Effect of Solutes on Zinc Complex Formation in Heated Green Vegetables

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The effect of solutes on the formation of zinc complexes of chlorophyll derivatives in processed green vegetables was studied using a pea puree model system containing added Zn^{2+} and heated at 121 °C for 30 min. Divalent cations decreased the pH of unheated purees and zinc complex formation after heating, suggesting that the pH-lowering effect of divalent cations may influence complex formation during heating. Malate, tartrate, citrate, phosphate, and EDTA anions decreased zinc complex formation because of their ability to chelate Zn^{2+}. Thiocyanate, benzoate, oleate, and caprylate anions increased complex formation, while sucrose, glucose, and fructose had no effect on the reaction. Zinc complex formation increased when sodium dodecyl sulfate detergent was added but decreased in the presence of Tween 80, cetyltrimethylammonium bromide, and Triton X-100. The results suggest that surface active anionic compounds facilitate zinc complex formation by adsorbing onto chloroplast membranes and increasing the negative surface charge.

INTRODUCTION

Rapid degradation of chlorophyll occurs during thermal processing of green vegetables as olive-colored pheophytin and pyropheophytin are formed (Gold and Weckel, 1959; Schwartz and von Elbe, 1983). Efforts to improve the quality of canned green vegetables through retention of chlorophyll have been extensive although unsuccessful (von Elbe and LaBorde, 1989). Formation of green metallocomplexes of chlorophyll derivatives during processing and storage has been reported (Fischbach, 1943; Schanderl et al., 1965; Decleire, 1966; Swirski et al., 1969) and offers a promising method for the preservation of green color.

Zinc salts were deliberately added to blanch solutions in a commercial process, known as Veri-Green, to improve the color of canned green vegetables (Segner et al., 1984). The greener color of Veri-Green-processed green vegetables was attributed by von Elbe et al. (1986) to the formation of zinc complexes of pheophytin and pyropheophytin. Veri-Green-processed green beans and spinach are currently marketed under provision by the Food and Drug Administration (FDA) that the concentration of Zn^{2+} in the product be no more than 75 ppm (*Federal Register*, 1986). Production of Veri-Green-processed peas, however, has not been successful because the amount of Zn^{2+} required to yield a satisfactory color after processing has resulted in Zn^{2+} concentrations above the FDA limit of 75 ppm.

Several factors that affect metallocomplex formation with chlorophyll derivatives have been identified. Derivatives of chlorophyll *a* form metallocomplexes more rapidly than does chlorophyll *b* (Jones et al., 1977; Berezin and Koifman, 1970). Pheophytin forms metallocomplexes more slowly than pyropheophytin and pheophorbide because of the presence of interfering substituent groups (Berezin and Koifman, 1970; Tonucci and von Elbe, 1992). Zinc complex formation increases with increasing pH and Zn^{2+} ion and pigment concentrations (LaBorde and von Elbe, 1990). High pH conditions, however, may result in retention of chlorophyll after heating and a reduction in the amount of chlorophyll derivatives available for the formation of zinc complexes (LaBorde and von Elbe, 1994).

The objective of this study was to determine the effects of several solutes on zinc complex formation with chlorophyll derivatives in heated green vegetables using a pea puree model system containing added $ZnCl_2$.

MATERIALS AND METHODS

Sample Preparation and Reaction Conditions. The effect of solutes on zinc complex formation was determined using a modification of the pea puree model system described by LaBorde and von Elbe (1994). Eight hundred grams (800.0 g) of peas and 200.0 mL of $ZnCl_2$ solution (1800 ppm of Zn^{2+}) were blended in a food processor for 30 s. One hundred gram (100.0 g) portions of the mixture were combined with 20.0 mL of solutions containing various salts, sugars, or detergents. Concentrations of solutes were based on the volume of the solute solution plus the volume of the $ZnCl_2$ solution added to the peas. The concentration of Zn^{2+} (1800 ppm) initially added to the peas resulted in a pea puree concentration of 300 ppm (w/w). pH values of purees were adjusted by adding 1 M HCl or NaOH, which increased the puree volume by no more than 1%.

Fifteen-gram samples were heated in thermal death time (TDT) cans (American Can Co., Milwaukee, WI) at 121 °C for 30 min (LaBorde and von Elbe, 1994).

Pigment Extraction and Analysis. Procedures for extraction and HPLC analysis of pigments have been described by LaBorde and von Elbe (1994). The HPLC system consisted of an ethyl acetate/methanol/water (4:5.4:1 w/v) isocratic mobile phase and a Nova-Pak C_{18} Radial-Pak cartridge HPLC column (Waters Associates Co., Milford, MA). Pigments were monitored at 658 nm on a Waters Model 440 dual-wavelength detector and integrated on a Waters 740 data module. Quantification of pigments was achieved using previously prepared standard curves.

Determination of pH. The pH values of purees before and after heating were determined using an Orion Model 601-A pH meter as described in LaBorde and von Elbe (1994).

RESULTS AND DISCUSSION

The combined concentrations of zinc pheophytin *a* and zinc pyropheophytin *a* (ZnP*a*) were used to follow zinc complex formation because both compounds contribute to the green color of vegetables processed in the presence of zinc (LaBorde and von Elbe, 1994). Only derivatives of chlorophyll *a* were followed because of their greater concentration in plant tissue and their greater reactivity compared to chlorophyll *b* (Jones et al., 1977).

Effect of Cations. Cations were studied because they occur in process waters or may be present as additives in canned foods. Chloride salts of each cation (0.1 M) were

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Table 1. Effect of Cations on Initial pH of Pea Puree Containing 300 ppm of Zn^{2+} and on pH and Zinc Complexes (ZnP*a*) Formed after Heating at 121 °C for 30 min

cation (M)	pH before heating[a]	pH after heating[b]	ZnP*a*[b,c] (nmol/g)
none	6.36	5.93 a	21.0 a
Na^+	6.29	5.91 a	20.2 a
Li^+	6.27	5.93 a	19.4 a
K^+	6.29	5.90 a	18.6 a
Mg^{2+}	5.95	5.60 b	15.9 b
Ca^{2+}	5.90	5.46 b	14.8 c
Ba^{2+}	5.71	5.48 b	13.0 d

[a] Standard deviation = ±0.03. [b] Average of three determinations. Numbers in columns followed by the same letter do not differ significantly using Fisher's *t*-test ($p = 0.05$). [c] Measurements made on a fresh weight basis.

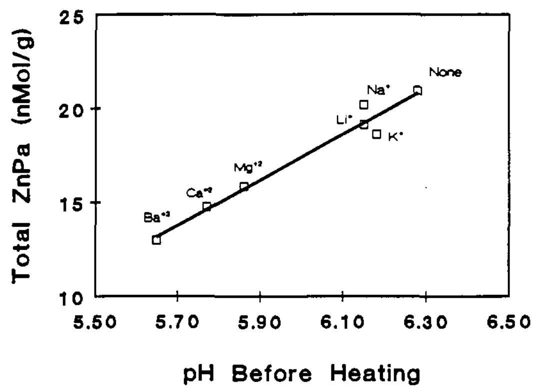

Figure 1. Total zinc complexes (ZnP*a*) formed in pea puree containing 300 ppm of Zn^{2+} and and 0.1 M Na^+, K^+, Li^+, Mg^{2+}, Ca^{2+}, or Ba^{2+} after heating at 121 °C for 30 min vs pH before heating (average of three determinations).

used to eliminate anion effects. The effect of cations on zinc complex formation in pea purees containing 300 ppm of Zn^{2+} and heated at 121 °C for 30 min is shown in Table 1. The amount of zinc complexes (ZnP*a*) formed in heated pea puree containing added monovalent cations (Na^+, K^+, and Li^+) was not significantly different ($p < 0.05$) from that in control purees containing no added salts. Zinc complex formation was significantly lower ($p < 0.05$), however, in pea purees containing added divalent cations. ZnP*a* concentrations in heated pea purees containing added Mg^{2+}, Ca^{2+}, and Ba^{2+} were 24, 30, and 38%, respectively, lower than the control samples. No chlorophyll *a* was detected in any of the samples.

The pH of pea puree before heating was decreased by divalent cations but was only slightly affected by monovalent cations (Table 1). Divalent cations are known to decrease plant tissue pH by binding with pectic material and displacing hydrogen ions and by hydrolysis of water (Sterling, 1968; Hughes et al., 1975). After heating, the pH was further reduced in all puree samples but remained lowest in samples containing divalent cations. Reduction in the pH of plant tissue may occur during heating because of the release of cellular acids, the formation of new acidic compounds, or the further interaction of cations with pectic materials (Clydesdale et al., 1972; Haisman and Clarke, 1975).

Figure 1 shows a linear relationship ($r = 0.981$) between the pH of unheated purees and zinc complex formation. A similar relationship was reported between the adjusted pH of pea puree before heating and zinc complex formation after heating (LaBorde and von Elbe, 1994). These results suggest that the pH lowering effect of divalent cations influences zinc complex formation during heating. Divalent cations are also known to have a protective effect

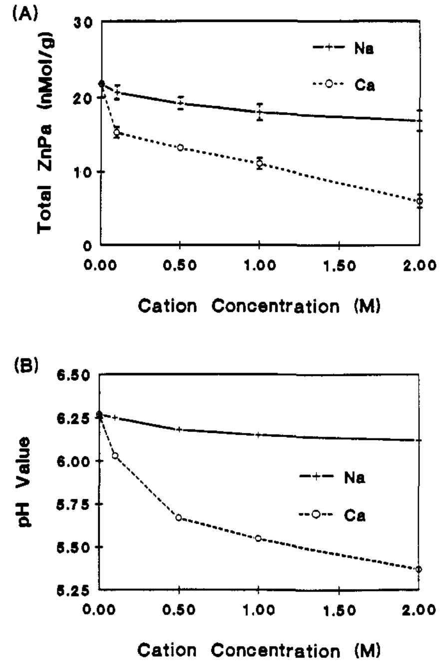

Figure 2. (A) Concentration of zinc complexes (ZnP*a*) formed in pea puree containing 300 ppm of Zn^{2+} and 0, 0.025, 0.50, 1.00, or 2.00 M Na^+ or Ca^{2+} after heating at 121 °C for 30 min (average of three determinations ± SD). (B) Initial pH of unheated pea puree containing 300 ppm of Zn^{2+} and 0, 0.025, 0.50, 1.00, or 2.00 M Na^+ or Ca^{2+} (SD = 0.03).

Table 2. Effect of Detergents on Zinc Complex (ZnP*a*) Formation, Chlorophyll *a* (Chl*a*) Retention, and pH in Pea Puree Containing 300 ppm of Zn^{2+} after Heating at 121 °C for 30 min

detergent	mol/L	Chl*a*[a,b] (nmol/g)	ZnP*a*[a] (nmol/g)	pH after heating[c]
none		0.0 a	15.6 b	5.79
Tween 80	0.1	4.8 b	7.9 a	5.79
CTAB	0.01	3.5 b	6.8 a	5.66
Triton X-100	0.1	10.7 c	2.8 a	5.75
SDS	0.1	0.0 a	37.9 c	5.87

[a] Average of three determinations made on a fresh weight basis. Numbers in a column followed by the same letter are not significantly different using Fisher's *t*-test ($p = 0.05$). [b] Chlorophyll *a* concentration before heating = 46.0 ± 1.4 nmol/g (average ± standard deviation). [c] Initial pH = 6.0. Pooled standard deviation = 0.04.

on cell membranes during heating (Toprover and Glinka, 1976) and may further decrease zinc complex formation by decreasing the permeability of Zn^{2+} into cells.

Figure 2A shows the dependence of cation concentration on zinc complex formation in pea puree containing 300 ppm of Zn^{2+} and heated at 121 °C for 30 min. ZnP*a* concentration after heating decreased from 21.8 ± 0.5 nmol/g ZnP*a* (control samples) to 17.0 ± 1.4 and 6.0 ± 0.9 nmol/g in samples containing 2.0 M Na^+ or Ca^{2+}, respectively. The pH of unheated purees similarly decreased with increasing cation concentration (Figure 2B). These results further suggest that zinc complex formation in the presence of added cations is caused by the pH-lowering effect.

Effect of Detergents. The effect of Tween 80, cetyltrimethylammonium bromide (CTAB), Triton X-100, and sodium dodecyl sulfate (SDS) on zinc complex formation was determined because these detergents have been shown to influence chlorophyll degradation. Table 2 shows zinc complex formation, chlorophyll retention, and pH in heated (121 °C, 30 min) pea puree containing 300 ppm of Zn^{2+}. All purees were adjusted to pH 6.0 before heating to minimize the effect of pH on zinc complex formation. Detergents were added to yield 0.1 M solutions except for CTAB, which, because of its lower solubility, was added to yield a 0.01 M solution. Compared to control puree that contained no detergent, Tween 80, CTAB, and Triton X-100 decreased the amount of ZnP*a* formed by 49, 56, and 82%, respectively. In the same purees, chlorophyll *a* retained after heating accounted for 10, 8, and 23%, respectively, of the initial chlorophyll *a* present before heating (46.0 ± 1.4 nmol/g). No chlorophyll *a* was present in control purees after heating. In contrast, SDS increased the amount of ZnP*a* formed after heating 2.4 times compared to control puree, and these purees contained no chlorophyll after heating. Since all samples in Table 2 were initially adjusted to pH 6.0 before heating and differences in pH value after heating are small, pH effects do not appear to explain the effects of detergents on zinc complex formation.

Triton X-100 and CTAB have previously been shown to increase chlorophyll retention in heated plant tissue (Clydesdale et al., 1970; Haisman and Clarke, 1975), while SDS increased pheophytin formation in plant tissue during heating (Smith, 1940; Haisman and Clarke, 1975). Haisman and Clarke (1975) recognized that chlorophyll is associated with chloroplast membranes and proposed that detergents affect chlorophyll degradation in heated plant tissue by adsorbing onto membranes and altering the surface charge. According to the authors, anionic detergents (i.e., SDS) increase the negative charge of chloroplast membrane surfaces, resulting in an accumulation of H^+ ions and an increase in pheophytin formation. Conversely, cationic detergents (i.e., CTAB) decrease the negative surface charge of membrane surfaces, repel H^+ ions, and therefore decrease chlorophyll degradation. The same study reported that neutral detergents (i.e., Triton X-100) increase chlorophyll retention during heating by displacing negatively charged phospholipids and proteins. The catalytic effect of surface charge on interfacial diffusion of ions across lipid monolayers has been reported (Davies, 1954; Britten and Blank, 1977) and is believed to play a role in the regulation of membrane permeability in biological systems (Blank, 1987).

The results from this study suggest that detergents similarly affect zinc complex formation in heated pea puree through their effects on membrane surface charge. Anionic detergents may increase the concentration of Zn^{2+} at membrane surfaces, facilitating the reaction of chlorophyll derivatives with Zn^{2+} ions. Conversely, cationic (CTAB) and neutral (Tween 80 and Triton X-100) detergents may reduce zinc complex formation by decreasing the membrane surface charge and, therefore, the concentration of Zn^{2+} at membrane surfaces. Lower concentrations of ZnP*a* in cationic and neutral detergent samples may also be attributed to chlorophyll retention after heating, thus reducing the amount of chlorophyll derivatives available for complex formation.

Effect of Sugars and Anions. Sugars and anions were studied because they may be added to canned foods to influence flavor, texture, appearance, or pH value. Anions were compared using their sodium salts to eliminate cation effects. In Table 3, the effect of anions and sugars (0.1 M) on zinc complex formation in heated (121 °C, 30 min) pea purees (300 ppm of Zn^{2+}, pH 6.0) is shown. Zinc complex formation was not significantly ($p < 0.05$) affected by sucrose, glucose, and fructose. Chloride, sulfate, lactate, acetate, and propionate ions also did not affect ($p < 0.05$) zinc complex formation. Malate, tartrate, citrate, phosphate, and EDTA, however, significantly decreased ($p < 0.05$) the amount of ZnP*a* formed by 19, 20, 84, 85, and 100%, respectively. In contrast, thiocyanate, benzoate, oleate, and caprylate ions increased ($p < 0.05$) zinc complex formation 1.3, 1.3, 2.7, and 3.0 times, respectively. Chlorophyll *a* was not detected in any of the samples.

Table 3. Effect of Solutes on Zinc Complex (ZnP*a*) Formation and pH in Pea Puree Containing 300 ppm of Zn^{2+} after Heating at 121 °C for 30 min

solute[a]	ZnP*a*[b] (nmol/g)	pH after heating[c]
control	15.6 c	5.79
sucrose	15.8 c	5.74
glucose	15.8 c	5.76
fructose	15.7 c	5.77
chloride	15.5 c	5.77
sulfate	15.6 c	5.82
lactate	14.3 c	5.70
malate	12.6 d	5.79
tartrate	12.4 d	5.78
citrate	2.5 e	5.86
phosphate	2.4 e	5.86
EDTA	0.0 e	5.87
acetate	16.5 c	5.80
propionate	17.0 c	5.79
thiocyanate	20.5 b	5.80
benzoate	20.8 b	5.78
caprylate	46.9 a	5.98
oleate	42.2 a	5.90

[a] 0.1 M solutions. Sodium salts used to determine anion effects. [b] Average of three determinations made on a fresh weight basis. Numbers followed by the same letter are not significantly different using Fisher's *t*-test ($p = 0.05$). [c] Initial pH = 6.0. Pooled standard deviation for pH = 0.04.

Differences in the pH of puree samples after heating were small and, therefore, do not appear to explain these results. Decreased zinc complex formation in samples containing malate, tartrate, citrate, phosphate, and EDTA may be attributed to the metal chelation properties of these compounds (Lindsay, 1985). Removal of Zn^{2+} ions from solution effectively decreases their availability for complexing with chlorophyll derivatives, thus resulting in lesser amounts of ZnP*a* formed. Chloride and sulfate anions and sucrose, glucose, and fructose do not form chelates with Zn^{2+} ions and therefore do not affect zinc complex formation. Lactic acid is a weak chelator and, therefore, had only a slight negative effect on complex formation.

Thiocyanate, benzoate, oleate, or caprylate anions, similar to the anionic detergent SDS, may increase zinc complex formation in heated pea puree by increasing the negative surface charge of membranes. Differences in the amount of ZnP*a* formed may be attributed to differences in the tendency of these compounds to adsorb onto cell membranes.

Increased formation of zinc complexes in purees containing SDS, sodium oleate, and sodium caprylate is concentration dependent (Figure 3). At 10^{-3} M, only sodium oleate had a significant effect ($p < 0.05$), increasing zinc complex formation 1.4 times compared to control samples. The amount of ZnP*a* formed after heating was increased 1.2, 1.8, and 1.4 times, respectively, when the concentration of the three chemicals was raised from 10^{-3} to 10^{-2} M. An additional 10-fold increase in concentration to 10^{-1} M increased ZnP*a* concentration 1.9, 1.1, and 2.0 times, respectively. This suggests that adsorption of surface active anionic compounds increases with increasing concentration and thus increases the negative surface charge at the membrane surface.

Conclusions. The concentration of Zn^{2+} in pea purees and the severity of the heat treatments used in these

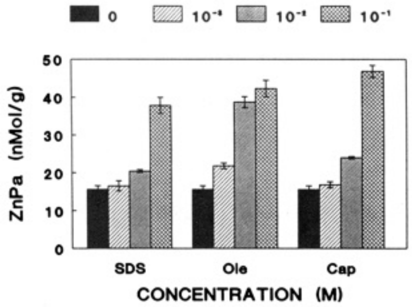

Figure 3. Total zinc complexes (ZnP*a*) formed in pea puree containing 300 ppm of Zn^{2+} and 0, 10^{-3}, 10^{-2}, or 10^{-1} M sodium dodecyl sulfate (SDS), sodium oleate (Ole), or sodium caprylate (Cap) after heating at 121 °C for 30 min (average of three determinations ± SD).

experiments were greater than would be used in a commercial process. The data obtained, however, demonstrate that compounds present during processing can have dramatically different effects on zinc complex formation. Use of calcium salts to improve the texture of zinc-processed green vegetables may adversely affect zinc complex formation at concentrations greater that 0.1 M. Addition of salt (NaCl) and sugars at concentrations typically used to flavor canned green vegetables, however, will not affect zinc complex formation. Organic acids added as acidulants or chelating agents may adversely affect zinc complex formation and the development of green color during processing. Surface active compounds may be useful in increasing zinc complex formation in zinc-processed green vegetables. Further research is needed to determine the practical applications of these compounds because their use is limited by regulatory and organoleptic considerations.

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