# Formation of Zinc-Chlorophyll-Derivative Complexes in Thermally Processed Green Pears (*Pyrus communis* L.)

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**ABSTRACT: The formation of zinc-chlorophyll-derivative complexes was investigated in peels-on green D'Anjou pears when subjected to blanching in zinc ion solution (1300, 2600, and 0 ppm) at 94 °C for 6, 12, or 18 min and then canning at 94 °C for 20 min. The peels removed from the pears were freeze-dried and ground into powders in liquid nitrogen for pigment extraction using ethyl ether. The visual absorption of the extracts was measured using a spectrophotometer along with identification and quantification of chlorophyll derivatives using reverse-phase HPLC method. Furthermore, pears with or without the peels were blanched in 2600 ppm of zinc solution for 12 min following the canning process in 10 °Brix syrup solution. Total antioxidant (TA) and total phenolic content (TPC) of the pear flesh and peels were evaluated using Folin-Ciocalteu's phenol and 1,1-diphenyl-2-picrylhydrazyl assays. Thermal processing destroyed chlorophylls on pear peels, in which pheophytins were found to be the major degraded compounds while an insignificant amount of pyropheophytins was also formed. In zinc blanched peels, Zn pheophytins a was the dominant green compound, and its amount increased about 100% and 144.4% in peels blanched in 1300 ppm zinc solution for 6 and 12 min, respectively. When blanching peels in 2600 ppm zinc solution for 6 and 12 min, the pigment increased about 118% and 242%, respectively. Significant reductions in TA and TPC were found on the peels of zinc treated pears, but the overall TA and TPC of whole fruits were not significantly affected by the treatments.**

**Keywords: antioxidant capacity, chlorophyll derivatives, D'Anjou green pears, thermal processing, zinc**

## Introduction

Chlorophyll a and b, 2 major forms of chlorophylls in plant materials, are partially or totally degraded to yellow-olive colored pheophytins and pyropheophytins during heat processing (Haisman and Clarke 1975; Schwartz and von Elbe 1983). Schwartz and von Elbe (1983) suggested a mechanism for the decomposition of chlorophyll during heat processing of vegetables as: Chlorophyll → Pheophytin → Pyropheophytin. The conversion of chlorophylls to pheophytins initiates at temperature of 60 °C or higher (Haisman and Clarke 1975; Weemaes and others 1999) as a result of increased permeability of hydrogen ions across cell membranes (Haisman and Clarke 1975). Pyrophephytins may be generated from pheophytins (Canjura and Schwartz 1991; von Elbe and Schwartz 1996) and accumulate, especially in severely heated vegetables (Schwartz and von Elbe 1983; von Elbe and Schwartz 1996). A technology for improving color of containerized green vegetables was invented by LaBorde and von Elbe (1996), through reacting zinc (Zn) ions with pheophytins and pyropheophytins generated in heated vegetables to form new green Zn complexes, Zn pheophytins and Zn pyropheophytins, for regreening the products (von Elbe and Schwartz 1996).

Recently, a technology for retaining green pigments in thermally processed green pears using zinc to form zinc-chlorophyll complex has been developed and patented by the authors (Ngo and Zhao 2005). Zinc was used as a processing aid in blanching process for retaining the green pigments in finally processed pears (Ngo and Zhao 2005), leading to the development of a new value-added peels-on canned pear product. Previous studies of retaining green pigments in canned peas and spinaches with zinc reported that the pigment retention was attributed to the formation of metallochlorophyll complexes, such as Zn pheophytins or Zn pyropheophytins (Jones and others 1977; von Elbe and others 1986; Canjura and others 1999). However, it is unknown what exact compounds are responsible for the new green pigments on the pear peels, thus the intention of this study.

Identification of chlorophyll and its derivatives is based on their spectral characteristics in the visual light region (von Elbe and Schwartz 1996). Simple determination of chlorophyll a and b in a whole pigment extract of green plant tissues can be efficiently done by spectroscopy or fluorometry when there are few pigments present in the samples (Schoefs 2001). However, the accuracy of the method depends on the type of device used, the ability to determine the absorbance maxima, and the accuracy of the absorption coefficient used for the calculation (Schoefs 2001; Lichtenthaler and Buschmann 2005a). Examination of the processes by which chlorophyll derivatives are formed requires a method that allows their individual determination in the presence of carotenoids (Mangos and Berger 1997). HPLC methods using normal-phase (NP) and reverse-phase (RP) have been developed and concentrated on the analysis of chlorophylls and their degradation products (Canjura and Schwartz 1991; Li and others 2002; Ferruzzi and Schwartz 2005). Similar methodology has also been developed for the analysis of Zn and Cu chlorophyll derivatives (Canjura and others 1999; Ferruzzi and Schwartz 2005; Scotter and others 2005). Compared to RP-HPLC, the NP-HPLC method

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doi: 10.1111/j.1750-3841.2007.00465.x

requires extra steps to eliminate water from the extracts (Canjura and Schwartz 1991), but can successfully separate the a and b forms of metallo-chlorophyll derivatives (Canjura and others 1999), while available RP-HPLC methods result in poor separation of compounds, such as Zn pyropheophytin a and pheophytin b (von Elbe and others 1986) or Zn pyropheophytin a and Zn pheophytin a' (LaBorde and vonElbe 1994). Fortunately, the a forms of chlorophyll occur naturally in higher quantities, have greater reactivity than the b forms (Jones and others 1977), and show very high absorbance at 658 nm detection as well (Canjura and others 1999). Thus, most studies on thermally processed green vegetables only have chlorophyll a derivatives been followed in the chromatography spectrum (LaBorde and vonElbe 1994; Canjura and others 1999).

Pigment identification and quantification require the lipids and lipoproteins of the chloroplast membrane to be solubilized in a solvent matrix (Hagerthey and others 2006). Current protocols for extracting green pigments from plant materials are to grind samples at room temperature (Canjura and others 1999; Lichtenthaler and Buschmann 2005b), in which heat generated during grinding may degrade the pigment and stimulate chlorophyllase activity (Hagerthey and others 2006). Rodriguez-Saona and Wrolstad (2005) described the use of liquid nitrogen to minimize the degradation of anthocyanins by lowering the temperature and providing a nitrogen environment. This technique has thus a potential application for chlorophyll extraction. The fine powders can maximize pigment recoveries as a result of their high surface area (Rodriguez-Saona and Wrolstad 2005). In a comprehensive study on the extraction methods of alga pigments, Hagerthey and others (2006) reported that freeze-drying samples prior to extraction increases the extraction of chlorophyls and its derivatives. Freeze-dried materials can be directly extracted with nonpolar solvents, such as diethyl ether (Lichtenthaler and Buschmann 2005b; Hagerthey and others 2006).

Since pear peels contain phenolics many times more than flesh (Amiot and others 1995; Galvis Sanchez and others 2003), the peels are generally considered as a positive attribute to enhance the nutritional value of peels-on processed pears compared to peeled ones (Ngo and Zhao 2005). However, thermal processing disrupts fruit cells and may change phenolic composition of the fruits (Renard 2005), hence a need to evaluate the total phenolics and antioxidant capacity of thermally processed fruits in a peels-on fruit model. The objectives of this study were to investigate the degradation of chlorophyll and formation of Zn-chlorophyll derivatives in thermally processed green pear peels while using zinc as a processing aid in blanching solutions for helping retain green pigment and to evaluate the total antioxidant activity and total phenolics of processed pears in a peels-on model.

## Materials and Methods

### Materials

D'Anjou green pears (*Pyrus communis* L.) and fresh spinach were purchased from a local grocery store in Corvallis, Oreg., U.S.A. Cane sugar was obtained from C&H Sugar Company (Crockett, Calif., U.S.A.). Zinc chloride and anhydrous sodium sulfate were obtained from EM Science (EM Industries Inc., N.J., U.S.A.) and zinc lactate from Purac America Inc., (Lincolnshire, Ill., U.S.A.). Acetone and ascorbic acid were obtained from J.T. Baker (Malinckrodt Baker Inc., Phillipsburg, N.J., U.S.A.), diethyl ether, ethyl acetate, and methanol from EMD Chemicals Inc. (Gibbstown, N.J., U.S.A.). Chlorophyll a standard, Folin-Ciocalteu's phenol reagent (FC), gallic acid, and sodium carbonate were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from TCI America (Portland, Oreg., U.S.A.).

### Preparation of pears

Pears were de-waxed using a sugar spraying system as described by Ngo and Zhao (2005) to remove the wax layer on the top of the pear peels. De-waxed fruits were immediately soaked in a 1% iced ascorbic acid solution for less than 10 min. The fruits were then drained and blotted with paper towel. The green areas of the pears peels were manually peeled using a dual peeler, immediately frozen in liquid nitrogen, and stored in the sealed 3-L glass jars at −22 °C overnight. For creating a standard mélange of the peels, peels from approximately 140 pears were manually blended and mixed on a tray under cold condition of −22 °C.

### Thermal process of pear peels in zinc solutions

Thirty grams of frozen peels were hot filled in 1-L straight-side glass jar (Richard Packaging Inc., Portland, Oreg., U.S.A.) filled with near-boiling solution containing 0, 1300, or 2600 ppm of zinc ions prepared from zinc lactate. For providing the chlorophylls in the peels a reaction condition close to what actually occurs on pears during canning process, approximately 330 g of de-waxed peels-on whole pears were added into the jar along with this 30 g of peels. The concentration of the zinc was chosen based on our previous findings that using 2600 ppm or less of zinc in blanching solution resulted in greenish products while observing the FDA temporary regulation of 75 ppm of zinc in final product (Ngo and Zhao 2005). The jars were sealed and immersed in 94 °C water in a 60-L steam kettle for a designed time of 6, 12, or 18 min. After blanching, pear peels and fruits were drained and washed off with boiling water, then immediately hot filled in glass jars with boiling water and thermally treated at 94 °C for 20 min. The thermally processed peels were collected, immediately frozen by liquid nitrogen, and stored at −22 °C for less than 4 h before freeze-drying under a 100-mmHg vacuum pressure for 48 h using a Consol 4.5 (The Virtis Co. Inc., Gardiner, N.Y., U.S.A.).

### Extraction of green pigments from pear peels

Freeze-dried peels were ground into powders in liquid nitrogen using a blender (Waring Products, Model 34B97, Dynamics Corporation of America, New Hartford, Conn., U.S.A.). About 1.5 g of powder were mixed with 15 mL of 100% ethyl ether. The mixture was sonicated, centrifuged, and the supernatant was collected. Three additional extractions of the residue were done with ethyl ether. Supernatants were combined and concentrated in a glass container under a flow of nitrogen gas before being transferred to a 10-mL volumetric flask. Ether was added into the flask to reach final volume of 10 mL and the extracts were stored at −22 °C until analysis. Approximately 2 g of the peel powder of each sample were dried until reaching constant weight in a vacuum dryer (Model 58401, Natl. Appliance Co., Portland, Oreg., U.S.A.) at 70 °C to determine the dry weight of the material.

### Visual spectroscopic properties of peels extracts

The visual spectroscopic properties of the ether extracts of fresh and thermal processed pear peels were recorded using a Shimazu UV160U spectrometer (Shimazu Corp., Kyoto, Japan) with 1-cm disposable cells at 5-nm intervals.

### Analysis and identification of pigments

Chlorophyll derivatives were separated by HPLC using an isocratic mobile phase of ethyl acetate/methanol/water (40:54:10

v/v/v). One milliliter of ether peel extract was evaporated to dry followed with addition of 0.2 mL of acetone. Duplicate 20-$\mu$L injections of extract in acetone were made onto an Altex Ultrasphere™ reverse phase column (ODS, 5 $\mu$, 4.6 × 150 mm) (Altex-Beckman, Berkeley, Calif., U.S.A.). All pigments were monitored at 656 and 436 nm on a Shimazu dual-wavelength detector. The HPLC apparatus consisted of a injection valve of 20-$\mu$L sample loop (Model 7725i, Rheodyne LLC, Rohnert Park, Calif., U.S.A.), 2 pumps (LC-10AS) controlled by a SCL-10A controller, and a SPA-20A UV/Vis Detector (Shimadzu Scientific Instruments Inc., Columbia, Md., U.S.A.). A Dell desktop computer (Dell Inc., Round Rock, Tex., U.S.A.) equipped with EZStart, version 7.2.1 SP1 (Shimadzu Scientific Instruments Inc.) was used to collect and integrate data.

Chlorophyll derivatives in the samples were identified via chromatographic retention time and spectroscopic properties by external standard procedures using purified chlorophyll a from a commercial source and unpurified pheophytins, pyropheophytins, and their zinc complexes prepared from spinach leaves. Spinach leaves are a rich source of chlorophylls and the detection method of spinach chlorophyll derivatives using reversed phase HPLC was well established (van Breeman and others 1991; LaBorde and von Elbe 1994). A photodiode array detector coupled with an Agilent HPLC (Agilent Technologies Inc., Palo Alto, Calif., U.S.A.) in conjunction with chromatography ChemStation software was used to determine the visual spectra of the resolved compounds from 20 L of prepared spinach standards or peel samples. The chromatographic method described earlier using isocratic mobile phase of ethyl acetate/methanol/water (40:54:10 v/v/v) and the Altex Ultrasphere column was used.

### Preparation of chlorophylls, chlorophyll derivatives, and their zinc complexes from spinach leaves

Pheophytins and pyropheophytins were prepared from liquid nitrogen ground spinach leaves using modified methods by Tonucci and von Elbe (1992). Chlorophylls were extracted from 50 g of frozen powders of spinach leaves with 80 mL of 100% acetone. The extracts were filtered through Whatman No. 1 papers into a 200-mL separatory funnel with the addition of 60 mL of 100% ethyl ether and 30 mL of MilliQ water. The ether layer was then separated and collected. Ten milliliters of acetone containing 15 $\mu$L of hydrochloric acid were added into 30 mL of ether extract. The pheophytins were formed within 2 min and the acid was removed by washing 3 times the mixture with water.

To prepare pyropheophytins, 50 g of frozen powders of spinach leaves were filled in 125 mL of near-boiling water contained in a 125 mL glass jar. The jar was loosely sealed, heated at 98 °C in a Precision water bath (Jouan Inc., Winchester, Va., U.S.A.) to inactivate chlorophyllases before thermally processed at 121 °C for 1 h in a Tomy Digital Autoclaves (Model SS-325, Peninsula Laboratories Inc., Belmont, Calif., U.S.A.). The powders in water were then filtered, drained of water, extracted with 80 mL of acetone, filtered again, and transferred into ether as done with chlorophylls.

Zinc-chlorophyll derivative complexes were prepared using a method described by Ferruzzi and Schwartz (2005). Fifteen milliliters of pheophytins or pyropheophytins in ether were dried under a stream of nitrogen gas. Twenty milliliters of acetone containing 3 g of zinc chloride were then added. After a reaction time of 30 and 5 min for pheophytin and pyropheophytin, respectively, the zinc complexes were re-extracted with 30 mL of ether and washed 3 times with MilliQ water.

The excess water dispersed in the ether extracts was dried off by anhydrous sodium sulfate before storage at −22 °C until analysis.

### Measurement of total phenolics and antioxidant capacity

One of the goals in this study was to evaluate the effect of thermal processing on the amount of health benefit compounds represented by antioxidant capacity and total phenolics, as well as these values on the peels of the pears. To process peels-on and peeled pears, whole pears were de-waxed or peeled and stored in 1% iced ascorbic acid solution for less than 2 min before use. Two peeled or de-waxed peels-on pears were hot filled in water inside glass jar and heated at 94 °C for 20 min. Two de-waxed peels-on whole pears were blanched in 2600 ppm zinc solution for 12 min following with canning process in 10 °Brix sugar solution at 94 °C for 20 min. The 10 °Brix syrup has been commonly used in canning Northwest pears as this level of sugar is similar to the sugar content in pears, thus avoiding osmotic dehydration of the fruit during process and storage. The jars of processed pears were immediately cooled to room temperature under the running tap water before set aside for 24 h before further analysis.

Processed pears and de-waxed fresh pears were peeled and sliced from the outer to the core into 1-cm thickness of slices. Peels and slices of pear flesh were frozen in liquid nitrogen. The flesh was powdered in liquid nitrogen using a blender, while the peels were freeze-dried before powdered. Approximately 5 g of flesh powders or about 1.4 g of dried peels were extracted 3 times for phenolic content using a method described by Rodriguez-Saona and Wrolstad (2005). About 30 g of flesh powders and about 8 g of dried peel powders were subjected to vacuum drying at 70 °C for determination of their moisture content. The total phenolic content and antioxidant activity were determined using FC and DPPH assay, respectively, as described in the previous study by Ngo and others (2007). The experiment was replicated 4 times. The peels from 2 different replicates were mixed together to make a larger quantity sufficient to powdering.

### Experimental design

A completely randomized design (CRD) with 2 factors (zinc concentration of 0, 1300, and 2600 ppm and blanching time of 6, 12, and 18 min) was duplicated. A CRD design with 1 factor (processed pears with peels-on and without peels) was applied to evaluate total phenolics and antioxidant activity. The general linear model (GLM) procedure performed by SAS version 10 (SAS Institute Inc., Cary, N.C., U.S.A.) was applied in testing differences among the different treatments. Least significant difference (LSD) multiple range test was used for the multiple mean comparisons .

## Results and Discussion

### Spectral properties of processed pear peels

Figure 1 and Table 1 represent 3 typical visual spectral properties of ether extracts from fresh peels, peels blanched, and canned without the use of zinc, and peels blanched with 2600 ppm zinc ions and then canned. Both blanching (18 min) and canning (20 min) processes were performed at 94 °C. Figure 1 shows that 3 peel extracts absorb mostly in the lower (blue) region and higher (red) region of visible light, which is typical for extracts containing chlorophylls and their derivative forms (von Elbe and Schwartz 1996). The broad and high absorption of the extracts in the blue region would partly be due to the presence of carotenoids in the peels that are known

to absorb visual light in the range from 430 to 480 nm (von Elbe and Schwartz 1996). There was a shift in the wavelength of maximum absorption among the peel extracts, suggesting color differences between fresh peels (green), peels treated with zinc (green) and those without zinc treatment (brown yellow). Table 1 shows a coincidence between the absorption maxima (abs max) of the 3 peel extracts and those of chlorophyll a derivatives reported in the literature. Absorption maxima of fresh peels were found at 660.5 and 430 nm, respectively, which corresponds fairly well with those of chlorophyll a at 660.5 and 428.5 nm. Similarly, peels thermally processed in water without the use of zinc had absorption maxima at 666 nm and 410 nm, respectively, very close to those of pheophytin a, at 667 and 409 nm. For peels blanched in 2600 ppm zinc solution, their extracts showed absorption maxima at 655.5 and 422 nm, respectively, corresponding well with those of 655 and 423 nm reported for Zn pheophytin a in literature (von Elbe and Schwartz 1996). These findings imply that the green color of fresh pear peels was dominated by chlorophyll a, while zinc treated green pear peels hold a greenness possibly attributed by Zn pheophytin a.

## Chlorophylls, chlorophyll derivatives, and their Zn complexes in fresh and processed pear peels

The analysis of the pear peel extracts revealed that, in the order of retention time on the column, chlorophyll a was present in the fresh peels (Figure 2A), zinc-pheophytin a and pheophytin a (Figure 2B and 2C) were found in the zinc treated samples.

Figure 3 shows the 3 typical reverse-phase HPLC chromatograms of the peel extracts. The elution order of all the presented compounds/peaks coincided well with what reported in previous studies for green bean and spinach extracts (von Elbe and others 1986; LaBorde and von Elbe 1994). Figure 3A corresponds to the fresh peel extracts, in which chlorophyll a (peak 2) and b (peak 1) are present. Pheophytin a (peak 5 in Figure 3A) appeared possibly as a result of freeze drying of peels before extraction. This is based on the observation that multiple extractions with acetone or ether of fresh peels that were not freeze dried before extraction yielded extracts contained only chlorophyll peaks (data not shown). As chlorophyll b is generally much less abundant than chlorophyll a, the spectral

**Table 1 — Spectral properties of ether extracts of fresh and processed pear peel pigments compared with chlorophyll a and its derivatives**

| Sample/compound | Absorption maxima (nm) "Red" region | Absorption maxima (nm) "Blue" region | Visual color |
|---|---|---|---|
| Fresh pear peels | 660.5 | 430 | Green |
| Pear peels canned (94 °C) without the use of zinc ions | 666 | 410 | Brown yellow |
| Pear peels blanched with 2600 ppm zinc ions at 94 °C and then canned at same temperature | 655.5 | 422 | Green |
| [a]Chlorophyll a | 660.5 | 428.5 | Green |
| [a]Pheophytin a | 667 | 409 | Olive yellow |
| [a]Zn pheophytin a | 655 | 423 | Bright green |

[a]Modified from von Elbe and Schwartz 1996.

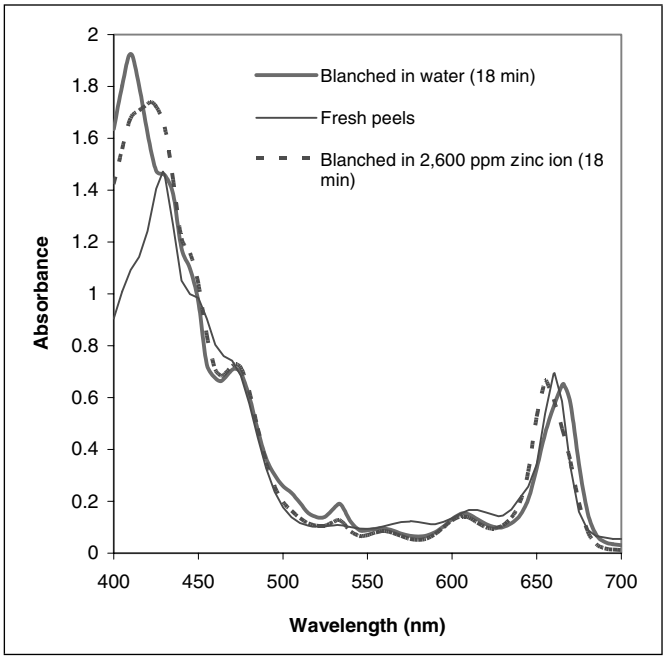


**Figure 1 — Absorbance spectra of ether extracts of fresh pear peels and canned pear peels with or without zinc blanching pretreatment (blanching in 2600 ppm of zinc ion solution at 94 °C for 18 min and canning at same temperature for 20 min)**

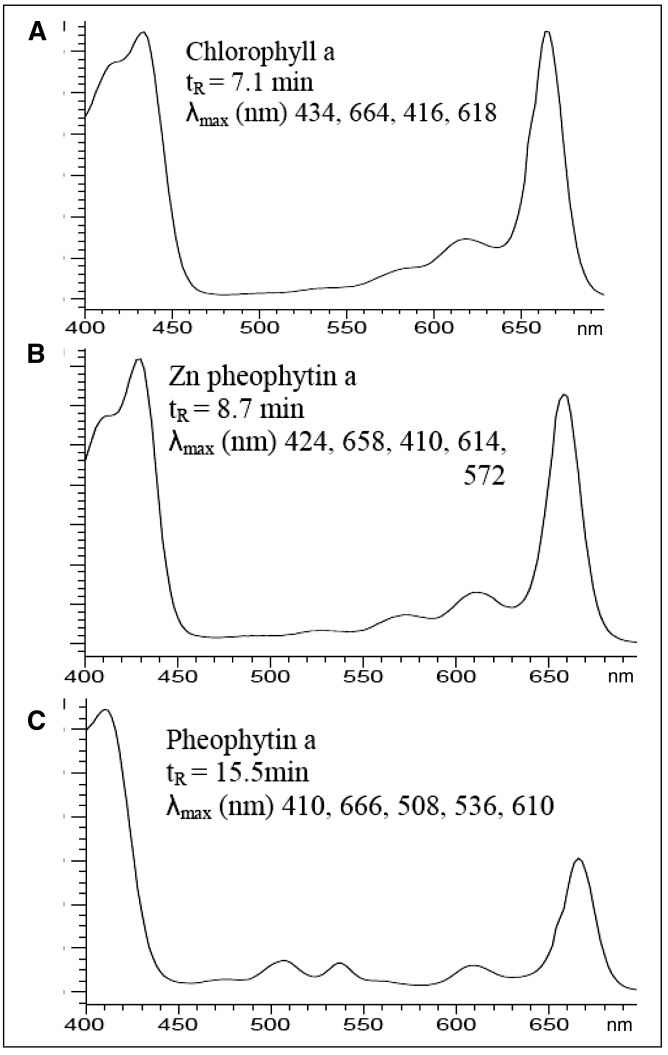


**Figure 2 — Visual spectra of major pigments in fresh and zinc-treated pear peel extracts eluted from an Altex Ultrasphere reverse phase column (ODS, 5 $\mu$, 4.6 × 150 mm) and detected by a diode array detector couple with a HPLC system using an isocratic mobile phase of ethyl acetate/methanol/water (40:54:10 v/v/v)**

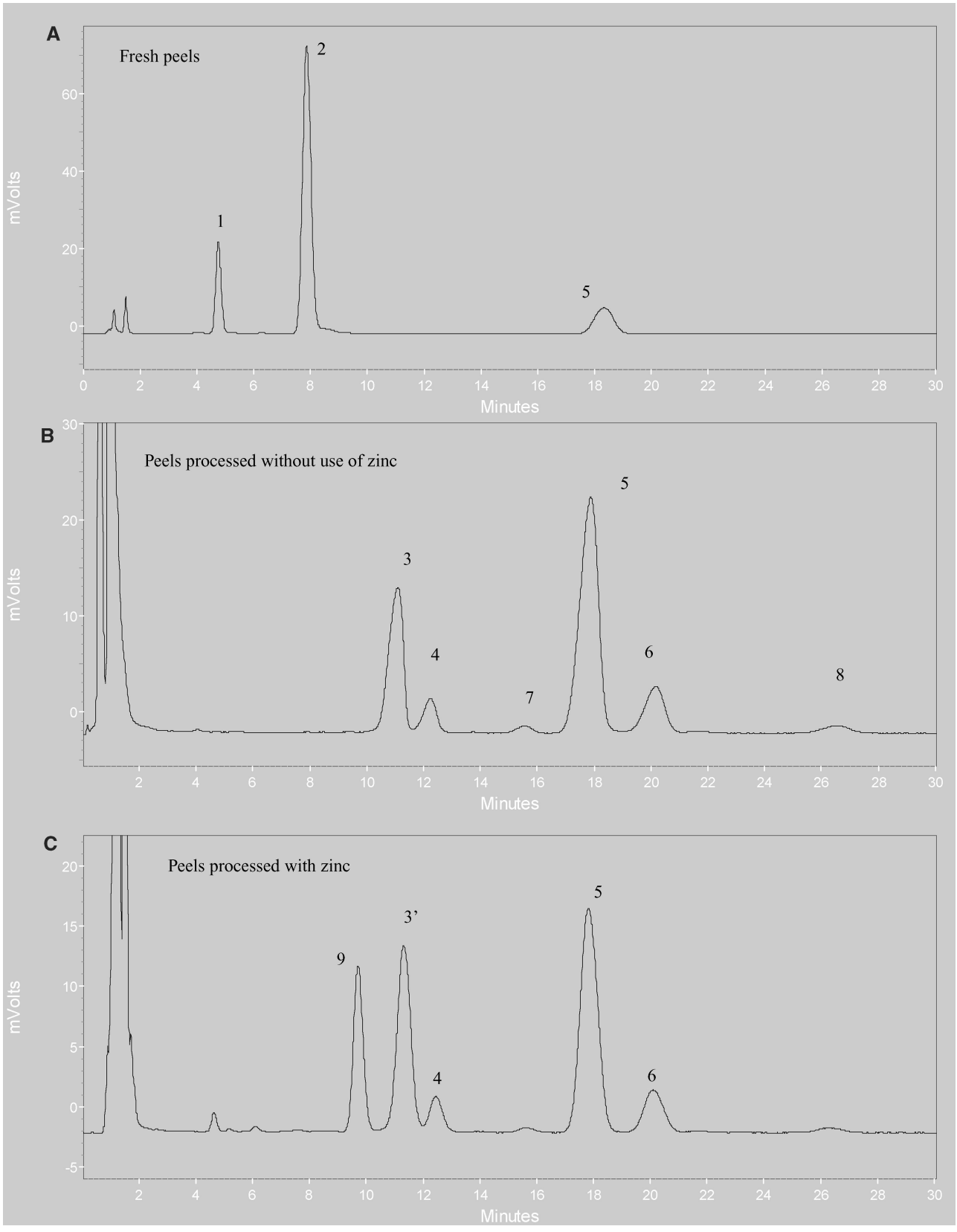


**Figure 3—Typical reversed-phase HPLC chromatograms detected at 656 nm on pear peels. (A) Fresh peels; (B) thermally processed peels (blanched in water at 94 °C for 6 min and canned at same temperature for 20 min); (C) peels processed with zinc blanching (blanched in 2600 ppm of zinc solution for 18 min followed with canning for 20 min at same temperature). Peak identification: 1, chlorophyll b; 2, chlorophyll a; 3 and 4: pheophytins b and b'; 5 and 6: pheophytins a and a'; 7 and 8: pyropheophytin b and a; 9: Zn pheophytin a; 3': Zn pheophytin a'/pheophytin b.**

absorption properties of the fresh peel extracts shared major absorbance maxima of chlorophyll a as described previously. In the chromatogram of thermally processed peel extract as shown in Figure 3B, pheophytin a, a', b, and b' (peak 5, 6, 3, and 4, respectively) and pyropheophytin a and b (peak 7 and 8, respectively) appeared, demonstrating that chlorophylls were all destroyed during the thermal process. Among the degraded products, pheophytins were the dominant ones while the pyropheophytins were negligible since the peak area of the later was of 2% or less of those of pheophytins detected at 656 nm (results not shown). The presence of pheophytin a' (peak 6, Figure 3B) in thermally treated peel extracts implied that a part of chlorophyll a was isomerized and formed into chlorophyll a' (von Elbe and Schwartz 1996), then further degraded into pheophytin a'. Chlorophyll isomerization may result in conversion of 5% to 10% of chlorophyll a and b into a' and b' after 10 min heating at 100 °C (von Elbe and Schwartz 1996). In this study, the peak area of pheophytin a' at 656 nm was approximately 20% of that of pheophytin a (Figure 3B).

Samples processed with zinc treatment showed a new peak of Zn pheophytin a (peak 9) and peak 3', possibly composed of Zn pheophytin a' (Figure 3C). Zn pheophytins were formed from their precursors, pheophytins, and zinc ions (LaBorde and vonElbe 1994). While the extent at which Zn pheophytin a' formed was unknown, its amount was believed to be much smaller than that of the 'a' form as there was more pheophytin a than phephtytin a' in thermally processed pear peels. The insignificant presence of Zn pheophytin a' was also supported by the fact that there was only a slight difference in peak height of the 3 or 3' peak relative to that of peak 4, representing pheophytin b' (Figure 3B and 3C). Thus, Zn pheophytin a would be the major compound contributing to the overall green color of the extract in zinc treated peels, which echoes the earlier findings from the spectral observation of the extracts (Table 1 and Figure 1). If Zn pyropheophytin was present in the extract, it would co-elute at this same peak 3, as shown in heated, zinc treated spinach extracts (data not shown). However, as described earlier, pyropheophytins were present in a negligible amount in heated pear peels and the amount of Zn pyropheophytins resulted from pyropheophytins were thus negligible as well. Zn pheophytins might be converted to Zn pyropheophytins during heating (von Elbe and Schwartz 1996). This process, however, was believed to be as slow as the formation of pyropheophytins from pheophytins and yielded very insignificant amount by considering the low temperature heat process in this study.

Based on the observation on the spectral characteristics and HPLC chromatograms of extracts from fresh peels, peels thermally treated with or without zinc pretreatment, it can be concluded that chlorophylls were completely destroyed by heating at 94 °C for a total time period of 26 to 38 min (blanching plus canning process). This agreed well with the remarks made by LaBorde and von Elbe (1994) that chlorophyll a decreased to trace level in peas after 20 min of heating at 121 °C. In this study, pheophytins were formed in a large amount compared to the trace amount of pyropheophytins when no zinc ion pretreatment was applied. The abundance in pheophytins was in favor of the formation of Zn complexes, Zn pheophytins, when peels entered in contact with zinc ions and the finished product color was dominated by the Zn pheophytin a. A similar finding was reported by Jones and others (1977) that Zn pheophytins is the sole compound responsible for the green color of spinach leaves processed at 100 °C. This is unlike the favored formation of Zn pyropheophytins reported by others (Canjura and others 1999) on vegetables processed at higher temperatures.

One thing worth to point here is about the amount of the chlorophyll reacting with zinc, that is, a relative proportion between the initial chlorophylls and their zinc derivatives. In this study, unpurified chlorophyll standards from spinach leaves were used in the HPLC analysis; hence, it was unable to quantify absolute amounts of chlorophyll derivatives formed, such as pheophytin a and Zn phyophytin a, and then using these numbers to calculate the ratio of chlorophyll degradation into zinc derivatives. This is a disadvantage of using unpurified standards, and future study needs to consider this limit.

## Evolution of major heat-generated pigments in treated pear peels

The kinetic data collected for canned peels previously blanched in different zinc solutions (0, 1300, or 2600 ppm) for 6, 12, or 18 min are presented in Figure 4. Note that the concentrations of Zn pheophytin a in heated peels blanched in 1300 ppm Zn solution for 6 min were set as 100%. For pheophytins a, b, and a', their concentrations in the heated peels blanched in water for 6 min were set at 100%. As pheophytin b, Zn pheophytin a', and Zn pyropheophytin a co-eluted, the calculated amount of pheophytin b in the peels treated with zinc ions would include the amount of Zn pheophytin a' and Zn pyropheophytin a, if present, as well.

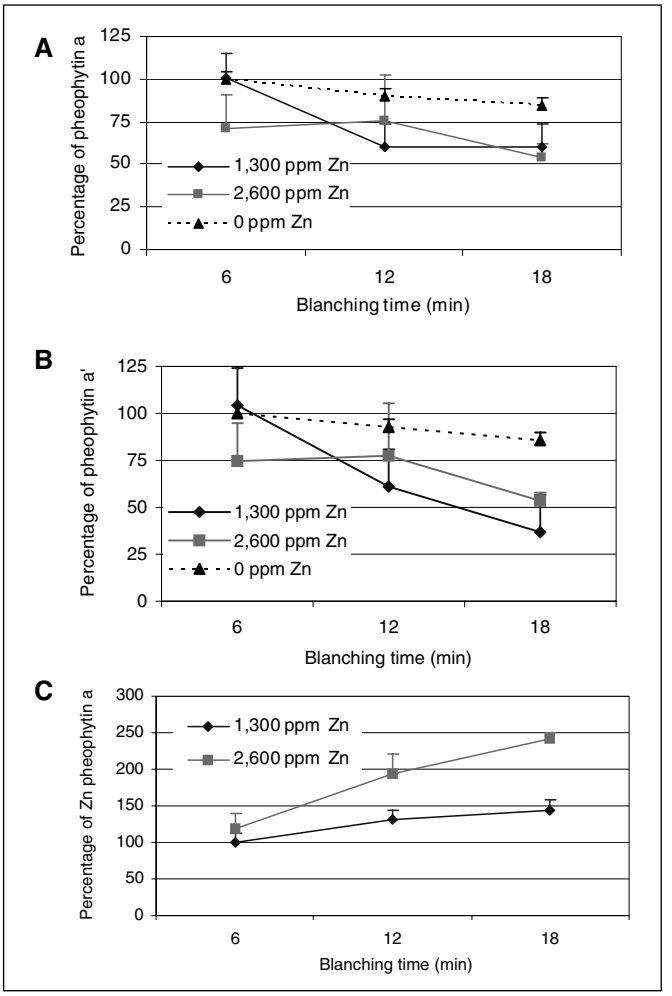


**Figure 4—Evolution of pheophytin a (A) and a' (B) and formation of Zn pheophytin a (C) (relative percentage of 100% as in canned pear peels previously blanched in water for 6 min) in pear peels previously blanched in 0, 1300, or 2600 ppm zinc solution at 94 °C for different blanching times. The pH values of the blanching solutions were about 4.55, 4.80, and 4.93, respectively, and finished product has a pH 4.4.**

Figure 4A and 4B show that the amount of pheophytins tended to decrease as the blanching time increased. Although this decrease was not statistically significant ($P > 0.05$) except for samples treated with low level of zinc ions (1300 ppm), both pheophytins a and a' followed the same trend of reduction (Figure 4A and 4B). Figure 4C shows the formation of Zn pheophytin a as a function of zinc concentration and blanching time. The increase of this green Zn-chlorophyll complex was higher at higher level of zinc ($P < 0.05$). This finding supports our previous observation on processed pear peels at a similar processing condition, where an increase in blanching time and/or zinc concentration favored the green color intensity of the canned pear peels (Ngo and Zhao 2005).

### Effects of peels-on model on the retention of antioxidant activity and total phenolics

Table 2 shows the antioxidant activity and the total phenolic content of thermally processed pears with or without the peels-on and/or zinc blanching pretreatment. Note that pears treated with zinc ions were subjected to a blanching step. Otherwise, pears peeled or with peels-on were processed by the conventional canning procedures, that is, no blanching, but hot filling with 10 °Brix syrup solution.

No significant difference in the total antioxidant activity between fresh and processed whole pears was detected, while the peels of processed pears had significantly lower antioxidant activity than the fresh peels ($P < 0.05$). The total phenolic content of fresh peels was also significantly higher than that of processed peels ($P < 0.05$), but there was no significant difference between fresh and processed whole pears. Note that the total phenolic content of fresh green D'Anjou pears obtained in this study was 4.63 ± 0.28 mg EGA/g (Table 2), about twice higher than the amount of 2.2 ± 0.18 mg EGA/g reported by Wu and others (2004) for green pears using the same method. This difference can be explained by differences in cultivars, environment factors, and so on, as it was unclear which specific green pear cultivars were tested by Wu and others (2004). Galvis Sanchez and others (2003) found that the small phenolic compounds, such as hydroxycinnamics and arbutin contribute more than 80% of the total phenolics in D'Anjou pear peels. These small phenolics could diffuse out of the peels into the surrounding solution, explaining the reduction in the total antioxidants and phenolic content of the peels of processed pears compared to fresh peels. As peels consist of only a small part of the fruits, this finding suggests that the overall antioxidant activity and the total phenolics of pears were not affected by the processing, no matter if there was an extra step of blanching for retaining the green pigments.

**Table 2—Antioxidant activity and total phenolic content of whole pear and pear peels of fresh and canned pears (based on dried weight of samples)[a]**

| Samples | Pears | Antioxidant capacity mg EAA/g[d] | Phenolics mg EGA/g[f] |
|---|---|---|---|
| Whole pears | Fresh pears | 2.1 ± 0.4a | 4.63 ± 0.28a |
| | Canned peeled pears[b] | 2.3 ± 0.4a | 4.98 ± 0.45a |
| | Canned with peels-on[c] | 2.8 ± 0.8a | 5.58 ± 0.46a |
| | Canned with peels-on and treated with Zn[d] | 2.1 ± 0.8a | 4.94 ± 0.77a |
| Peels | Fresh peels | 3.9 ± 0.2b | 12.09 ± 0.85b |
| | Canned with peels-on | 1.7 ± 0.1c | 2.59 ± 0.01c |
| | Canned with peels-on and treated with Zn | 1.0 ± 0.1c | 1.82 ± 0.08c |

[a]Pear peels were de-waxed before analysis. Means ± standard deviations derived from 4 replications for whole pears and 2 replications for peels with 2 pears per replication. Means within the same column followed by the same letter were not significantly different (LSD test, $P < 0.05$).
[b]Pears were peeled.
[c]Pears were de-waxed with peels-on.
[d]Pears were de-waxed and blanched at 94 °C for 12 min in 2600 ppm of zinc solution.
[e]Antioxidant activity was expressed as milligram of equivalent ascorbic acid per gram of dried sample.
[f]Phenolic content was expressed as milligram equivalent gallic acid per gram of dried sample.

## Conclusions

The zinc ion reactions with chlorophyll lead the formation of zinc chlorophyll complex, Zn pheophytin a and a', compounds contributing to the green color of thermally processed peels-on green pears. Hence, zinc can be used as a processing aid in the blanching step before canning process for this purpose. Zinc ion concentration of 1300 ppm or above and blanching time up to 12 min are necessary for the formation and retention of Zn pheophytin a and a'. In the context of this research, the peels-on pears did not show enhanced antioxidant activity in comparison with fresh and conventional canned pears.

## Acknowledgments

The authors thank Dr. James Kennedy and Mr. Seth D. Cohen for their help with DAD-HPLC analysis. This research was partially funded by the Green & Green Inc., Corvallis, Oreg., U.S.A.

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