

Zinc Complex Formation in Heated Vegetable Purees

Luke F. LaBorde and Joachim H. von Elbe*

Department of Food Science, University of Wisconsin—Madison, Madison, Wisconsin 53706

Zinc complex formation with pyropheophytin *a* was studied on vegetable purees in order to further understand metallo complex formation during thermal processing. Zinc pyropheophytin *a* formation was dependent on added Zn^{2+} ion, pigment concentration, and pH. Pea puree heated 60 min at 121 °C with 150 and 300 ppm added Zn^{2+} ion contained 3 and 9 times more zinc pyropheophytin *a* than puree containing 75 ppm Zn^{2+} ion. Spinach puree with 75 ppm Zn^{2+} ion added, with 12 times the pigment concentration compared to pea puree, contained approximately 40 times more zinc complex than similar pea puree heated at 121 °C for 60 min. Increasing the pH value of the spinach puree from 4.0 to 8.5 resulted in an 11-fold increase in zinc pyropheophytin *a* formed after heating at 121 °C for 90 min. Green color of the puree, as measured by Hunter color value ($-a$), followed zinc complex formation.

Chlorophyll degradation during thermal processing of green vegetables leads to serious losses in quality and consumer appeal. Rapid formation of olive-colored pheophytins during heating is initiated by the release of cellular acids (Haisman and Clarke, 1975) and the synthesis of new acids (Clydesdale et al., 1972). Formation of pheophytin in processed vegetables is increased at lower tissue pH values and at higher process temperatures (Gold and Weckel, 1959). Further heating results in the formation of pyropheophytin (Schwartz et al., 1981); the amount formed is dependent on the heat severity of the process (Schwartz and von Elbe, 1983).

Attempts to preserve the color of processed green vegetables with alkalizing agents in process solutions (Blair and Ayres, 1943), high-temperature short-time processing (Tan and Francis, 1962), and a combination of both (Gupte and Francis, 1964) have produced good results immediately after processing through rapid color loss occurred during storage. Improved color stability of processed spinach blanched at low temperature (70 °C) has been attributed to the enzyme-induced conversion of chlorophyll to phytol-free chlorophyllide (Loef and Thung, 1965). However, vegetables processed in this way quickly lose their color during storage (Clydesdale and Francis, 1968). Incorporation of all of these methods into a single process has also failed to maintain green color during storage (Clydesdale et al., 1970).

Green metallo complexes of chlorophyll derivatives formed during thermal processing have been observed and offer a promising solution to the problem of color loss. Spontaneous regreening of commercially canned okra was reported by Fischbach and Newburger (1943) and attributed to the presence of zinc-containing pigments (Fischbach, 1943). Copper or zinc ions present in plant tissues during processing have been associated with improvement in the color of processed green beans (Declaire, 1966) and Brussels sprouts (Swirski et al., 1969). Zinc and copper complexes of pheophytin *a* and *b* have been associated with regreening of pea puree during storage (Schanderl et al., 1965).

The dependence of metallo complex formation on the nature and concentration of reactants has been shown. Copper salts were shown to be 30 times more reactive than zinc salts in heated spinach puree (Jones et al., 1977). In refluxed pea puree, copper and zinc ion concentrations of 1–2 and 25 ppm, respectively, were required for complex formation (Schanderl et al., 1965). The more

rapid formation of metal complexes of pheophytin *a* compared to those of pheophytin *b* has been reported by Jones et al. (1977) and Berezin and Koifman (1970) using zinc and copper ions, respectively.

Metallo complex formation in heated vegetables appears to be pH-dependent. Jones et al. (1977) heated fresh spinach in zinc chloride brines and reported a nearly 40% increase in zinc complex formation between initial pH values of 6.8–8.5. However, the rate of copper complex formation was unaffected between pH 3.8 and 8.5.

The use of metallo complexes as food colorants continues to be of interest today. Copper complexes of chlorophyll derivatives are available commercially as food colorants in most European countries, although their use in the United States is not permitted (Humphrey, 1980). Recently, a commercial process has incorporated zinc salts in process solutions in an effort to improve the color of thermally processed green vegetables (Segner et al., 1982). The increase in green color has been primarily attributed to the presence of zinc pheophytin *a* and zinc pyropheophytin *a* (von Elbe et al., 1986). The Food and Drug Administration has responded by limiting the amount of added zinc in canned vegetables to 75 ppm and has granted temporary permits to test market canned green beans to a number of processors (*Food Chem. News*, 1984, 1988).

The objective of this study was to investigate zinc complex formation in heated green vegetables and to study the effect of zinc ion and pigment concentrations and pH value on the rate of complex formation in vegetable purees containing only pyropheophytin.

MATERIALS AND METHODS

Sample Preparation and Reaction Conditions. Commercially canned pea and spinach samples were heated in a retort at 121 °C for 75 min, drained, and blended for 30 s in a food processor. Complete conversion of chlorophyll to pheophytin followed by conversion to pyropheophytin was confirmed by HPLC. The purees (1 part) were mixed with an appropriate concentration of zinc chloride solution (2 parts) to yield the desired fresh weight concentration of Zn^{2+} ion (Table I). The pH values of the purees were adjusted with 1 N HCl and 1 N NaOH, increasing the volume by no more than 3%. Pea and spinach purees mixed with distilled water (2:1, w/v) served as controls.

Fifteen-gram samples from each treatment were added to 25-mL thermal death time (TDT) cans (American Can Co., Milwaukee, WI) and exhausted in a steam tunnel for 3 min at 82

Table I. Zinc Ion Concentration, Initial pH Value, and Heating Time Interval for Vegetable Purees Processed at 121 °C

vegetable	Zn ²⁺ concn, ppm	init pH	proc time interval, min
pea	75	6.0	15, 30, 45, 60
	150	6.0	15, 30, 45, 60
	300	6.0	10, 20, 30, 40, 60
spinach	75	6.0	12, 24, 36, 48, 60
	75	4.0	20, 40, 60, 90
	75	5.5	20, 40, 60, 90
	75	7.0	20, 40, 60, 90
	75	8.5	20, 40, 60, 90
	75	10.0	20, 40, 60, 90

°C before sealing. Cans were heated in a laboratory thermal death time retort at 121 °C according to the schedule listed in Table I. Process time intervals began after a come-up time of approximately 30 s. At the end of each heating interval, cans were immediately cooled in ice water and stored at -15 °C until analysis.

Pigment Analysis. The contents of each duplicate TDT can containing pea puree were mixed with 50 mL of acetone and 5 g of Celite. The mixture was filtered under vacuum through Whatman No. 1 and No. 42 filter papers, and the residue was washed with an additional 75 mL of acetone until colorless. The pigments were transferred to ethyl ether with a sodium sulfate solution (5%). The ether extract was dried over anhydrous sodium sulfate, concentrated under vacuum until nearly dry, and brought to volume with acetone in a 25-mL volumetric flask for HPLC analysis.

Spinach puree samples were analyzed in the same manner except the acetone filtrate was directly brought to volume in 200-mL flask for HPLC analysis. Duplicate control samples from each puree were analyzed by the same methods.

HPLC separation of chlorophyll derivatives was achieved on a Nova-Pak C₁₈ Radial-Pak cartridge column (Waters Associates Co., Milford, MA) according to the method of Schwartz and von Elbe (1983). All pigments were monitored at 658 nm on a Waters Model 440 dual-wavelength detector set at 0.01 AUFS and integrated on a Waters 740 data module.

Standard curves were prepared for the quantification of pyropheophytin *a* and zinc pyropheophytin *a* and were based on the spectral characteristics of pyropheophytin *a* (Pennington et al., 1964) and zinc pheophytin *a* (Jones et al., 1968), respectively. Values reported represent the mean of duplicate samples ± 1 standard deviation.

Determination of pH and Color. A third replicate TDT can was used to measure the pH value and to obtain objective color values. The pH values of the purees were determined at each heating time interval. Color was measured with a Hunter Lab model D-25A-9 colorimeter using the procedure of von Elbe et al. (1986).

RESULTS AND DISCUSSION

Purees containing only pyropheophytin were chosen to eliminate both the formation of zinc complexes of pheophytin and the conversion of chlorophyll to pheophytin to pyropheophytin during the heating periods used in the experiment. Only the formation of zinc pyropheophytin *a* was followed because of the greater concentration of *a* derivatives present in plant tissue and their greater reactivities (Jones et al., 1977).

Zinc pyropheophytin *a* formation in heated pea puree with initial Zn²⁺ ion concentrations of 75, 150, and 300 ppm at pH 6.0 and heated at 121 °C is shown in Figure 1. After 60 min of heating, the amount of zinc pyropheophytin *a* formed increased from 3.0 \pm 0.2 nmol/g of puree in samples containing 75 ppm Zn²⁺ ion to 10.4 \pm 0.2 and 27.8 \pm 0.2 nmol/g in samples containing 150 and 300 ppm Zn²⁺ ion, respectively. These results show that successive doubling of Zn²⁺ ion increased the amount of zinc complex formed by approximately 3 and 9 times.

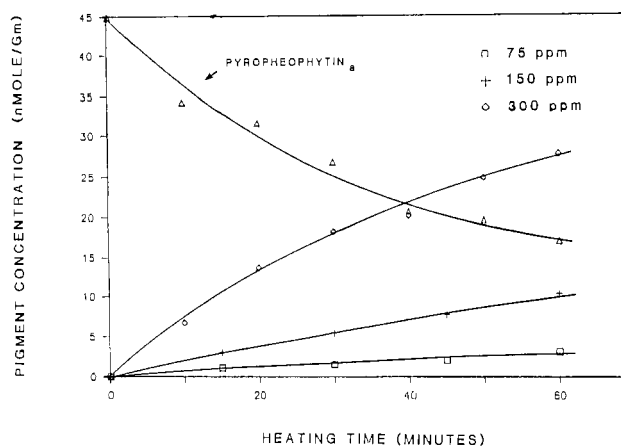


Figure 1. Formation of zinc pyropheophytin *a* in pea puree containing 75, 150, and 300 ppm Zn²⁺ ion and simultaneous reaction of pyropheophytin *a* in puree containing 300 ppm Zn²⁺ ion at pH 6.0 and heated at 121 °C. Average coefficient of variation 6.5%.

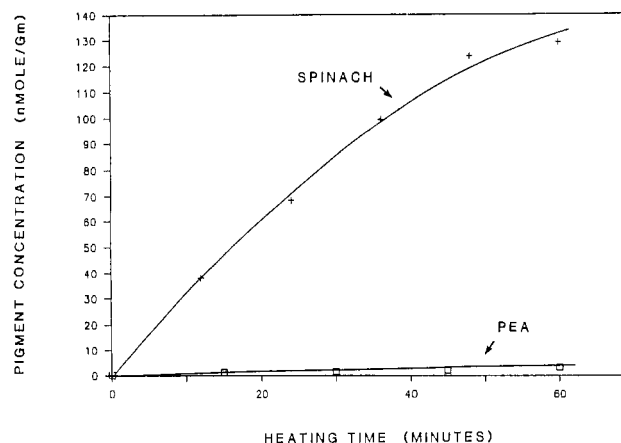


Figure 2. Formation of zinc pyropheophytin *a* in pea puree and spinach puree containing 75 ppm Zn²⁺ ion at pH 6.0 and heated at 121 °C. Average coefficient of variation 7.8%.

Also indicated in Figure 1 is the decrease in pyropheophytin *a* concentration in the samples initially containing 300 ppm Zn²⁺ ion. The loss of pyropheophytin *a* approximately equaled the formation of its zinc complex.

Spinach and pea purees containing 75 ppm Zn²⁺ ion at pH 6.0 and heated at 121 °C were used to demonstrate the effect of pigment concentration on the formation of zinc pyropheophytin *a* (Figure 2). Unheated spinach puree contained 479 \pm 2 nmol of pyropheophytin *a* while pea puree contained 44 \pm 1 nmol/g of puree or nearly 12 times the pyropheophytin *a* concentration. After 60 min of heating, the spinach puree samples contained 129 \pm 4 nmol/g of zinc pyropheophytin *a*, compared to 3.0 \pm 0.2 nmol/g in pea puree or an approximately 43 times greater concentration of zinc complex. These results clearly demonstrate that the formation of zinc complexes of chlorophyll derivatives is pigment concentration dependent. Furthermore, the difference in the pigment ratios for the two vegetable purees suggests that the complex reaction in peas is affected by other pea puree constituents capable of binding zinc, such as protein and phytic acid.

Interference with zinc complex formation in pea puree is also illustrated in Figure 1. Successive halving of zinc concentration from 300 ppm Zn²⁺ should result in similar reductions in zinc pyropheophytin *a* concentration.

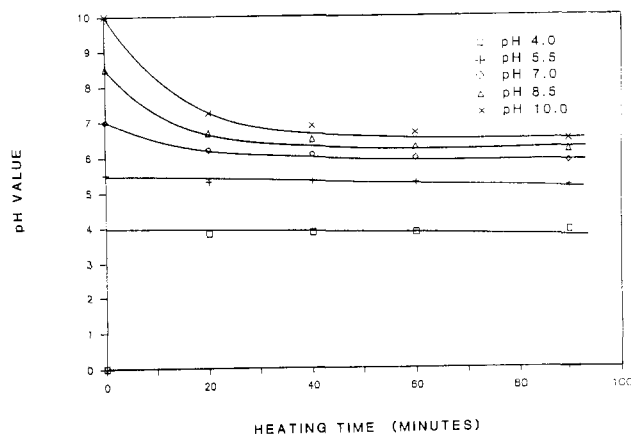


Figure 3. Formation of zinc pyropheophytin *a* in spinach puree containing 75 ppm Zn^{2+} ion at pH 4.0, 5.5, 7.0, 8.5, and 10.0 and heated at 121 °C. Average coefficient of variation 3.9%.

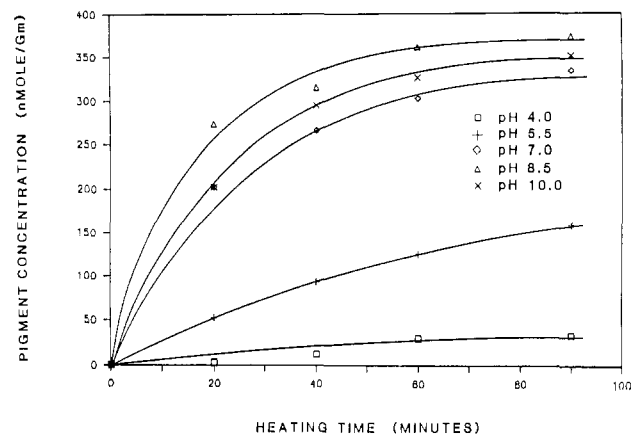


Figure 4. Increase in the color of spinach puree containing 75 ppm Zn^{2+} ion at pH 4.0, 5.5, 7.0, 8.5, and 10.0 and heated at 121 °C.

However, greater than 50% reductions in zinc complex formation were observed after 60 min of heating, further suggesting an interaction between pea puree constituents and zinc ions.

The effect of pH on the formation of zinc pyropheophytin *a* in spinach puree containing 75 ppm Zn^{2+} ion at pH 4.0, 5.5, 7.0, 8.5, and 10.0 and heated at 121 °C is shown in Figure 3. The amount of zinc complex formed increased 11-fold as the pH was increased from pH 4.0 to 8.5 but decreased at pH 10.0. After 90 min of heating, zinc pyropheophytin *a* concentrations at pH 4.0, 8.5, and 10.0 were 33.1 ± 1.2 , 372 ± 5 , and 350 ± 1.7 nmol/g, respectively. These results show that the formation of zinc complexes is increased up to a maximum pH value. Decreases in the reaction rate at higher pH values may be attributable to the lower solubility of zinc chloride at higher pH values and the effective removal of Zn^{2+} ions from the reaction medium.

Figure 4 shows the increase in green color values, as measured by the increase in Hunter *-a* values, in spinach puree samples containing 75 ppm Zn^{2+} ion and heated at 121 °C. The increase in *-a* values followed the formation of zinc pyropheophytin *a*. The values increased as the pH increased from 4.0 to 8.5 and decreased when the pH was raised to 10.0. At pH values of 4.0, 8.5, and 10.0, the *-a* value changed from the initial value of 0.5 (canned spinach color) to 1.5, 5.2, and 3.5, respectively. The colors of all samples containing Zn^{2+} ion and heated for 90 min at 121 °C were definitely considered more

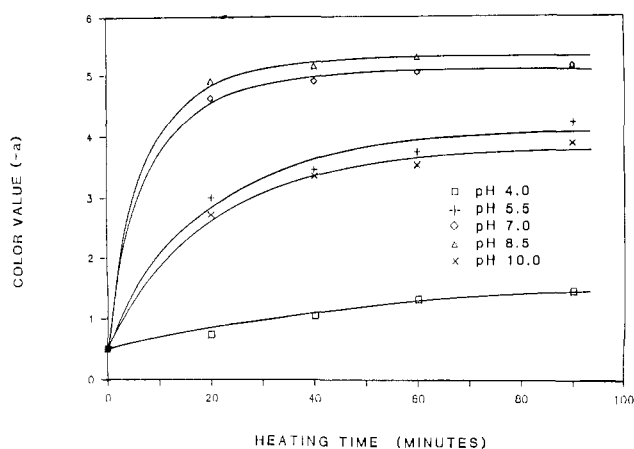


Figure 5. Change in the pH value of spinach puree containing 75 ppm Zn^{2+} ion at pH 4.0, 5.5, 7.0, 8.5, and 10.0 and heated at 121 °C.

green than controls containing no Zn^{2+} ion. It should be pointed out that the *-a* value of fresh ground spinach leaves blanched for 2 min in boiling water was 11.2.

The results of the effect of pH on the formation of zinc pyropheophytin *a* and the development of green color value show that the reaction rate decreased after the first 20 min of heating. Since the reaction was shown to be pH-dependent, the decrease in rate suggests that the rate of reaction changes as the pH value of the puree changes during processing. Figure 5 shows the change in the pH value of spinach puree containing 75 ppm Zn^{2+} ion at initial pH of 4.0, 5.5, 7.0, 8.5, and 10.0 and heated at 121 °C. Although those samples with the highest initial pH values retained the highest pH values after heat processing, the samples with initial pH of 7.0, 8.5, or 10.0 decreased during heating, with the most change occurring in the first 20 min. Samples with an initial pH of 4.0 or 5.5 retained their pH throughout the heating periods. Similar decreases in the pH of pH-elevated vegetable tissues during processing have been observed (Blair and Ayres, 1943; Clydesdale and Francis, 1968). The change in pH of the puree samples suggests that formation of zinc pyropheophytin *a*, and therefore green color, in the pH-elevated samples is slowed by the drop in pH that occurs within the first 20 min of heating.

Formation of zinc complexes during heat processing will, therefore, be most rapid in those vegetables containing the greatest amount of chlorophyll and processed with greater concentrations of zinc salts. Given the FDA limit of 75 ppm Zn^{2+} ion in canned vegetables, the success of this process may be a function of the species and quality of the fresh vegetable. The high-pH conditions that have been shown in this study to promote zinc complex formation are the same that decrease the formation of pheophytin and pyropheophytin from chlorophyll during processing. Therefore, optimal regreening would be achieved by processing and storage in buffered process solutions at pH values that allow both reactions to occur at a maximum rate and with minimal zinc salt precipitation. The heat treatment chosen for these experiments in the case of spinach, which heats by conduction, is within the range of commercial thermal severity of spinach products. However for peas, which heat mainly by convection, the heating times are most likely in excess. Therefore, optimization of the factors favoring metallo complex formation in peas becomes especially critical.

ACKNOWLEDGMENT

This research was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin—

Madison, and Continental Food Packaging, a Division of Continental Can Co., Inc.

LITERATURE CITED

- Berezin, B. D.; Koifman, O. I. Effect of substituents on the reactivity of pheophytin in reaction with metal salts in ethanol. *Russ. J. Phys. Chem.* **1970**, *44*, 677-679.
- Blair, J. S.; Ayres, T. B. Protection of natural green pigment in the canning of peas. *Ind. Eng. Chem.* **1943**, *35*, 85-95.
- Clydesdale, F. M.; Francis, F. J. Chlorophyll changes in thermally processed spinach as influenced by enzyme conversion and pH adjustment. *Food Technol.* **1968**, *22*, 793-796.
- Clydesdale, F. M.; Fleischmann, D. L.; Francis, F. J. Maintenance of color in processed green vegetables. *Food Prod. Dev.* **1970**, *4*, 127-138.
- Clydesdale, F. M.; Lin, Y. D.; Francis, F. J. Formation of 2-pyrrolidone-S-carboxylic acid from glutamine during processing and storage of spinach puree. *J. Food Sci.* **1972**, *37*, 45-47.
- Decleire, M. Etude du reverdissement obtenu lors de la sterilisation de haricots prealablement conserves en saumure. *Rev. Ferment. Ind. Aliment.* **1966**, *21*, 95-98.
- Fischbach, H. Microdeterminations for organically combined metal in pigment of okra. *J. Assoc. Off. Agric. Chem.* **1943**, *26*, 139-143.
- Fischbach, H.; Newburger, S. H. Spectrophotometric study of the green color in okra. *J. Assoc. Off. Agric. Chem.* **1943**, *26*, 134-139.
- Food Chem. News.* Temporary permits granted for canned green beans with zinc chloride. *Food Chem. News* **1984**, *26* (29), 32.
- Food Chem. News.* Canned green beans standard amendment submitted. *Food Chem. News* **1988**, *30* (25), 46.
- Gold, H. J.; Weckel, K. G. Degradation of chlorophyll to pheophytin during sterilization of canned green peas by heat. *Food Technol.* **1959**, *13*, 281-286.
- Gupte, S. M.; Francis, F. J. Effect of pH adjustment and high-temperature short-time processing on color and pigment retention in spinach puree. *Food Technol.* **1964**, *18*, 1645-1658.
- Haisman, D. R.; Clarke, M. W. The interfacial factor in the heat induced conversion of chlorophyll to pheophytin in green leaves. *J. Sci. Food Agric.* **1975**, *26*, 1111-1126.
- Humphrey, A. M. Chlorophyll. *Food Chem.* **1980**, *5*, 57-67.
- Jones, I. D.; White, R. C.; Gibbs, E.; Denard, C. D. Absorption spectra of copper and zinc complexes of pheophytins and pheophorbides. *J. Agric. Food Chem.* **1968**, *16*, 80-83.
- Jones, I. D.; White, R. C.; Gibbs, E.; Butler, L. S.; Nelson, L. A. Experimental formation of zinc and copper complexes of chlorophyll derivatives in vegetable tissue by thermal processing. *J. Agric. Food Chem.* **1977**, *25*, 149-153.
- Loef, H. W.; Thung, S. B. Ueber den Einfluss von Chlorophyllase auf die Farbe von Spinat waehrend und nach der Verwertung. *Z. Lebensm. Untersuch. Forsch.* **1965**, *126*, 401-406.
- Pennington, F. C.; Strain, H. H.; Svec, W. A.; Katz, J. J. Preparation and properties of pyrochlorophyll a, methyl pyrochlorophyll a, pyropheophytin a, and methyl pyropheophorbide a derived from chlorophyll by decarbomethoxylation. *J. Am. Chem. Soc.* **1964**, *80*, 1418-1426.
- Schanderl, S. H.; Marsh, G. L.; Chichester, C. O. Color reversion in processed vegetables. I. Studies on regreened pea puree. *J. Food Sci.* **1965**, *30*, 312-316.
- Schwartz, S. J.; von Elbe, J. H. Kinetics of chlorophyll degradation to pyropheophytin in vegetables. *J. Food Sci.* **1983**, *48*, 1303-1306.
- Schwartz, S. J.; Woo, S. L.; von Elbe, J. H. High performance liquid chromatography of chlorophylls and their derivatives in fresh and processed spinach. *J. Agric. Food Chem.* **1981**, *29*, 533-535.
- Segner, W. P.; Ragusa, T. J.; Nank, W. K.; Hoyle, W. C. Process for the preservation of green color in canned vegetables. U.S. Patent 4,473,591, Dec 15, 1982.
- Swirski, M. A.; Allouf, R.; Guimard, A.; Cheftel, H. A water-soluble, stable green pigment, originating during processing of canned brussels sprouts picked before the first autumn frosts. *J. Agric. Food Chem.* **1969**, *17*, 799-801.
- Tan, C. T.; Francis, F. J. Effect of processing temperature on pigments and color of spinach. *J. Food Sci.* **1962**, *27*, 232-240.
- von Elbe, J. H.; Huang, A. S.; Attoe, E. L.; Nank, W. K. Pigment composition and color of conventional and Veri-Green canned beans. *J. Agric. Food Chem.* **1986**, *34*, 52-54.

Received for review January 30, 1989. Accepted October 24, 1989.

Registry No. Zn, 7440-66-6; zinc pyropheophytin a, 15333-64-9.