Prevention of Warmed-Over Flavor in Cooked Beef: Effect of Phosphate Type, Phosphate Concentration, a Lemon Juice/Phosphate Blend, and Beef Extract

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This study examined the effect of (a) 0.5% Lemophos (a commercial blend of lemon juice and sodium tripolyphosphate), (b) 3.0% commercial beef extract, (c) five different phosphates (0.5%) [disodium phosphate (Pi), tetrasodium pyrophosphate (PP), sodium tripolyphosphate (TPP), sodium tetrapolyphosphate (TTPP), and sodium hexametaphosphate (HMP)], and (d) four TPP concentrations on the thiobarbituric acid reactive substances (TBARS) values and warmed-over flavor (WOF) of cooked ground beef during 6 days of refrigerated storage. PP, TPP, TTPP, and Lemophos completely inhibited WOF as determined by both TBARS values and sensory evaluation. With HMP, TBARS values increased slightly with increasing storage time; however, the WOF scores did not show a corresponding increase. Pi and beef extract slightly reduced TBARS values and WOF scores; the beef extract also partly masked the WOF. The minimum TPP concentration required to inhibit WOF completely was 0.5%; 0.375% TPP prevented the increase in WOF scores but not the increase in TBARS values.

An off-odor and flavor known as warmed-over flavor (WOF) develops rapidly in cooked meat products (Tims and Watts, 1958; Siu and Draper, 1978). WOF is the result of the spontaneous oxidation of the unsaturated fats present in meat. This oxidation occurs rapidly in cooked meat because iron released from the denatured hemoglobin and myoglobin during cooking catalyzes the oxidation reaction (Igene et al., 1979). WOF is a major problem in the prepared food industry where meat is precooked, cooled, distributed refrigerated, and then reheated at the restaurant or the place where it is consumed. If WOF develops, the reheated meat has a flavor that is not typical of freshly cooked meat.

The lipids most important in the development of WOF are the highly unsaturated phospholipids, sphingolipids, glycolipids, and other membrane lipids rather than the more saturated triglycerides from the fat depots (Gray and Pearson, 1987). The oxidized lipids (i.e., the hydroperoxides) do not themselves cause WOF. The hydroperoxides, however, are unstable and react further to produce secondary oxidation products such as alcohols, acids, ketones, lactones, and unsaturated hydrocarbons, which produce the WOF (Sato and Herring, 1973). Many of these secondary oxidation products are highly odoriferous and have flavor thresholds of less than 1 ppb. Of these compounds, hexanal, 2,3-octanedione (St. Angelo et al., 1987), pentanal, 2-pentylfuran (Bailey et al., 1980), and 2-octenal (Josephson and Lindsay, 1987) have been found to be highly correlated with the development of WOF.

WOF development can be retarded by many ingredients commonly used in meat products. Vitamin E (Bishov and Henick, 1972) and the phenolic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ) effectively inhibit WOF. Unfortunately, these antioxidants have limited application in cooked intact muscle roasts because of their low water solubility. Other ingredients permitted in meat products that have antioxidant properties include (a) phosphates (Tims and Watts, 1958), (b) a phosphate/lemon juice blend (Haymon et al., 1976), and (c) beef extracts that contain browning reaction products (Sato et al., 1973; Bailey, 1985). Using these ingredients in cooked meat products is more practical than using the phenolic antioxidants because these additives are commonly used in meat products to improve other properties such as flavor, texture, and juiciness. In addition, these additives have a more "natural" connotation than the phenolic antioxidants.

It is not known how effective these different food additives are as antioxidants in cooked refrigerated beef. Tims and Watts (1958) originally showed that several different types of phosphates inhibit WOF in cooked pork, but it is not known if all types and concentrations of phosphates currently permitted in meat products inhibit WOF in cooked beef. Lemophos, a commercial combination of tripolyphosphate and lemon juice, has been shown to prevent the development of WOF in cooked frozen beef (Haymon et al., 1976). It has not been determined whether Lemophos is effective with cooked refrigerated beef, however. Similarly, laboratory-prepared beef extracts that contain browning reaction compounds such as reductic acid, maltol, and γ-pyrone have been shown to prevent the development of WOF (Sato et al., 1973). But it is not known whether commercially prepared beef extracts also inhibit WOF.

Hence, the objective of this study was to determine the effect of (a) Lemophos, (b) a commercial beef extract, (c) five different phosphate types, and (d) four different TPP concentrations on the TBARS values and WOF scores of cooked ground beef.

MATERIALS AND METHODS

Meat and Additives. The meat used in this study was top round obtained 48 h postmortem from commercially slaughtered steers. It was trimmed of all visual fat, ground through a 0.4-cm plate with a Kitchen Aid grinder fitted with a stainless steel plate and blade (Model K45SS; Hobart Co., Troy, OH),

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and stored at 1 °C until use later that day. The average composition of the meat was as follows: pH, 5.55; moisture, 71.4%; fat, 5.8%.

The additives used in the study were analytical reagent grade sodium chloride, deionized water, beef extract (Hormel Inc., Austin, MN), Lemophos (Stauffer Chemical Co.), and the following food-grade phosphates: disodium phosphate (Pi), tetrasodium pyrophosphate (PP), sodium tripolyphosphate (TPP), sodium tetrapolyphosphate (TTPP), and sodium hexametaphosphate (HMP) (courtesy of FMC Corp., Philadelphia, PA). All the phosphates were analyzed for number-average chain length (n) by the titration method outlined by Lowenheim (1973). The number-average chain length of the phosphates was as follows: Pi, 1.0; PP, 2.0; TPP, 3.0; TTPP, 5.7; HMP, 12.9.

Treatments. *Experiment 1.* This study examined the effect of five phosphate types (Pi, PP, TPP, TTPP, HMP), Lemophos, beef extract, and no additives on the WOF and TBARS values of cooked ground beef stored for 0, 1, 2, 3, and 6 days. The phosphates and Lemophos were used at 0.5%; the beef extract was used at 3.0%. All treatments, including the treatment with no additives, contained 10.0% deionized water and 1.5% sodium chloride.

Experiment 2. This study examined the effect of five concentrations of TPP (0.000, 0.125, 0.250, 0.375, 0.500%) and 0.625% Lemophos (to give a TPP concentration of 0.5%) on the WOF and TBARS of cooked ground beef stored for 0, 1, 2, 3, and 6 days. All treatments contained 10.0% deionized water and 1.5% sodium chloride.

Sample Preparation and Heating. Ground beef (350 g), deionized water, and all nonmeat additives were mixed for 2 min on speed 2 in a Kitchen Aid mixer. All nonmeat ingredients were added to the meat during the initial stages of mixing; the sodium chloride was added dry, and the other ingredients were dissolved in all of the deionized water. The meat mixture was packed into 500-mL glass jars, sealed, and stored at 2 °C overnight to equilibrate.

The ground beef was heated for 90–95 min in a thermostatically controlled water bath (water temperature 72.0 ± 0.5 °C) to an internal temperature of 70 °C. The internal temperature was monitored by a Digisense Thermistor digital thermometer (Model No. 8522-10; Cole Parmer, Chicago, IL) with the probe placed in the geometric center of a jar that had been handled the same way as the treatment jars. The jars were cooled in ice slush to an internal temperature of 10 °C. Then, the cooked ground beef was ground through a 0.4-cm plate, the separated liquid was reincorporated, and the meat was stored in glass jars at 2 °C until analyzed for WOF and TBARS. On day 0, the samples were analyzed within 30 min of grinding.

Sensory Evaluation. WOF aroma was evaluated by a 10-member trained sensory panel. The panelists, who were experienced in sensory evaluation, were trained for 1–2 weeks on samples that had extremely strong, moderate, and no WOF (AMSA, 1978). The samples were heated to approximately 50 °C for evaluation. Twenty-gram samples of each treatment were weighed into 250-mL tall-form, small-mouthed jars, which were sealed with Parafilm and heated for 30 s on defrost setting in a microwave oven (Sanyo Cuisine Master x-80). The parafilm was replaced by aluminum foil, and the jars were held in a 50 °C water bath while being evaluated. WOF aroma was evaluated on a scale of 1–8 (1 = no WOF; 8 = extremely strong WOF). The panelists evaluated one sample from each treatment for each of the three replications.

Thiobarbituric Acid Reactive Substances (TBARS) Analysis. TBARS values were determined in triplicate by the modified procedure of Tarladgis et al. (1960) as outlined by Ke et al. (1984). The modifications were as follows: (1) 100-mg portions of both propyl gallate and ethylenediaminetetraacetic acid (EDTA) were added to the sample before distillation to prevent further oxidation from occurring during distillation, and (2) 95 mL of 4 N HCl was added to the sample before distillation to ensure complete release of the malonaldehyde. A standard curve was prepared against 1,1,3,3-tetraethoxypropane (TEP) [malonaldehyde bis(diethyl acetal)]. TBARS values were expressed as milligrams of malonaldehyde equivalent per kilogram of sample.

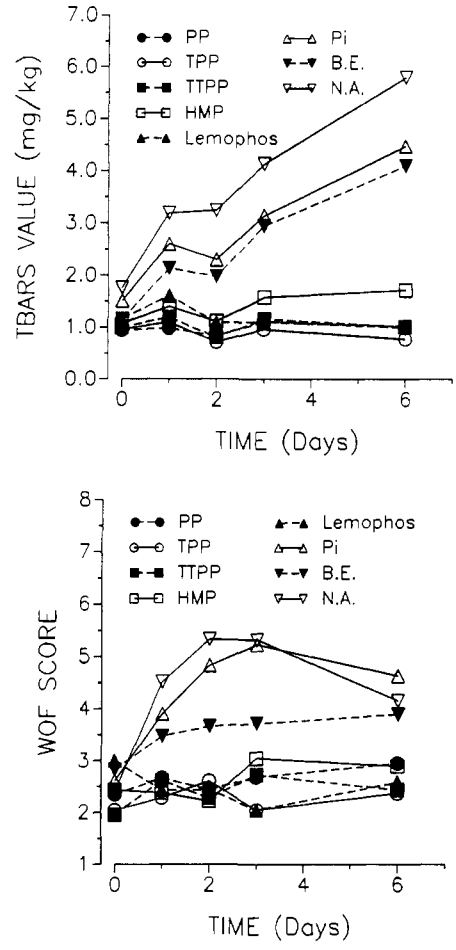

Figure 1. Effect of phosphate type, Lemophos, beef extract (B.E.), no additives (N.A.), and storage time on (a) TBARS values and (b) WOF scores of cooked beef. $LSD_{(5\%)}$ values for comparison between treatment means are 0.40 and 1.01 for TBARS values and WOF scores, respectively.

Proximate Analysis. The pH was determined by blending 13 g of sample with 130 mL of deionized water at high speed in an Osterizer Imperial blender (John Oster Manufacturing Co., Milwaukee, WI) as previously described (Trout and Schmidt, 1986). Fat and moisture concentrations were determined by the AOAC (1970) procedures.

Statistical Analysis. Data were analyzed by analysis of variance using a split-block design as described by Steel and Torrie (1980), with treatments as the main effect and storage time as the subunit. When F values were significant ($P < 0.05$), either Fischer's least significant difference test ($P < 0.05$) ($LSD_{(5\%)}$) or orthogonal contrasts ($P < 0.05$) were used to locate differences between treatment means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Experiment 1. *TBARS Values.* Phosphate type had a significant effect on both the initial TBARS values of the cooked beef and the rate at which the TBARS values increased with storage time (Figure 1). The initial TBARS values of the cooked beef that contained no additives were significantly greater ($P < 0.01$) than those of the other treatments, and the values increased rapidly with increasing storage time (0.7 mg/kg per day). All additives except Pi prevented the initial lipid oxidation that had occurred either during cooking or during the time between cooking and analysis with the samples that contained no additives. PP, TPP, TTPP, and Lemophos completely inhibited lipid oxidation during the 6 days of refrigerated storage; the TBARS values of these treatments were relatively low initially (=1.0 mg/kg) and did

not increase ($P > 0.05$) during storage. HMP did not inhibit lipid oxidation as effectively as the four phosphates just mentioned; the TBARS values increased slightly but linearly ($P < 0.01$) with increasing time (0.1 mg/kg per day). As a result, after 6 days of storage the TBARS values of the samples containing HMP were higher ($P < 0.01$) than those of the four most effective phosphates. Pi was the least effective phosphate used in this study; TBARS values of the samples that contained Pi increased only slightly less rapidly (0.5 mg/kg per day) than the samples that contained no additives. The beef extract had a slight antioxidative effect; the TBARS values of the samples that contained beef extract increased at the same rate as the samples that contained Pi (0.5 mg/kg per day).

The antioxidative properties of most of the compounds tested in this study were similar to those previously reported. Pi, PP, and TPP gave results similar to those reported by Tims and Watts (1958) and Shahidi et al. (1988) for cooked pork. Lemophos had antioxidative properties similar to those determined previously with cooked frozen beef (Haymon et al., 1976). Moreover, as was also shown by Haymon et al. (1976), our results indicated that the antioxidative properties of the Lemophos were not due solely to the TTP since the TPP concentration in ground beef that contained 0.5% Lemophos is 0.4% and it was subsequently shown in experiment 2 that a similar concentration of TPP (0.375%) does not completely prevent lipid oxidation. The commercial beef extract had much lower antioxidative properties than the laboratory-prepared beef extract studied by Sato et al. (1973). TTPP has not been previously studied for its antioxidative properties in cooked meat.

HMP did not show the same antioxidative ability with cooked beef as was previously shown with cooked pork. However, the results from the two previous studies on its antioxidative effect are conflicting. An early study by Tims and Watts (1958) showed that HMP completely inhibits lipid oxidation in cooked pork, whereas a latter study by Shahidi et al. (1988) found that HMP had virtually no antioxidative properties.

There are several reasons for the conflicting results. One reason is that Shahidi et al. (1988) used 0.3% HMP instead of 0.5% HMP that was used in this and the earlier study by Tims and Watts (1958). Another reason is that the HMP may not have been the same in all three studies since HMP is not a clearly defined compound. HMP is a mixture of straight-chain polyphosphate with an average chain length between 10 and 15 (Ellinger, 1972). Phosphates sold commercially as HMP may have an average chain length anywhere between 4 and 100. Neither of the two earlier researchers clearly indicated the chain length of the HMP they used. Although Tims and Watts (1958) indicated that the HMP they used had the formula $Na_6P_6O_{18}$, they were probably in error though since this is the formula for a true hexametaphosphate (a cyclic six-membered polyphosphate), which is what HMP was erroneously believed to be at that time (Ellinger, 1972).

The differences in concentration and chain length of the HMP between studies are significant since phosphates act as antioxidants by chelating free iron. Moreover, the chelating ability of phosphates is directly proportional to the concentration of phosphate and indirectly proportional to the chain length of the phosphate (Irani and Morgenthaler, 1963). Hence, a possible explanation for the earlier difference in results is that, in addition to the lower HMP concentration, Shahidi et al. (1988) used a HMP with a longer chain length than that used by Tims and Watts. In the present study, TTPP, which may also be described a short chain length HMP, completely inhibited lipid oxidation. The lower antioxidant ability of HMP in this study with cooked beef is not unexpected, since beef has a higher myoglobin concentration than pork and, presumably, more iron is released from the myoglobin during cooking. If HMP cannot completely chelate the additional free iron released from the cooked beef, then it will not completely inhibit lipid oxidation as it does with pork.

WOF Scores. For most treatments, the WOF scores (Figure 1) followed essentially the same trends as the TBARS values ($r = 0.724$, $p < 0.01$). There were, however, several differences. One difference was that the cooked beef that contained beef extract had much lower WOF scores than indicated by its TBARS values. To illustrate this point, the correlation coefficient of the correlation between TBARS values and WOF score increased from 0.724 to 0.790 when the beef extract results were excluded from the calculation. One reason for the lower WOF values with beef extract was that the beef extract, a meat flavor enhancer, masked the WOF odor. However, the odor produced by beef extract was not typical of fresh cooked beef. Another possible reason for the lower WOF values is that components in the beef extract themselves oxidized during storage, thereby increasing the TBARS values but without increasing WOF.

A second difference was that WOF score of the cooked beef that contained HMP did not increase with increasing storage time ($P > 0.05$), in contrast to the TBARS values, which increased slightly but significantly ($P < 0.05$) during the same time. This difference occurred presumably because even though the lipid oxidation products detected by the TBARS test increased with storage time, not all of these compounds contribute to the WOF.

A third difference was that the WOF scores of treatments that had the highest TBARS values (i.e., those that contained no additives and Pi) plateaued after 2 days of storage even though the TBARS values of these treatments increased linearly with increasing storage time. The most likely reason for this difference is that not all the lipid oxidation products detected by the TBARS test contribute to the WOF.

Although the WOF scores were determined by a well-trained sensory panel, the scores do not completely reflect the level of WOF in the samples. It was expected that WOF scores would range from 1 for the best samples on day 0 to 8 for the worst samples on days 4–6, since the panelists were trained to give freshly prepared samples a score of 1 and samples stored for at least 4 days a score of 8. However, the mean panel scores ranged from 2.0 to 5.5. This is most likely because, even though they were well-trained, the panelists were reluctant to use the two extremes on the evaluation scale (Stone and Sidel, 1985). Thus, in reality, the lowest score of 2.0 would represent a sample with no WOF and the highest score of 5.5 would represent a sample that had extremely strong WOF.

Experiment 2. *TBARS Values.* TPP concentration had a significant effect on both the initial TBARS values of the ground beef and the rate at which the TBARS values increased with increasing storage time (see Figure 2). Samples that contained no TPP had higher initial TBARS values ($P < 0.01$) than those that contained the two higher concentrations of TPP (0.375, 0.500%). Moreover, the TBARS values of the samples that contained no TPP increased rapidly (0.62 mg/kg per day) with increasing storage time ($P < 0.01$). As a generalization, the rate at which the TBARS values increased

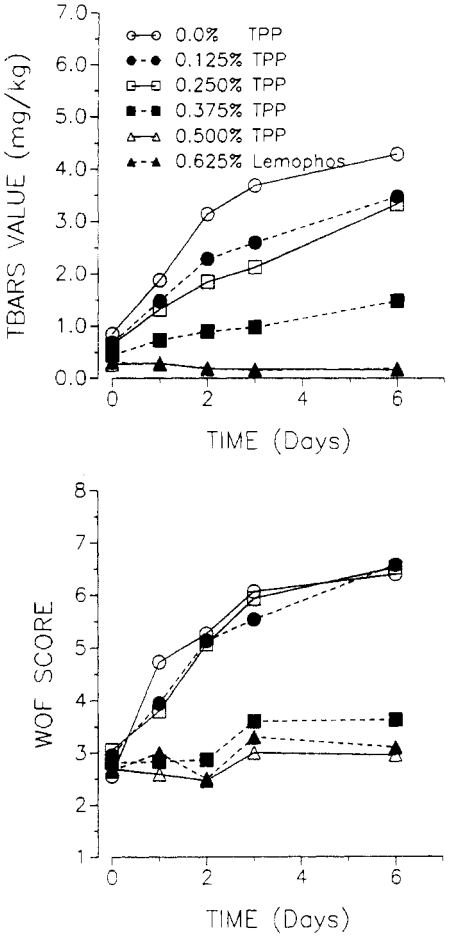

Figure 2. Effect of tripolyphosphate concentration, Lemophos, and storage time on (a) TBARS values and (b) WOF scores of cooked beef. $LSD_{(5\%)}$ values for comparison between treatment means are 0.67 and 1.13 for TBARS values and WOF scores, respectively.

with storage time decreased progressively with increasing TPP concentration. However, only the highest TPP concentration (0.500%) completely prevented TBARS values from increasing during the 6 days of storage.

Lemophos at a concentration of 0.625% also completely inhibited lipid oxidation during the 6 days of storage (the Lemophos and 0.500% TPP gave identical results; consequently, in Figure 2 the data points for Lemophos are obscured by those of the 0.500% TPP). The Lemophos was used at 0.625% so that its effectiveness could be compared to that of TPP when both additives were used at the maximum permissible phosphate concentration of 0.5%. The TPP concentration in Lemophos is only 80%.

WOF Scores. The changes in WOF scores with increasing storage time were very similar to the changes in TBARS values, and the two values were highly correlated (r = 0.828, $p < 0.01$). There were, however, some minor differences between the two results. First, the WOF scores of samples that contained the two lowest concentrations of TPP were the same as those that contained no additives ($p > 0.05$), even though these samples had significantly lower ($p < 0.05$) TBARS values. Second, the WOF scores of the samples that contained 0.375% TPP did not increase with increasing storage time even though the TBARS values of these samples increased significantly ($p < 0.05$) with storage time. Presumably, in both cases, either the panelists were not sensitive enough to detect the minor difference in WOF levels indicated by the TBARS values or the TBARS values measured lipid oxidation products other than those responsible for WOF.

CONCLUSION

PP, TPP, TTPP, and Lemophos used at 0.5% completely inhibited WOF in cooked beef during 6 days of refrigerated storage as measured by both TBARS values and sensory evaluation. HMP did not completely inhibit WOF as indicated by TBARS values; however, the sensory panelists did not detect an increase in WOF levels. Pi and beef extract were only slightly effective at inhibiting WOF, although the beef extract partly masked the WOF odor. The minimum TPP concentration required to completely inhibit WOF was 0.5%; 0.375% TPP inhibited the increase in WOF scores but not the increase in TBARS values. This research indicates that to be completely effective at inhibiting WOF in cooked beef, the concentration and type of phosphate must be such that it completely chelates any free iron that is present in the meat.

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Effect of Acetylation on Emulsifying Properties of Glycinin

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Emulsifying properties of acetylated glycinin were checked on samples of 0%, 45%, and 90% lysine residue modification. Sonication at 15 W was employed for making emulsion. At an oil to protein solution volume ratio of 2.5:7 (v/v), pH 7.6, 90% acetylated glycinin showed an over 70% increase in emulsifying activity over native protein. Above the isoelectric point (p*I*) of the protein, emulsifying activity of acetylated protein was superior to that of native glycinin. Below the p*I*, native glycinin showed better emulsifying activity. Emulsion stability of the glycinin was significantly improved by acetylation. Changes in emulsifying properties of acetylated glycinin are discussed in terms of such intrinsic properties of the protein as surface hydrophobicity and flexibility. Effects of the presence of NaCl, $CaCl_2$, and β-mercaptoethanol and browning as the result of incubation with glucose are also discussed.

There have been numerous studies on functional properties of soy protein (Shiga and Nakamura, 1987; Townsend and Nakai, 1983; Deeslie and Cheryan, 1988), and chemical modification was proposed as one of the means for improving functional properties of the protein (Barman et al., 1977; Sung et al., 1983a; Kim and Kinsella, 1986). The primary objectives of modification are (a) to learn more about the functional properties in terms of the intrinsic properties of the protein and (b) to improve functionality for wider usage in the food industries. Acylation (Franzen and Kinsella, 1976; Barman et al., 1977; Brinegar and Kinsella, 1980; Sung et al., 1983a) and phosphorylation (Sung et al., 1983b) are known to increase solubility, emulsifying activity, and foaming capacity of the protein. Proteins showing poor functional properties could be effectively used in food processing following acylation (Barber and Warthesen, 1982; Eisele and Brekke, 1981).

More recently, attempts were made to correlate functional properties to physicochemical properties exhibited by the protein. In protein-based emulsion the protein forms an interfacial film. The physical properties of this film matrix and its surface characteristics determine its capacity to form and stabilize emulsion. Kato and Nakai (1980) reported that a significant correlation existed between surface hydrophobicity and emulsifying properties of food protein. Graham and Phillips (1979, 1980) attributed the higher rate of surface pressure development for β-casein than bovine serum albumin and lysozyme to greater flexibility of the β-casein, which increased its facility of unfold at the interface. Stability of protein emulsion was influenced by the properties of the interfacial film material and the viscosity of the continuous phase.

There have been numerous reports on emulsifying properties of soybean protein (Yasumatsu et al., 1972; McWatters and Cherry, 1977; Aoki et al., 1981, 1984; Voutsinas et al., 1983), but still more research is needed in order to apply the protein in the development for use as food. In our previous report (1989), we discussed the effect of acetylation on physicochemical properties of glycinin, such as conformation, surface hydrophobicity, and flexibility. In this paper, emulsifying properties of native and acetylated glycinin are discussed in the absence and presence of NaCl, $CaCl_2$, and β-mercaptoethanol. The effect of storage with glucose, which results in a Maillard reaction, was also determined. Glycinin was chosen because of ease in purification of a large quantity, availability of

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