

# Chlorophyll Degradation and Zinc Complex Formation with Chlorophyll Derivatives in Heated Green Vegetables

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Chlorophyll degradation and zinc complex formation with chlorophyll derivatives in processed green vegetables were studied using pea puree containing added  $Zn^{2+}$  and heated at 121 °C for up to 150 min. Chlorophyll *a* decreased to trace levels after 20 min as zinc complexes of pheophytin *a* and pyropheophytin *a* were formed. Further heating decreased zinc pheophytin *a* and increased zinc pyropheophytin *a* concentrations. Zinc pyropheophytin may form by decarbomethoxylation of zinc pheophytin or by reaction of pyropheophytin with  $Zn^{2+}$ . pH effects were determined using pea puree adjusted to pH between 4.0 and 10.0 and heated at 121 °C for 30 min. Zinc complex formation increased in purees between pH 4.0 and 6.0 but decreased at pH values of 8.0 or greater. Chlorophyll *a* was retained at pH 8.0 or higher, suggesting that high pH processing may decrease zinc complex formation by reducing the amount of chlorophyll derivatives available for the reaction.

## INTRODUCTION

Loss of green color in thermally processed green vegetables is of concern to processors as consumer preference shifts toward more fresh-appearing products. Degradation of chlorophyll and formation of olive-colored pheophytin occur when cellular acids are released during heating (Haisman and Clarke, 1975). Heat treatments required to achieve commercial sterility further result in decarbomethoxylation of pheophytin to form similarly colored pyropheophytin (Schwartz and von Elbe, 1983).

Efforts to preserve the color of green vegetables after processing have been reviewed by von Elbe and LaBorde (1989). Alkalizing agents in blanch and brine solutions have been used to raise the pH of green vegetables and, therefore, retain chlorophyll after processing (Blair and Ayres, 1943). Color preservation after processing has also been demonstrated using high-temperature short-time processing (Tan and Francis, 1962; Schwartz and Lorenzo, 1991) and enzymatic conversion of chlorophyll to chlorophyllide (Loef and Thung, 1965). None of these methods, however, have successfully retained green color after prolonged storage.

Formation of green metal complexes of chlorophyll derivatives during thermal processing, known as regreening, has emerged as a promising method to preserve the color of canned green vegetables. Spontaneous regreening of green vegetables during processing, long known by canners as a nonuniform color defect, was attributed by Fischbach (1943) to zinc-containing pigments. Sporadic bright green color in processed green beans (Declaire, 1966) and Brussels sprouts (Swirski et al., 1969) was associated with  $Cu^{2+}$  or  $Zn^{2+}$  ions present as contaminants in process solutions. Formation of zinc and copper complexes of pheophytins *a* and *b* during storage of pea puree has also been reported (Schanderl et al., 1965).

A commercial process, Veri-Green (Segner et al., 1984), has been developed to improve the color of canned green vegetables by adding metal ions to blanch solutions. von Elbe et al. (1986), using high-performance liquid chromatography (HPLC), have attributed the improved color of canned green beans blanched in a  $ZnCl_2$  solution to the presence of zinc complexes of pheophytin and pyropheophytin. The U.S. Food and Drug Administration (FDA) has temporarily amended the standard of identity for canned green beans to allow  $ZnCl_2$  on the list of optional

ingredients with the provision that the concentration of  $Zn^{2+}$  in the product be no more than 75 ppm (*Federal Register*, 1986). Production of Veri-Green-processed peas has not been successful because the concentration of  $Zn^{2+}$  required to yield a satisfactory color after processing is above the FDA limit of 75 ppm.

Limited information is available about the formation of metal complexes with chlorophyll derivatives during thermal processing. Copper complexes of chlorophyll derivatives are formed more rapidly compared to zinc complexes (Schanderl et al., 1965; Jones et al., 1977). Zinc complex formation, however, is of greater interest because of the toxic nature of  $Cu^{2+}$  ions. Derivatives of chlorophyll *a* form metal complexes more rapidly than does chlorophyll *b* (Jones et al., 1977; Berezin and Koifman, 1970). Pheophytin forms metal complexes more slowly than pyropheophytin and pheophorbide because of the presence of interfering substituent groups (Berezin and Koifman, 1970; Tonucci and von Elbe, 1992).

LaBorde and von Elbe (1990) used previously heated vegetable purees in which chlorophyll had initially been entirely converted to pyropheophytin to show the effects of chlorophyll derivative,  $Zn^{2+}$  concentrations, and pH value on zinc complex formation in heated green vegetables. Zinc complex formation in pea purees containing 150 and 300 ppm of added  $Zn^{2+}$  was 3 and 9 times, respectively, greater than in puree containing 75 ppm of added  $Zn^{2+}$ . Spinach puree that had 12 times the concentration of chlorophyll derivatives compared to pea puree contained 40 times more zinc complexes after identical heating. In spinach purees containing equal concentrations of  $Zn^{2+}$ , an increase in the initial pH from 4.0 to 8.5 resulted in an 11-fold increase in zinc complex concentration after heating. Decreased zinc complex formation in pH 10.0 puree was attributed to formation of insoluble  $Zn(OH)_2$ .

The objectives of this study were to (1) follow the degradation of chlorophyll and formation of chlorophyll derivatives during heating in pea puree containing added  $ZnCl_2$  and (2) determine the effect of pH on these reactions.

## MATERIALS AND METHODS

**Sample Preparation and Reaction Conditions.** A pea puree model system was prepared using commercially obtained frozen peas which were thawed in distilled water for 20 min and allowed

to drain. Zinc was added by blending peas and  $ZnCl_2$  solution (900 ppm of  $Zn^{2+}$ ) (2:1 w/v) for 30 s in a food processor to yield a final  $Zn^{2+}$  concentration of 300 ppm (w/w). The pH of purees was adjusted using 1 M HCl or NaOH; the amounts added increased the volume by no more than 3%.

Fifteen-gram samples from each treatment were placed into 25-mL thermal death time (TDT) cans (American Can Co., Milwaukee, WI), which were mechanically sealed and heated in a laboratory thermal death time retort (LaBorde and von Elbe, 1990) at 121 °C for appropriate time intervals. At the end of heating periods, the cans were immediately cooled under flowing cold water and stored at -15 °C until analysis.

**Analysis of Pigments.** Pigments contained in pea puree were extracted by mixing the content of each TDT can with 75 mL of acetone and 5 g of Celite. The mixture was filtered under vacuum through Whatman No. 1 and No. 42 filter papers. The residue was washed with an additional 20 mL of acetone until colorless and brought to volume in a 100-mL volumetric flask.

Chlorophyll derivatives were separated by high-performance liquid chromatography (HPLC) using an isocratic mobile phase of ethyl acetate/methanol/water (4:5.4:1 v/v/v). Duplicate 40- $\mu$ L injections were made onto a Nova-Pak  $C_{18}$  Radial-Pak cartridge HPLC column (Waters Associates Co., Milford, MA). All pigments were monitored at 658 nm on a Waters Model 440 dual-wavelength detector and integrated on a Waters 740 data module.

Standard curves for the quantification of each pigment were prepared using pure compounds obtained by preparative HPLC (Tonucci and von Elbe, 1992). Pigment concentrations were calculated on the basis of reported spectral characteristics of chlorophyll (Strain et al., 1963), pheophytin *a* and pyropheophytin *a* (Pennington et al., 1964), and zinc pheophytin *a* (Jones et al., 1968). The spectral characteristics of zinc pheophytin *a* and zinc pyropheophytin *a* were assumed to be identical.

**Determination of pH.** The pH of purees was determined using an Orion Model 601-A pH meter calibrated with pH 4.00 and 7.00 buffer. After each reading, the pH meter was recalibrated in pH 7.00 buffer.

## RESULTS AND DISCUSSION

Zinc complex formation was studied using a pea puree model system because of the inferior color that Veri-Green-processed peas have compared to that of green beans and spinach. In a previous study on zinc complex formation in vegetable purees (LaBorde and von Elbe, 1990), a simplified model system was used in which chlorophyll had been entirely converted to pyropheophytin before heating with  $Zn^{2+}$ . The pea puree used in this study was not initially heated and, therefore, did contain chlorophyll. Data obtained, therefore, more closely describe zinc complex formation in thermally processed fresh green vegetables. 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).

Figure 1 shows an HPLC chromatogram of a pea puree extract containing chlorophyll and chlorophyll derivatives. Identification of peaks was confirmed by comparing retention times obtained using pure compounds. The elution pattern of the zinc complexes and their parent compounds is identical to that obtained using purified extracts (Schwartz, 1984) and zinc-processed green beans (von Elbe et al., 1986). Secondary peaks of chlorophyll *a* (Chla) and pheophytin *a* (Phea), designated Chla' and Phea', respectively, have been identified as C-10 isomers by Schwartz et al. (1981). The absence of shoulders adjacent to peaks representing pyropheophytin *a* (Pyr) and zinc pyropheophytin *a* (ZnPyr) is the result of the heat-induced removal of the C-10 carbomethoxy group, which eliminates the chiral center and the possibility of isomerization (Schwartz et al., 1981). The C-10 isomer of zinc pheophytin *a* (ZnPya') coeluted with zinc pyropheo-

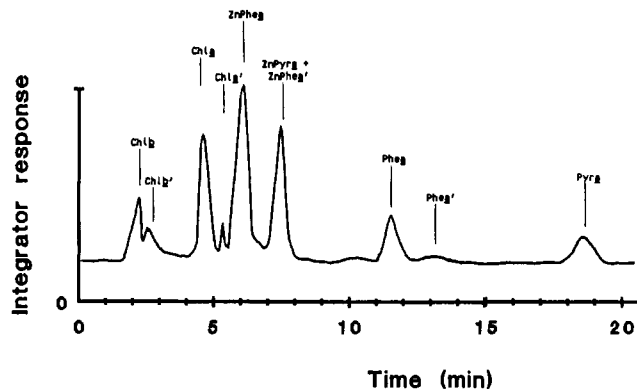


Figure 1. HPLC chromatogram of pigments in pea puree (pH 9.0) containing 300 ppm of  $Zn^{2+}$  after heating at 121 °C for 30 min.

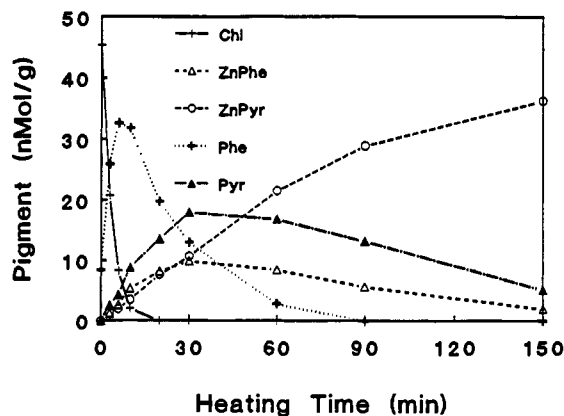


Figure 2. Pigments in pea puree containing 300 ppm of  $Zn^{2+}$  after heating at 121 °C for up to 150 min.

phytin *a* (ZnPyr). Conversion of Chla to Chla' in heated plant tissue is approximately 10% (Bacon and Holden, 1967). It is assumed, therefore, that the proportion of ZnPhe' compared to ZnPhe is similar and that the error involved in quantifying zinc complexes of chlorophyll derivatives is minimal.

**Reaction Sequence.** Chlorophyll degradation and formation of chlorophyll derivatives in pea puree containing 300 ppm of added  $Zn^{2+}$  and heated at 121 °C for up to 150 min are shown in Figure 2. The pea puree initially contained  $45.4 \pm 2.2$  nmol/g chlorophyll *a*. Small amounts of pheophytin *a* ( $8.4 \pm 1.0$  nmol/g) were detected in frozen peas because they had been blanched. Chlorophyll *a* degraded rapidly to trace levels after only 20 min of heating as pheophytin *a* increased to  $32.6 \pm 0.8$  nmol/g during the first 10 min. Further heating resulted in decreases of pheophytin *a*. Pyropheophytin *a* was detected after 3 min of heating, increasing to a maximum of  $18.0 \pm 0.5$  nmol/g after 30 min of heating. These results are consistent with the chlorophyll to pheophytin to pyropheophytin reaction sequence demonstrated by Schwartz and von Elbe (1983) using heated spinach puree. Zinc complexes of pheophytin *a* and pyropheophytin *a* appeared after 3 min of heating, increasing to  $9.9 \pm 0.4$  nmol/g and  $10.7 \pm 0.3$  nmol/g, respectively, after 30 min. With further heating, zinc pheophytin *a* decreased to  $2.0 \pm 0.1$  nmol/g after 150 min, while the concentration of zinc pyropheophytin *a* increased to  $36.3 \pm 0.8$  nmol/g.

The changes in pigment concentration shown in Figure 2 suggest that the chemical reactions in Figure 3 occur in heated plant tissue containing added  $Zn^{2+}$ . When plant tissue is heated in the presence of  $Zn^{2+}$ , chlorophyll reacts with tissue acids to form pheophytin, which may then

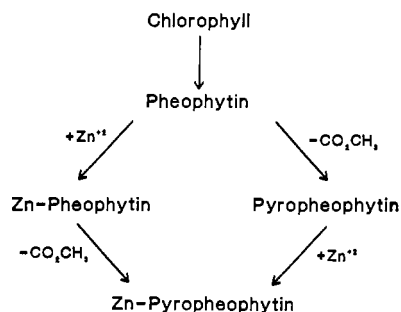


Figure 3. Chemical reactions occurring in heated green vegetables containing  $Zn^{2+}$ .

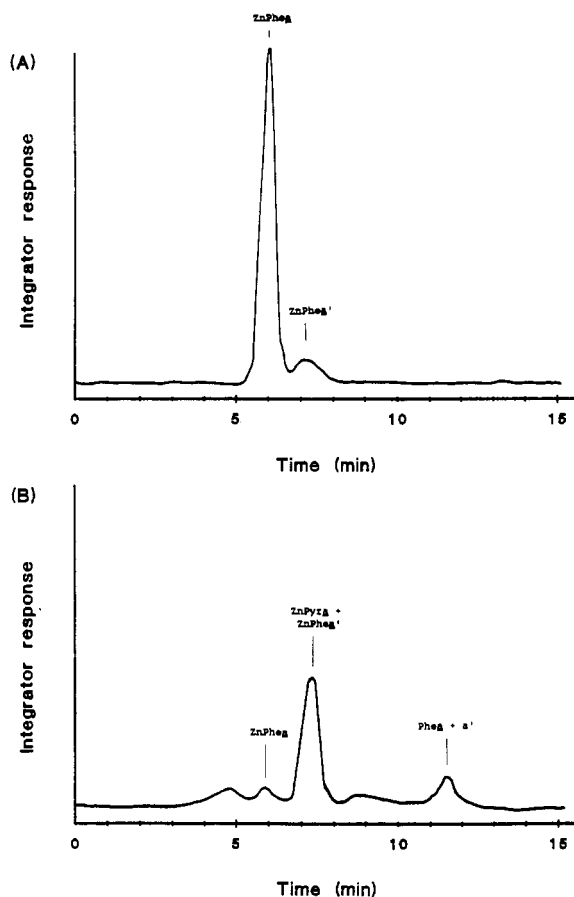


Figure 4. HPLC chromatogram of zinc pheophytin *a* and zinc pyropheophytin *a* before (A) and after (B) heating in collidine at 100 °C for 6 h. Zinc pheophytin *a* (ZnPhea, ZnPhea'); zinc pyropheophytin *a* (ZnPyra); pheophytin *a* (Phea, Phea').

combine with  $Zn^{2+}$  to form zinc pheophytin or be decarbomethoxylated to form pyropheophytin. The observed increase in zinc pyropheophytin *a* over the entire heating period suggests that zinc pyropheophytin can be formed by decarbomethoxylation of zinc pheophytin as well as by the reaction of pyropheophytin with  $Zn^{2+}$ .

Decarbomethoxylation of zinc pheophytin during heating was confirmed by heating zinc pheophytin *a* in collidine at 100 °C. Thermal decarbomethoxylation of chlorophyll in pyridine (Pennington et al., 1964), of pheophytin in collidine (2,4,6-trimethylpyridine) (Schwartz et al., 1981), and of pheophorbide in collidine (Kenner et al., 1973) has previously been demonstrated. Figure 4 shows HPLC chromatograms taken before (A) and after (B) zinc pheophytin *a* was heated (100 °C) for 6 h. The peak representing zinc pheophytin *a* (A) decreased with heating time as the peak representing zinc pyropheophytin *a* (B) increased. It is likely that this peak includes only a small

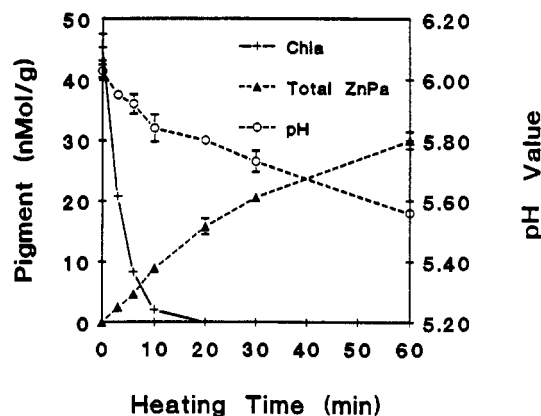


Figure 5. Conversion of chlorophyll *a* (Chla) to total zinc complexes (ZnPa) and change in pH in pea puree heated at 121 °C for up to 60 min (average of three determinations  $\pm$  standard deviation).

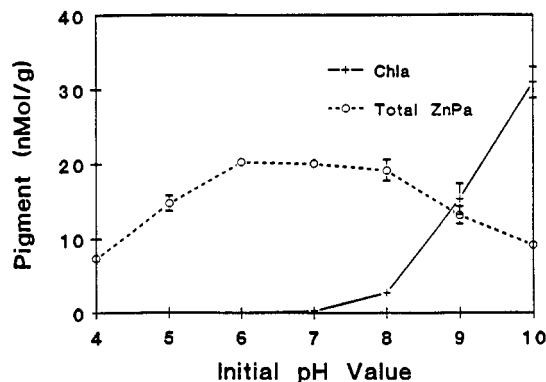


Figure 6. Chlorophyll *a* (Chla) retention and zinc complex (ZnPa) formation in pea puree adjusted to pH 4, 5, 6, 7, 8, 9, and 10 and containing 300 ppm of  $Zn^{2+}$  after heating at 121 °C for 30 min (average of three determinations  $\pm$  standard deviation).

amount of ZnPhea', given the low conversion of chlorophyll derivatives to isomers in heated plant tissue. Other peaks appear to indicate the presence of pheophytin *a* as well as other degradation products formed during heating.

Because both zinc pheophytin *a* and zinc pyropheophytin *a* contribute to the green color of zinc-processed green vegetables (von Elbe et al., 1986), the sum of these pigments, designated ZnPa, can be used to follow the degree to which regreening occurs during heating. Degradation of chlorophyll *a*, formation of total zinc complexes (ZnPa), and pH changes in pea puree (300 ppm of  $Zn^{2+}$ ) heated at 121 °C for up to 60 min are shown in Figure 5. The amount of ZnPa formed increased rapidly to  $20.6 \pm 0.2$  nmol/g during the first 30 min of heating, after which time the rate of formation decreased, reaching a concentration of  $30.0 \pm 1.4$  nmol/g after 60 min. The pH value of the purees after heating decreased during the same interval from  $6.03 \pm 0.02$  before heating to  $5.56 \pm 0.01$  after heating. Similar decreases in the pH of heated vegetable tissue have been observed (Clydesdale et al., 1972; Schwartz and von Elbe, 1983) and may limit the amount of zinc complexes formed (LaBorde and von Elbe, 1990).

**Effect of pH.** In Figure 6, the effect of pH on retention of chlorophyll *a* and formation of total zinc complexes (ZnPa) in heated green vegetables was determined using pea puree (300 ppm of  $Zn^{2+}$ ) adjusted to between pH 4.0 and 10.0 and heated at 121 °C for 30 min. Zinc complex formation increased between pH 4.0 and 6.0, reaching a maximum between pH 6.0 and 8.0. Zinc complex formation, however, decreased at pH values greater than 8.0.

The amounts of zinc complexes formed after 30 min of heating at pH 4.0, 6.0, and 10.0 were  $7.4 \pm 0.3$ ,  $20.3 \pm 0.5$ , and  $9.2 \pm 0.4$  nmol/g, respectively. Chlorophyll *a* decreased from  $46.6 \pm 3.2$  nmol/g before heating to trace levels in samples with initial pH values of 7.0 or less. The amount of chlorophyll *a* remaining in pH 8.0 samples after heating, however, was  $2.8 \pm 0.3$  nmol/g and increased to  $31.0 \pm 2.1$  nmol/g in pH 10.0 samples. These results confirm previous studies demonstrating the greater stability of chlorophyll under high pH conditions (Blair and Ayres, 1943; Gold and Weckel, 1959; Sweeney and Martin, 1961).

Zinc complex formation in heated pea puree containing chlorophyll is, therefore, increased to an optimum at pH values between 6.0 and 8.0, beyond which further increases in ZnPa do not occur. In a previous study by LaBorde and von Elbe (1990) using previously heated spinach puree containing only pyropheophytin, zinc complex formation increased as the initial pH of purees was raised to 8.5. The lower optimal pH value for zinc complex formation in the present study suggests that retention of chlorophyll under high pH conditions decreases the amount of ZnPa formed during heating because the amount of chlorophyll derivatives available for the reaction is reduced.

**Conclusions.** The concentration of  $Zn^{2+}$  in pea purees and the heat treatments used in this experiment were greater than would occur during commercial processing of peas ( $F_0 = 7.5$  min). These data, however, have demonstrated that zinc complex formation in thermally processed green vegetables must be preceded by the degradation of chlorophyll to form pheophytin. Green vegetables containing added zinc and processed under high pH conditions retain chlorophyll and, therefore, may contain lesser amounts of zinc complexes of chlorophyll derivatives. It can be expected that color improvement in green vegetables processed this way may only be temporary because of the lower stability of chlorophyll compared to that of zinc complexes.

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