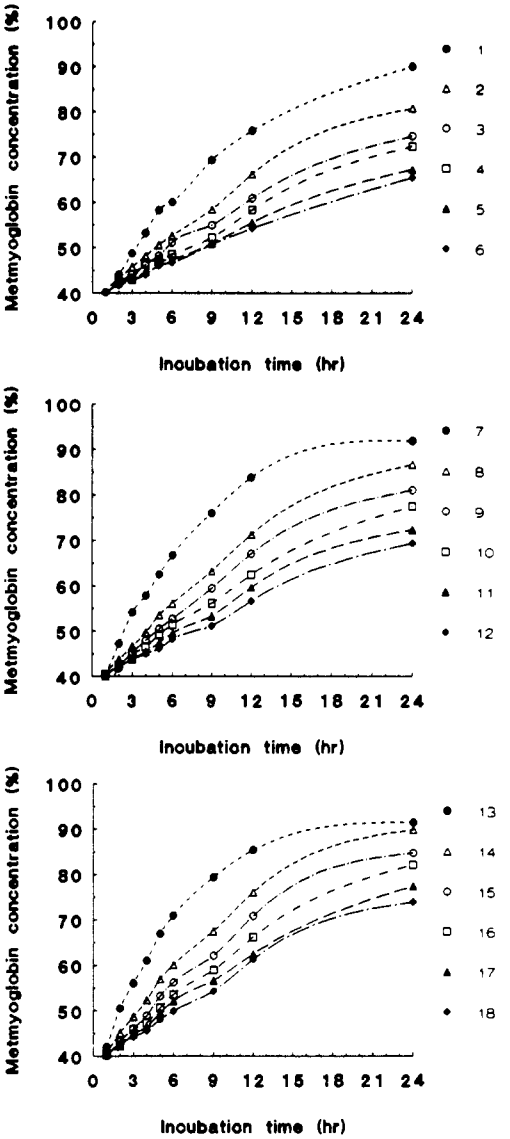

Figure 2. Effect of NaCl and STP on metmyoglobin formation. Treatment: 1, control (no additives); 2, 0.1% STP; 3, 0.2% STP; 4, 0.3% STP; 5, 0.4% STP; 6, 0.5% STP; 7, 1% NaCl; 8, 1% NaCl + 0.1% STP; 9, 1% NaCl + 0.2% STP; 10, 1% NaCl + 0.3% STP; 11, 1% NaCl + 0.4% STP; 12, 1% NaCl + 0.5% STP; 13, 2% NaCl; 14, 2% NaCl + 0.1% STP; 15, 2% NaCl + 0.2% STP; 16, 2% NaCl + 0.3% STP; 17, 2% NaCl + 0.4% STP; 18, 2% NaCl + 0.5% STP (SEM = 0.7% metmyoglobin).

(Cl^-, F^-, CN^-) increase the rate of oxymyoglobin oxidation (Wallace et al., 1982). Asghar et al. (1990) pointed out that the oxidation rate of heme iron depends upon the concentration and nucleophilic characteristics of anions. Since chloride is the least nucleophilic ion, chloride concentration could play a major role in myoglobin oxidation. Wallace et al. (1982) proposed that NaCl increases myoglobin oxidation via an anion-promoted autoxidation process which is directly proportional to the concentration of chloride ion. A curvilinear increase in the rate of metmyoglobin formation due to salt concentration was observed by Trout (1990) in ground beef. In the present study the rate of metmyoglobin formation was the same in either 1 or 2% NaCl. Interactions within the meat system of components not found in a purified myoglobin solution could explain this difference.

NaCl/STP Ratio Study. The effect of practical (from a processing standpoint) NaCl/STP ratios (from 0.5 to 4.0 in 0.5 increments) on the rate of metmyoglobin formation in purified bovine myoglobin solutions was also studied.

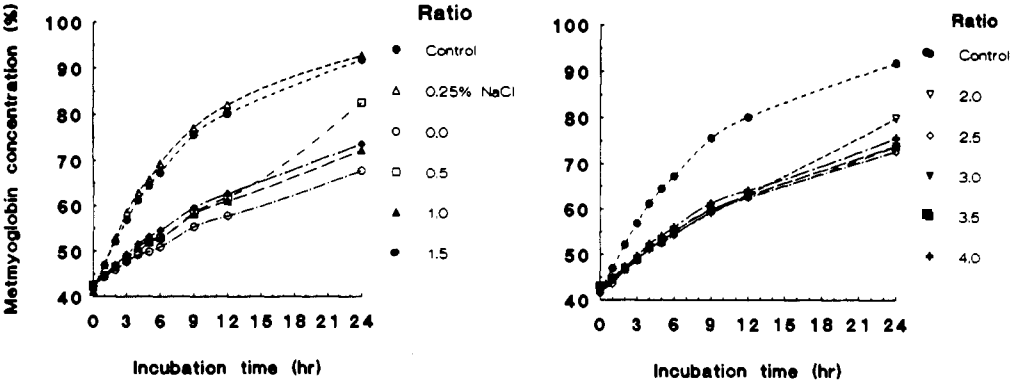

Figure 3. Effect of ratio of NaCl to STP on metmyoglobin formation (SEM = 0.7% metmyoglobin). Control (no additives, ●); +0.25% NaCl (△); +0.5% STP (○); 0.25% NaCl + 0.5% STP (ratio 0.5, □); 0.5% NaCl + 0.5% STP (ratio 1, ▲); 0.75% NaCl + 0.5% STP (ratio 1.5, ◆); 1% NaCl + 0.5% STP (ratio 2, ▽); 1.25% NaCl + 0.5% STP (ratio 2.5, ◇); 1.5% NaCl + 0.5% STP (ratio 3, ▼); 1.75% NaCl + 0.5% STP (ratio 3.5, ■); 2% NaCl + 0.5% STP (ratio 4, +).

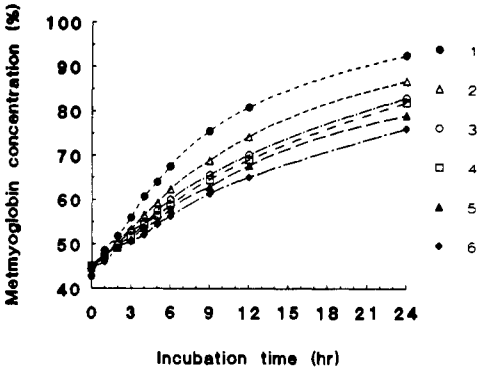

Figure 4. Effect of NaCl and STP concentration on metmyoglobin. Treatment: 1, control (no additives); 2, 0.35% NaCl + 0.1% STP; 3, 0.70% NaCl + 0.2% STP; 4, 1.05% NaCl + 0.3% STP; 5, 1.40% NaCl + 0.4% STP; 6, 1.75% NaCl + 0.5% STP (SEM = 0.3% metmyoglobin).

Metmyoglobin concentration increased ($P < 0.05$) for all treatments over the 24-h incubation period (Figure 3). The control myoglobin solutions containing no NaCl or STP or solutions containing 0.25% NaCl had greater ($P < 0.05$) metmyoglobin concentrations than solutions containing NaCl and STP at various ratios. No differences ($P > 0.05$) were found among the myoglobin solutions containing both NaCl and STP for metmyoglobin concentration over the first 12 h of incubation. STP (0.5%) slowed the rate of metmyoglobin formation regardless of NaCl concentration. There were no differences ($P > 0.05$) in myoglobin oxidation rates between the control (no NaCl or STP) and solutions containing 0.25% sodium chloride over the incubation period.

NaCl and STP Concentration Study. Since the ratio of NaCl to STP did not affect the rate of myoglobin oxidation, a ratio of NaCl/STP of 3.5 with varying amounts of the two components was used to study the rate of metmyoglobin formation. This NaCl/STP ratio is commonly used in the manufacture of restructured products. The rate of metmyoglobin formation decreased linearly ($P < 0.05$) as the concentrations of NaCl and STP increased (Figure 4). Neer and Mandigo (1977) reported similar results in restructured products where increases in salt and STP levels resulted in increased redness in the product.

Results from these STP studies agree with previous work by Huffman et al. (1981), Ande (1985), and Chu et al. (1987), who reported that STP reduces the rate of metmyoglobin formation during storage. It has been postulated that the protective effect of STP on myoglobin oxidation could be the result of one of the following or their combinations: (1) increased pH (Hagler et al., 1979; Trout, 1990); (2) chelation of free multivalent ions (Liu, 1970; Livingston and Brown, 1981); (3) reduction of lipid oxidation rate (Smith and Bowers, 1972; Matlock et al., 1984); and/or (4) unspecific intrinsic properties of the phosphate (Chu et al., 1987).

Binder Profile Study. Metmyoglobin formation was affected ($P < 0.05$) by various binders (Figure 5). Initially, myoglobin solutions with isolated soy protein (pH 6.0) or whey protein concentrate (pH 5.8) had greater ($P < 0.05$) metmyoglobin formation rates than the control (pH 5.5) or solutions with other binders (myosin, pH 5.6; calcium alginate, pH 6.3; wheat gluten, pH 5.6; surimi, pH 5.7; and carrageenan, pH 5.6). Earlier studies have shown that nonmeat additives such as isolated soy protein or whey protein concentrate may contain metal ions or salt that can affect product color (Seideman, 1982; Smith, 1982; Terrell et al., 1982).

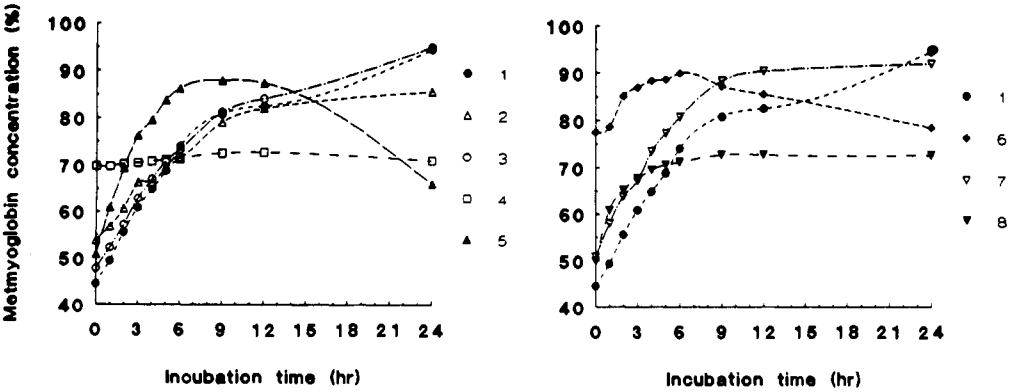

Figure 5. Effect of various binders on metmyoglobin formation. Treatment: 1, control (no binder); 2, calcium alginate; 3, crude myosin extract; 4, whey protein; 5, wheat gluten; 6, isolated soy protein; 7, surimi; 8, carrageenan (SEM = 2.1% metmyoglobin).

Metmyoglobin concentrations for myoglobin solutions increased ($P < 0.05$) over the incubation period except myoglobin solutions containing isolated soy protein, whey protein concentrate, or wheat gluten. Metmyoglobin concentration of myoglobin solutions containing whey protein did not change ($P < 0.05$) over the incubation period. The explanation of this observation is not clear. This may be due to some intrinsic properties of the whey protein concentrate.

Metmyoglobin concentrations increased through the 9-h incubation period and then decreased in myoglobin solution containing isolated soy protein or wheat gluten. Visual color changes were also observed in these two treatments from yellow brown to light pink during incubation. Possible reasons for these decreases may be due to some myoglobin reduction reaction in these two treatments. One possible explanation for this observation is that the oxygen tension of the myoglobin solution may have been reduced by the wheat gluten and isolated soy protein under the experimental conditions used. Myoglobin solutions containing carrageenan were not different ($P > 0.05$) from solutions with whey protein concentrate after 6 h of incubation and did not increase with further incubation. This is probably due to an antioxidant property of carrageenan (Glicksman, 1982). Solutions with myosin or surimi had metmyoglobin formation rates similar to those of control solutions. These results indicate that use of surimi and myosin as binders in meat products does not influence myoglobin oxidation.

The pH of myoglobin solutions for each treatment after 24 h of incubation was varied. In general, the pH of myoglobin solutions increased after incubation except myoglobin solutions with surimi and carrageenan. The pH of myoglobin solutions for each treatment after a 24-h incubation period was as follows: control, +0.06; calcium alginate, +1.0; myosin extract, +0.32; whey protein, +0.11; wheat gluten, +0.25; soy isolate protein, +0.5; surimi, −0.38; and carrageenan, −0.51. These pH changes from initial to final measurement do not reflect consistent changes in metmyoglobin formation found in the first study (pH effect). Therefore, intrinsic characteristics of each binder may play important roles in the autoxidation of purified myoglobin solutions.

Binder, NaCl, and STP Study. The concentration of metmyoglobin increased ($P < 0.05$) for all combinations of binder, NaCl, and STP used (Figure 6). Addition of STP to myoglobin solutions slowed the rate of metmyoglobin formation. After 5 h of incubation, myoglobin solutions with 0.1% NaCl plus 0.05% STP (T4) had higher ($P < 0.05$) metmyoglobin concentrations than myoglobin solutions containing 0.5% STP in combination with different levels of NaCl (T2, T3, and T5). There were no differences ($P > 0.05$) among T2, T3, and T5 for metmyoglobin concentration at any incubation period. This confirmed the finding shown in Figure 3 that 0.5% STP slowed the rate of myoglobin formation regardless of NaCl concentration. STP (0.05%) with 0.1% NaCl (T4) protected myoglobin against oxidation as well as 0.5% STP (T2, T3, or T5) for the first 3 h of incubation.

Initially, solutions containing calcium alginate with (T6) or without lactate (T7) had higher ($P < 0.05$) metmyoglobin concentrations than the other treatments. After 3 h of incubation, solutions with calcium alginate and lactate had higher ($P < 0.05$) metmyoglobin concentrations than those with calcium alginate alone. Addition of lactate decreased the pH from 5.24 to 5.07 for T6 and from 6.8 to 6.3 for T7, resulting in more rapid metmyoglobin formation. After 6 h of incubation, solutions containing

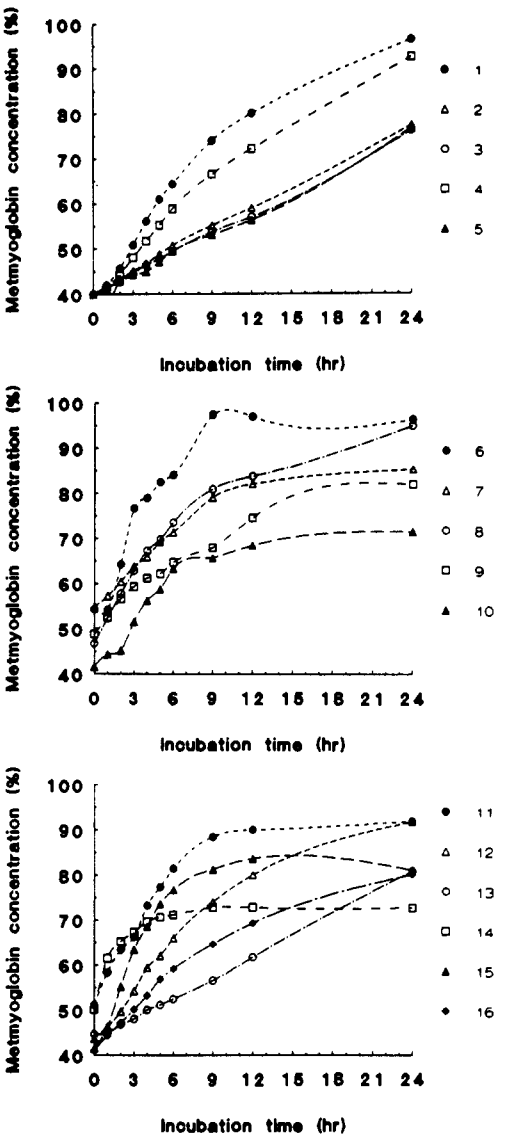

Figure 6. Effect of NaCl, STP, and selected binders on metmyoglobin formation. Treatment: 1, control (no additive); 2, 1% NaCl + 0.5% STP; 3, 0.5% NaCl + 0.5% STP; 4, 0.1% NaCl + 0.05% STP; 5, 0.1% NaCl + 0.5% STP; 6, 0.1% calcium alginate + 0.5% lactate; 7, 0.7% calcium alginate; 8, 8.5% crude myosin; 9, 8.5% crude myosin + 0.1% NaCl + 0.05% STP; 10, 8.5% crude myosin + 0.1% NaCl + 0.5% STP; 11, 1.5% surimi; 12, 1.5% surimi + 0.1% NaCl + 0.05% STP; 13, 1.5% surimi + 0.1% NaCl + 0.5% STP; 14, 0.5% carrageenan; 15, 0.5% carrageenan + 0.1% NaCl + 0.05% STP; 16, 0.5% carrageenan + 0.1% NaCl + 0.5% STP (SEM = 1.8% metmyoglobin).

calcium alginate had lower ($P < 0.05$) metmyoglobin concentrations than controls (T1) or those containing surimi. The protective effect was less than that of solutions containing 0.1% NaCl, myosin, or surimi with 0.05% STP addition, up to 6 h of incubation.

The rates of metmyoglobin formation were similar in controls and in solutions containing crude myosin extract or surimi. Solutions containing carrageenan (T14) were similar to solutions containing calcium alginate (T7) for the first 6 h of incubation and had a slower ($P < 0.05$) metmyoglobin formation rate thereafter. The protective effect of carrageenan was superior to that of calcium alginate.

Solutions with crude myosin in combination with 0.1% NaCl and 0.05 or 0.5% STP (T9 and T10) had lower ($P < 0.05$) metmyoglobin concentrations than solutions with only myosin (T8) after 1 h of incubation. The protective

effect of STP was observed in solutions containing surimi (T11–T13). These results indicate that addition of STP (0.05 or 0.5%) in combination with 0.1% NaCl in solutions with selected binders (myosin or surimi) was effective in slowing metmyoglobin formation compared to solutions with the binder alone (T8 and T11). The protective effect of STP increased as the concentration of STP increased from 0.05 to 0.5%. The protective effect of STP was observed in solutions with carrageenan in combination with 0.1% NaCl plus 0.5% STP. Solutions with carrageenan alone (T14) had lower ($P < 0.05$) metmyoglobin formation rates after 6 h of incubation than those with 0.05% STP in combination with 0.1% NaCl (T15 or T16). The reason for this difference is not clear; however, the viscosity of carrageenan solutions could be decreased by the addition of 0.1% NaCl, disrupting the protective environment originated from carrageenan addition. The reduction of the viscosity of hydrocolloid gels by salt has been reported (Glicksman, 1982). Solutions with higher levels of STP (0.5%) had lower ($P < 0.05$) metmyoglobin concentrations at any time period.

The addition of STP to solutions containing myosin or surimi decreased metmyoglobin formation. This protective effect increased as STP levels increased from 0.05 to 0.5%. Addition of 0.1% NaCl and 0.05% STP to solutions containing carrageenan increased metmyoglobin formation when compared to solutions containing carrageenan alone. Solutions containing carrageenan with 0.5% STP had similar ($P < 0.05$) metmyoglobin concentrations to solutions containing myosin or surimi with 0.5% STP. The protective effect derived from carrageenan was superior to that of calcium alginate. Myoglobin solutions containing calcium alginate alone had higher metmyoglobin formation initially and then leveled off after 6 h of incubation. Solutions containing calcium alginate with lactate exhibited the most rapid rate of metmyoglobin formation, probably because of the low pH induced by lactate.

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