

Color Improvement and Metallo-chlorophyll Complexes in Continuous Flow Aseptically Processed Peas

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ABSTRACT

Fresh and frozen green peas were blanched in zinc solution (50 to 500 mg/L) and thermally processed in a particle cell reactor, which simulates a continuous flow aseptic processing system. The thermal process temperatures ranged from 121 to 145 °C at holding times from 0 to 20 min. The degradation of chlorophyll and the resulting formation of Zn-pheophytin *a* and Zn-pyropheophytin *a* were monitored. Quantitative analysis of the metallo-chlorophyll complexes was performed by high-performance liquid chromatography. Complex formation increased during heat processing and was dependent on the zinc concentration absorbed within the peas during blanching. At 130 to 145 °C, the formation of Zn-pyropheophytin *a* increased and processing between 121 to 125 °C promoted the formation of Zn-pheophytin *a*. Improvements in color relative to control samples suggested that the process might be applicable to two-phase continuous aseptic processing of vegetables.

Key Words: chlorophyll, metallo-chlorophyll complexes, zinc, aseptic processing, peas

INTRODUCTION

DURING HEAT PROCESSING OF VEGETABLES, chlorophylls *a* and *b* are partially or totally degraded to yellow-olive colored pheophytins and pyropheophytins (Buckle and Edwards, 1970; Schwartz and von Elbe, 1983). Efforts to preserve the green chlorophyll have included pH control, enzymatic changes (Clydesdale and Francis, 1968), and high-temperature short-time treatments (HTST) (Tan and Francis, 1962; Malecki, 1978; Hekal and Erlandson, 1984), but none has been adopted for industrial use.

A regreening has been observed, which involved the formation of metallo-chlorophyll complexes. Metals, such as zinc and copper, have been long known to be introduced into the chlorophyll porphyrin ring. The metal chlorophyll derivatives form a firm bond that is more resistant to acid and heat than naturally-occurring magnesium (Mg^{2+}) chlorophyll complexes (Humphrey, 1980). Well-known historic studies with processed okra and pea puree in glass jars and stored in the dark confirmed such metal complexation reactions.

The metallo-chlorophyll reaction has been characterized to some extent. The for-

mation of copper complexes occurred more readily than zinc complexes (Jones et al., 1977; von Elbe et al., 1986; Minguez-Mosquera et al., 1995). LaBorde and von Elbe (1994) proposed that metal ions reacted only with chlorophyll derivatives and not with native chlorophyll. It has long been known that in a pure solution, a ratio of 1:1 between complexed metal and chlorophyll could be achieved; the complex has also been known to form in pea puree stored at 37 °C (Schanderl et al., 1965a, b). Pheophytin *a*, pyropheophytin *a*, and pheophorbide *a* react with zinc. However, the *b* forms of these chlorophyll derivatives have been reported to be less reactive (von Elbe et al., 1986; LaBorde and von Elbe, 1990). Tonucci and von Elbe (1992) also reported that pheophytin *a* did not react to form the complex as rapidly as did pyropheophytin *a*. LaBorde and von Elbe (1994) found that Zn-pyropheophytin *a* could be formed via 2 reactions, either from the further reaction of Zn-pheophytin *a* to form Zn-pyropheophytin or through zinc complexation with pyropheophytin *a*.

Metallo-chlorophyll complexes have potential use in the food industry to preserve the desirable fresh green color of vegetables. Studies have indicated that a color stabilization process could be implemented in traditional retorting of chlorophyll-containing vegetables. A patent has been issued for the preservation of green color in canned vegetables (Segner et al., 1984) and further investigations using alka-

line conditions and HTST processing have been patented (Hekal and Erlandson, 1984, 1986). Another patent was issued (Leake and Kirk, 1992) for a process that included blanching of vegetables in an aqueous $ZnCl_2$ solution followed by several thermal treatments. Another patent (LaBorde and von Elbe, 1996) led to color improvement of green vegetables through the addition of an aqueous packing solution containing zinc or copper ions. Further studies have indicated that the formation of the complexes depended on the Zn^{2+} concentration (Food and Drug Administration limits Zn ion content to 75 mg/kg in the final product.), pigment concentration, and pH (LaBorde and von Elbe, 1990).

Research efforts have focused on the preservation of chlorophyll in aseptically processed green vegetables, which may be subjected to less severe thermal treatments for sterilization (Schwartz and Lorenzo, 1991). Aseptic processing does not degrade as much chlorophyll as traditional canning, but eventually the increase in acidity during storage slowly degrades remaining chlorophyll. However, studies on the influence of zinc treatments on vegetables and the formation of chlorophyll-metal complexes during aseptic processing have not been reported. The objective of this study was to investigate chlorophyll zinc complex formation in aseptically processed particulates (green peas) and to monitor any color improvements in the treated samples.

MATERIALS & METHODS

Preparation of samples

Fresh and frozen green peas (*Pisum sativum* spp. *I*) were obtained from local markets. The Zn^{2+} ion was introduced into the vegetable tissue by 2 methods. In the first procedure, 200 g of peas were blanched in 500 mL of $ZnCl_2$ (50 to 500 mg/L Zn) solution for 5 min at 83 °C. Samples were rinsed with fresh water, cooled to room temperature (about 23 °C), and thermally processed in a Particle Cell Reactor (PCR, described below). In the second procedure, 200 g of peas were placed into the PCR, and the cell was filled with $ZnCl_2$ (50 to 500 mg/L Zn) solution. Peas were then thermally processed with a continuous fresh supply of heated $ZnCl_2$ solution.

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Extraction of pigments

Peas were blended in a food processor to provide a homogeneous puree. Pea puree (10 g) was extracted 3 times with 20 mL of acetone using a Tissuizer, model TR-10Z (Tekmar Co., Cincinnati, Ohio, U.S.A.). After each extraction step, the sample was centrifuged ($4302 \times g$, 5 min) to allow for sedimentation of starch. The filtrates were combined in a separatory funnel and the aqueous-acetone layer was re-extracted three times with 30 mL of hexane. The upper phase was retained, and excess hexane was evaporated under reduced pressure. The residue was brought to volume with hexane/isopropanol (98.3/1.7 v/v) in a 25-mL volumetric flask.

Analysis of pigments

High-performance liquid chromatography (HPLC) was used with a normal-phase silica column, 4.6 mm i.d. \times 250 mm (MacMod Analytical Inc., Chadds Ford, Pa., U.S.A.) and a gradient solvent system of hexane/isopropanol. The HPLC analysis began with isocratic conditions, using hexane/isopropanol (98.3/1.7 v/v). The isopropanol fraction was increased linearly from 9 to 11 min to yield a mobile phase with equal parts of hexane/isopropanol (98.3/1.7 v/v) and hexane/isopropanol (97.0/3.0 v/v). Conditions were isocratic for 3 min, and then the hexane/isopropanol (97.0/3.0 v/v) fraction was linearly increased to 100 % over the next 5 min. After a 2-min hold time at these conditions, the system was returned to original conditions. The HPLC apparatus consisted of a U6K injector, two model 510

pumps controlled by a 680 gradient controller (Waters Assoc., Milford, Mass., U.S.A.) and a Linear UVIS 200 detector (Linear Instruments Co., Reno, Nev., U.S.A.) set at 658 nm. A Macintosh Plus personal computer (Apple Computer Inc., Cupertino, Calif., U.S.A.), equipped with *Dynamax HPLC Method Manager, version 1.2* (Rainin Instrument Co., Woburn, Mass., U.S.A.) was used to collect and integrate data.

Identification of pigments

Chlorophyll pigments were identified via chromatographic retention and spectroscopic properties. A 990 photodiode array detector equipped with NEC APCIV computer and chromatography software (Waters Assoc., Milford, Mass., U.S.A.) was used to determine the ultraviolet spectra of the compounds as they eluted from the chromatographic column (Canjura and Schwartz, 1991). The identities of Zn-pheophytin *a* and Zn-pyropheophytin *a* were confirmed from their molecular weights using fast atom bombardment mass spectrometry (FAB-MS) (van Breemen et al., 1991).

Thermal treatments

Peas were heat-treated in the PCR, which held the solid pieces of food stationary while temperature controlled fluids were moved at a flow rate of 1.2 L/min through the reactor and around the particles. Water was pumped through the PCR at temperatures of 121 to 145 °C using a Laboratory Scale Thermal Processing System (LSTPS). The LSTPS consisted of a laboratory scale tubular (indirect) ultra-

high temperature (UHT) sterilizer and a HTST pasteurization system (North Carolina State Univ.) (Fig. 1). Time-temperature profiles for peas were obtained, using type T thermocouples connected to a computer-based data acquisition system (Labtech-notebook, Laboratory Technologies Corp., Wilmington, Mass., U.S.A.). Temperatures and times of treatment were: 121 °C (0.0, 5.4, 10.2, 13.7, 20.0 min), 125 °C (0.0, 3.2, 6.3, 9.5, 12.9, 15.4 min), 130 °C (0.0, 1.3, 2.0, 4.2, 6.5, 9.5 min), 135 °C (0.0, 1.2, 3.0, 4.1, 5.2, 9.5 min), 140 °C (0.0, 1.2, 3.4, 7.5 min), and 145 °C (0.0, 1.0, 1.6, 2.0, 2.9, 4.6 min).

Color stability test

After thermal processing of samples in the LSTPS-PCR, they were subjected to a stability test to determine if the zinc-treated peas maintained greenness during accelerated storage conditions. Zinc-treated samples (50 g) were packed in plastic bags and incubated in a water bath at 55 °C for 24 h. Under these conditions, any chlorophylls remaining in the samples would be converted to olive-brown pheophytins. Color changes in comparison to untreated controls were evaluated by reflectance colorimetry.

Colorimetric analysis

Reflectance measurements of pea samples were performed using a Pacific Scientific Color System (Silver Spring, Md., U.S.A.). Hunter L, a, and b values were calculated, using Spectrograd software (Version 2.0). Duplicate measurements for each sample were made. Total color differences (DE) were calculated as $DE = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$.

Zinc analysis

Whole peas were dried and ground to a fine powder. Zinc analyses were performed at the Soil Science Dept. of North Carolina State Univ. by inductively coupled plasma emission spectrophotometry (ICP), using a Perkin Elmer ICP instrument Model Plasma II. The results are reported on a wet weight basis.

RESULTS & DISCUSSION

THE THERMAL HISTORY OF A TYPICAL processed sample (Fig. 2) shows heating, holding, and cooling times representative for a sample processed at 135 °C for 180 s. The come-up time at each temperature was determined and, as expected, increased with increasing temperature.

The amount of zinc absorbed by each sample was dependent on the procedure used to introduce the ion into the pea tissue. A relationship was found (Fig. 3) between the zinc added and the zinc absorbed by the pea. After blanching, a linear increase in zinc concentration in the tissue was observed; while using the continuous procedure, an additive effect was found.

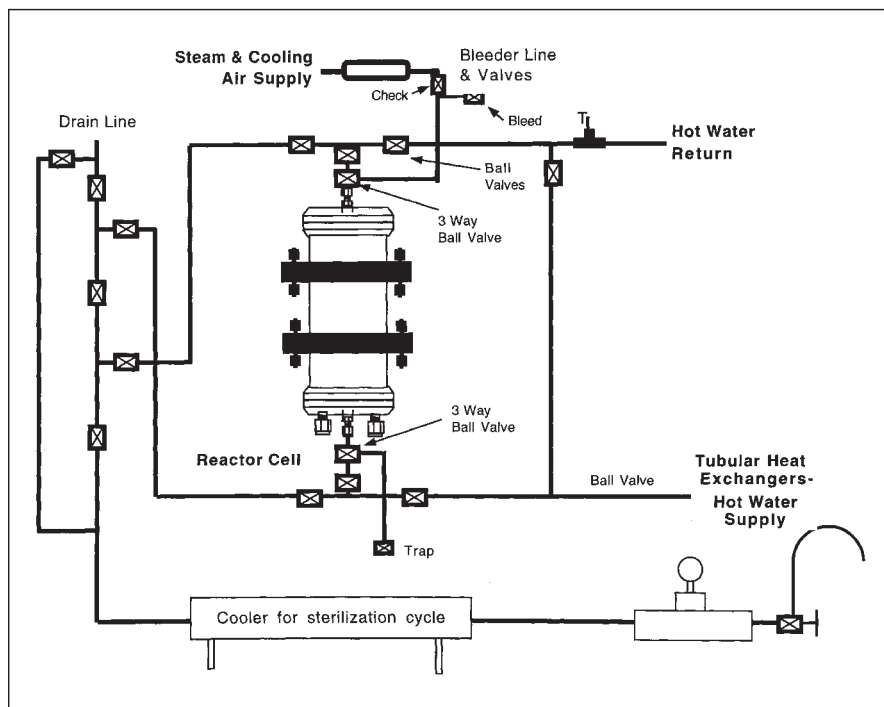


Fig. 1—Schematic of particle cell reactor (PCR) and laboratory scale thermal processing system (LSTPS).

These results were related to the design of the PCR in which the peas remained stationary, and fresh, heated zinc solution was continuously pumped around the peas. This enabled the concentration of the zinc to accumulate over time. Not all of the zinc that was absorbed into the pea tissue was available for reaction with chlorophyll molecules. As suggested by LaBorde and von Elbe (1990), zinc absorbed into the tissue may interact with other constituents, and only a fraction may react with chlorophyll to form Zn-chlorophyll complexes.

Five typical normal-phase HPLC chromatograms were compared (Fig. 4) for frozen and blanched pea samples, which had been thermally processed at 125, 130, or 145 °C. Blanching was performed at 83 °C for 5 min in ZnCl₂ solution with a concentration of 300 mg/L Zn. Normal-phase chromatography was used in order to resolve the zinc derivatives from the native magnesium complexes. The lower chromatogram corresponds to a frozen pea sample extract in which pheophytin *a* (peak 1), chlorophyll *a* (peak 2), and chlorophyll *b* (peak 6) were

present. Generally, chlorophyll *b* occurred naturally in lower quantities relative to chlorophyll *a*, and absorption of the *b* compound, at 658 nm detection, was almost 5 times lesser than the absorption of chlorophyll *a* at that wavelength. Thus, our results focused on the *a* complexes, and the *b* derivatives were not labeled on the chromatogram. Samples processed at 125 °C for 1.3 min showed formation of additional chlorophyll derivatives such as pyropheophytin *a* (peak 3) and Zn-pheophytin *a* (peak 4). Zn-pheophytin *a* was both formed and degraded during the thermal treatments (Fig. 4, 7A), while Zn-pyropheophytin *a* (peak 5) was only formed as heating time and temperature increased.

A reaction scheme of the major chlorophyll compounds degrading and forming during thermal processing of green peas (Fig. 5) indicates the suggested mechanism for the formation of Zn-pheophytin *a* and Zn-pyropheophytin *a* for our process. This mechanism is similar to that presented by LaBorde and von Elbe (1994); however, at aseptic processing temperatures, Zn-pyropheophytin *a* formation would be favored via the Zn-pheophytin *a* derivative. The pro-

posed mechanism for the formation of the various chlorophyll derivatives is supported by kinetic data (Fig. 6, 7), which represent data for 3 of 6 heat treatments. Note that the samples had previously been heated during the come-up period (Fig. 2) to reach the hold temperatures, which were isothermal. Therefore, concentrations of chlorophyll derivatives at the beginning of the holding time were different for each hold temperature and were set as 100%. The relative change in quantities of pheophytin *a* (A) and pyropheophytin *a* (B) was a function of holding times and temperatures (Fig. 6). Likewise, the relative amount of the Zn-complexes was also a function of holding times and temperatures (Fig. 7). Plot A illustrates the formation of Zn-pheophytin *a* and its subsequent conversion to Zn-pyropheophytin *a* at all processing temperatures (121 to 145 °C). Plot B shows the formation of Zn-pyropheophytin *a* as a function of processing temperatures.

The compound appeared to be formed by the contribution of both the conversion of Zn-pheophytin *a* and the reaction of pyropheophytin *a* with Zn²⁺. Kinetic studies by Tonucci and von Elbe (1992) suggested that pheophytin *a* in solution reacted more slowly than purified pyropheophytin *a* at lower (20 to 35 °C) temperatures. However, our data suggest that pyropheophytin *a* did not readily react with Zn to form the complex. Pyropheophytin *a* only formed at 121 °C (Fig. 6B) and increased with heating time. In contrast, Zn-pheophytin *a* reacted relatively rapidly at 121 °C to form Zn-pyropheophytin *a*. Thus, of the two pathways, conversion to Zn-pyropheophytin *a* from the metallo-pheophytin derivative would be the more favored reaction.

In comparison to controls, all Zn-treated tissues showed color improvement. Hunter color values at one temperature (135 °C) as an example were summarized (Table 1). L, a, b, and calculated total color difference (DE) values were compared before and after an accelerated storage stability test. Changes in reflectance values confirmed that as holding time increased, sample greenness increased. After the stability test, all chlorophylls had been degraded, and the green color was due predominantly to Zn-complexes. Those samples that were heat-treated for short holding times (0, 1.2, and 3.0 min) still contained notable quantities of native green chlorophylls and insignificant quantities of the metal complexes. Subjecting those samples to accelerated storage caused the bright green chlorophylls to degrade to olive-colored pheophytins. For the zinc treated samples at all temperatures, a decrease in the DE value over time was observed, indicative of regreening and color improvement.

CONCLUSIONS

ZINC ABSORPTION INTO PEA TISSUE WAS dependent upon the concentration of avail-

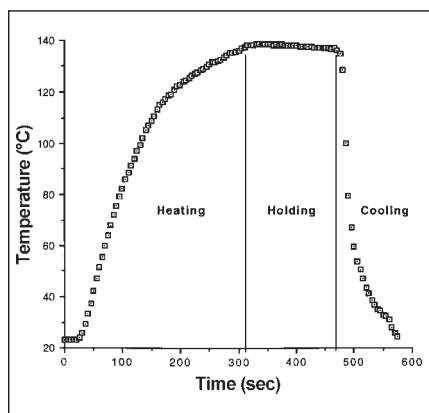


Fig. 2—Time-temperature curve of peas processed at 135 °C and 3 min hold time (thermocouple placed at the particle center).

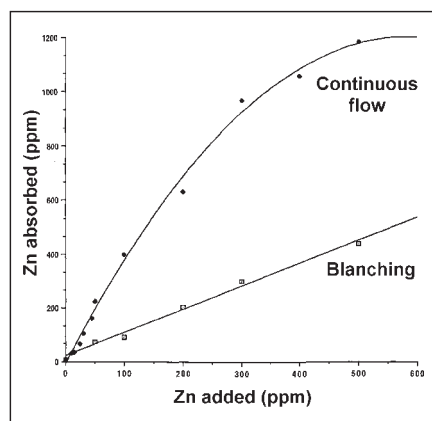


Fig. 3—Zinc ion absorbed by the pea during blanching and continuous processing at 121 °C and 10 min hold time. (Results are reported in mg/kg peas on a wet weight basis).

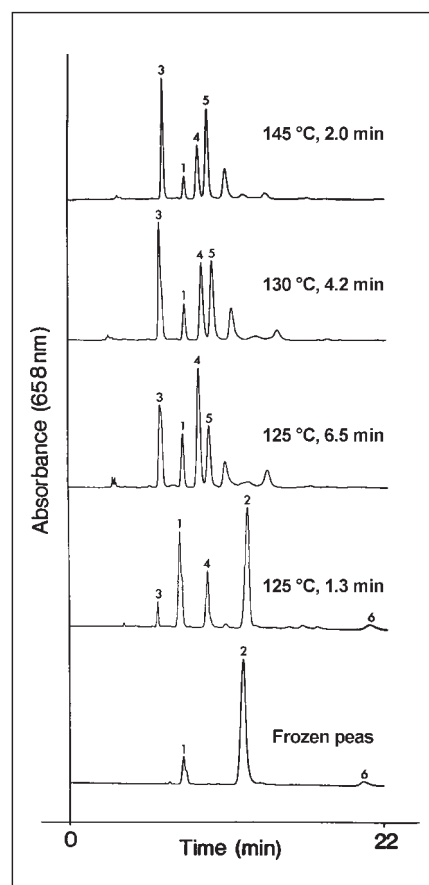


Fig. 4—Typical normal-phase HPLC chromatograms for the separation of Zn-chlorophyll derivatives. 1 = pheophytin *a*, 2 = chlorophyll *a*, 3 = pyropheophytin *a*, 4 = Zn-pheophytin *a*, 5 = Zn-pyropheophytin *a*, 6 = chlorophyll *b*, unlabeled peaks = chlorophyll *b* derivatives.

Table 1—Hunter color L, a, and b values of untreated and zinc treated green peas before (₀) and after (₁) accelerated storage at 55 °C for 24 h

Sample	L ₀	L ₁	a ₀	a ₁	b ₀	b ₁	DE ₀ ^a	DE ₁
Fresh peas	37.94		-8.34		13.30			
Control ^b	38.50	42.00	+0.75	+1.05	12.61	14.50	9.13	10.20
Processed at 135 °C (min)								
0.0 ^c	37.29	41.51	-2.98	-1.86	12.57	14.40	5.47	7.70
1.2	38.35	40.93	-3.39	-2.03	11.53	13.28	5.27	6.98
3.0	38.14	39.97	-3.56	-2.58	12.23	12.79	4.90	6.13
5.2	37.29	38.93	-3.65	-3.13	12.04	12.13	4.89	5.42
9.5	36.58	38.15	-4.27	-3.42	11.30	12.64	4.73	4.96

^aDE: total color difference value.

^bProcessed without Zn-treatment at 135 °C for 5.6 min.

^cProcessed to reach 135 °C and cooled.

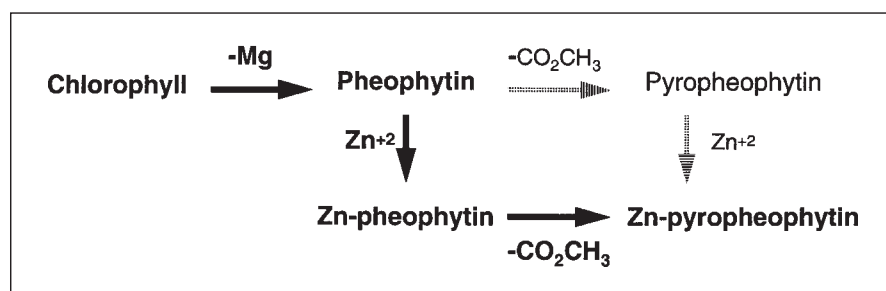


Fig. 5—Reaction pathway for the formation of Zn-pheophytin *a* and Zn-pyropheophytin *a*.

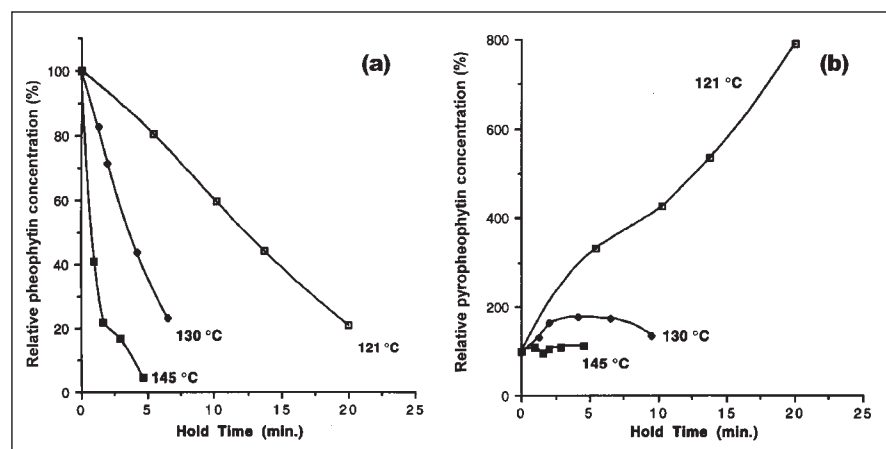


Fig. 6—Pheophytin *a* degradation (A) and pyropheophytin *a* formation (B) at different processing temperatures vs isothermal hold times. Zinc was applied to the peas by blanching at 83 °C for 5 min in ZnCl₂ solution with a concentration of 300 mg/L Zn.

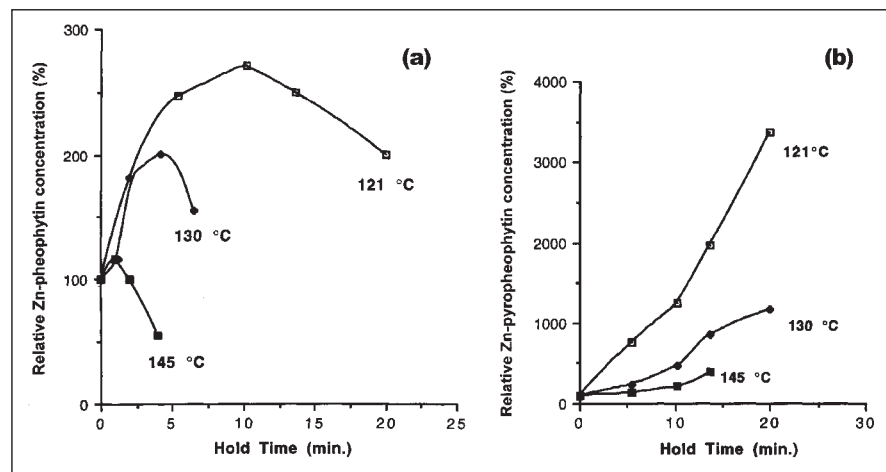


Fig. 7—Zn-pheophytin *a* (A) and Zn-pyropheophytin *a* (B) changes in concentration at different processing temperatures vs isothermal hold times. Zinc was applied to the peas by blanching at 83 °C for 5 min in ZnCl₂ solution with a concentration of 300 mg/L Zn.

able ion, duration of contact between the Zn²⁺ solution and pea tissue, and temperature. The complexation reaction leading to the formation of Zn-pyropheophytin *a* appeared to occur largely from the conversion of Zn-pheophytin *a* rather than from pyropheophytin *a*. The chlorophyll derivatives produced by the addition of ZnCl₂ during aseptic thermal processing of peas showed improved green color in contrast to traditionally processed peas. Thus, the use of metal salts coupled with continuous flow aseptic processing technology may be viable for the preservation of green color in vegetables.

REFERENCES

- Buckle, K.A. and Edwards, R.A. 1970. Chlorophyll, color and pH changes in HTST processed green pea puree. *J. Food. Technol.* 5: 173-186.
- Canjura, F.L. and Schwartz, S.J. 1991. Separation of chlorophyll compounds and their polar derivatives by high-performance liquid chromatography. *J. Agric. Food Chem.* 39: 1102-1105.
- Clydesdale, F.M. and Francis, F.J. 1968. Chlorophyll changes in thermally processed spinach as influenced by enzyme conversion and pH adjustment. *Food Technol.* 22: 793-796.
- Hekal, I.M. and Erlandson, P.M. 1984. Process for improving the organoleptic properties of canned green vegetables. U.S. patent 4,478,860.
- Hekal, I.M. and Erlandson, P.M. 1986. Coating and container for retention of green color of vegetables. U.S. patent 4,615,924.
- Humphrey, A.M. 1980. Chlorophyll. *Food Chem.* 5: 57-67.
- Jones, I.D., White, R.C., Gibbs, E., Butler, L.S. and Nelson, L.A. 1977. Experimental formation of zinc and copper complexes of chlorophyll derivatives in vegetable tissue by thermal processing. *J. Agric. Food Chem.* 25(1): 149-153.
- LaBorde, L.F. and von Elbe, J.H. 1990. Zinc complex formation in heated vegetable purees. *J. Agric. Food Chem.* 38: 484-487.
- LaBorde, L.F. and von Elbe, J.H. 1994. Chlorophyll degradation and zinc complex formation with chlorophyll derivatives in heated green vegetables. *J. Agric. Food Chem.* 42: 1100-1103.
- LaBorde, L.F. and von Elbe, J.H. 1996. Method for improving the color of containerized green vegetables. U.S. patent 5,482,727.
- Leake, L.H. and Kirk, L.K. 1992. Method for color preservation in canned green vegetables. U.S. patent 5,114,724.
- Malecki, G.J. 1978. Processing of green vegetables for color retention in canning. U.S. patent 4,104,410.
- Minguez-Mosquera, M.I., Gallardo-Guerrero, L., Hornero-Mendez, D., and Garrido-Fernandez, J. 1995. Involvement of copper and zinc ions in green staining of table olives of the variety gordal. *J. Food Prot.* 58(5): 564-569.
- Schanderl, S.H., Marsh, G.L., and Chichester, C.O. 1965a. Color reversion in processed vegetables. I. Studies on regreened pea puree. *J. Food Sci.* 30: 312-316.
- Schanderl, S.H., Marsh, G.L., and Chichester, C.O. 1965b. Color reversion in processed vegetables. II. Model system studies. *J. Food Sci.* 30: 317-324.
- Schwartz, S.J. and Lorenzo, T.V. 1991. Chlorophyll stability during continuous aseptic processing and storage. *J. Food Sci.* 54: 1059-1062.
- Schwartz, S.J. and von Elbe, J.H. 1983. Kinetics of chlorophyll degradation to pyropheophytin in vegetables. *J. Food Sci.* 48: 1303-1306.
- Segner, W.P., Ragusa, T.J., Nank, W.K., and Hoyle, W.C. 1984. Process for the preservation of green color in canned vegetables. U.S. patent 4,473,591.
- Tan, C.T. and Francis, F.J. 1962. Effects of processing temperature on pigments and color in spinach. *J. Food Sci.* 27: 232-241.
- Tonucci, L.H. and von Elbe, J.H. 1992. Kinetics of the formation of zinc complexes of chlorophyll derivatives. *J. Agric. Food Chem.* 40: 2341-2344.
- van Breemen, R.B., Canjura, F.L., and Schwartz, S.J. 1991. Identification of chlorophyll derivatives by mass spectrometry. *J. Agric. Food Chem.* 39: 1452-1456.
- von Elbe, J.H., Huang, A.S., Attoe, E.L., and Nank, W.K. 1986. Pigment composition and color of conventional Veri-Green® canned beans. *J. Agric. Food Chem.* 34: 52-54.

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