AN ABSTRACT OF THE DISSERTATION OF

Thao Xuan Ngo for the degree of Doctor of Philosophy in Food Science and Technology presented on August 22, 2007.

Title: Understanting the Principles and Procedures to Retain Green and Red Pigments in Thermally Processed Peels-On Pears (*Pyrus communis* L.)

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Abstract approved:

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Yanyun Zhao

Thermal stabilization of chlorophyll and anthocyanin on pear peels during thermal process was investigated in this study. The green color of peels-on thermally processed (canned) pears was successfully retained by complexation of magnesium-free chlorophyll with zinc ions, and the red pigments were stabilized through anthocyanin-stannous complexation and polymerization of pear peel materials

Blanching de-waxed green pears (Bartlett and Comice) in zinc solution (1,300-5,200 ppm) prior to canning resulted in attractive green color on the peels-on canned pears. Zinc pheophytin a was found to be the major zinc chlorophyll derivative created from pheophytin a and zinc ions in finished canned pears. The higher the zinc concentration in blanching solution and the longer the blanching time (6-18 min), the higher the chromacity of the color in canned green pears. The color of canned pears was highly stable during storage and did not show significant change after 19 wk of storage at 38 °C or at least 35 wk of storage at 10 °C under intensive illumination.

Red D'Anjou pears were tested with various individual metallic ions in aqueous solutions for pigment complexation with anthocyanins. A formula containing stannous, hydrochloric acid, formalin, and tannic acid was then developed as a pretreatment for stabilizing anthocyanins on the peels of red pears during thermal process. The stabilization was achieved through complexation with stannous ions which integrated the pigments in a cross-linked macromolecular matrix. Tannic acid and formaldehyde were the key reagents for the polymerization of pear peels. The effectiveness of the developed formula was found to be significantly enhanced by steam-heating of treated red pears prior to canning.

This study significantly improved our understandings on the principles of pigment retention during thermal process, especially for red anthocyanins in processed fruits and vegetables. Developed procedures can be used to stabilize green and red pigments on pears, as well as other fruits with pigmented peels, contributing in developing high value processed fruit and vegetable products.

Understanding the Principles and Procedures to Retain Green and Red Pigments in Thermally Processed Peels-on Pears (*Pyrus communis* L.)

by
Thao Xuan Ngo

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented August 22, 2007

Commencement June 2008

Doctor of Philosophy dissertation of Thao Xuan Ngo presented on August 22, 2007.

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Thao Xuan Ngo, Author

ACKNOWLEDGEMENTS

It is a pleasure to thank the many people who made this thesis possible.

My special thanks to Dr. Yanyun Zhao, my major professor and model of my future career. Without her support, flexibility, and guidance, I would never have extended myself so far. Throughout my research and study in Food Science at OSU, she provided encouragement, sound advice on my research and managed to read many versions of my papers and dissertation writing so thoroughly.

I am specially obliged to Dr. Ronald Wrolstad who has taught me on chemistry of pigments and color measurement, kept eyes on the progress of my work and always was available when I needed his advice. I would also like to thank Dr. James Kennedy who gave great insight to my work. I thank you all for taking effort in reading and providing me with valuable comments on earlier versions of my thesis. I would also like to thank Dr. Vincent Remcho and Dr. John Selker for serving on my Graduate Committee. I am indebted to Dr. Mike Penner, who was always available to my varied questions on food chemistry and to Dr. Robert McGorrin for his constant encouragement and interest in my work.

I would like to recognize Bob Durst and Seth Cohen for advising me on extraction techniques and analysis of phenolics and pigments and their precious time to run the HPLC. I am grateful to Brian Yorgey and Jeff Clawson for assisting me in many different ways in my research and teaching in the pilot plant. Special thanks to Eric Wilhelmsen for all the hard work to create the blasting system and to James Batdorff who patiently taught me about electrical things on various machines I used in the lab and put up with chemical and equipment needs and mishaps. Many thanks to Bob Durst and Dan Smith for being around to help answer my many and varied questions.

Thank you to everyone from the Value-Added Fruit and Vegetable Products lab (old and new) for putting up with me for years. Your friendship was irreplaceable.

I am also indebted to my many student colleagues for providing a stimulating and fun environment in which to learn and grow.

I'd like to extend a special thanks to Vietnamese Ministry of Education and Training that have sponsored my study and to Dennis Anderson, CEO of Green & Green, Inc., Corvallis and Northwest Pear Commissions for funding the research. I am also grateful for the department of food science and technology for providing me an excellent work environment during the past four years.

Finally, I would like to thank those closest to me. These are, among others, Robert and Kim Handerson, Tuan and Khanh Truong, Quang Dao, Hien Tran, Tin Huynh, Hao Hong, Chien and Phuong Nguyen, and Hang Le for all the emotional support, camaraderie, entertainment, and caring they provided. Most of all, I feel a deep sense of gratitude for my parents for their absolute confidence in me and I am forever grateful for the unconditional love and support of my wife Ly and our daughter.

CONTRIBUTION OF AUTHORS

Dr. Yanyun Zhao assisted with the experimental design, data analysis, and writing of each chapter. Dr. Ronald Wrolstad and Dr. James Kennedy provided assistance in the writing of Chapter 2 as well as the experimental design and analytical methods in Chapter 5 and 6. Mr. Dennis Anderson and Mr. Eric Wilhelmsen provided the sugar blasting system used throughout this study for de-waxing the pears. Mr. Brian Yorgey assisted with making jam and canning strawberries. Mr. Bob Durst and Mr. Seth Cohen assisted with the collection of HPLC – DAD data collection.

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CHAPTER 1

Thesis Introduction

More than 95% of the U.S. pears (*Pyrus communis* L.) are grown in Washington, Oregon, and California (NASS 2006) and about two-thirds of the fruit are processed and the remainings are sold in the fresh market (Seavert 2005). Canned pears are among the top four canned fruits consumed in the United States (Pollack and Perez 2006). Unfortunately, the consumption of canned fruits in 2006 fiscal year hit the lowest amount in the last 25 years, which can be partially attributed to the lower usage of canned pears (Pollack and Perez 2006). The decline in sales partially explains why the fruit bearing acreage of pears decreased from 81,000 acres to 62,200 acres from 1980 to 2005 in the U.S. (Pollack and Perez 2006).

A number of factors contribute to the decline of the canned fruit industry, including lost markets both domestically and internationally, increased labor costs and escalating transportation rates. One of the main challenges that pear producers face is to increase the value of the fruit, i.e., developing new value-added pear products.

For nutritional and aesthetic reasons, it is desirable to prepare colorful thermally processed peels-on pears. This study was driven by the need to develop a new value-added pear product: ready-to-eat, color-stabilized thermally processed peels-on pears packed in clear glass or plastic packaging to generate higher consumer appeal. Firstly, the current knowledge on natural pigments provides a sufficient scientific base to develop methods to retain the pigments on pear peels, particularly true for green and red pears. Secondly, the effort contributes to increase the competitive quality of U.S. pears as colored canned pears have not been developed

anywhere else yet. Thirdly, it helps to reduce the amount of material waste during production as the peels are left on the fruit.

Pears are normally thermally processed to assure microorganism stability. Unfortunately, green chlorophylls and red anthocyanins on the pear peels are readily degraded during the conventional process, resulting in dark brown products. The discoloration is directly linked to the extremely unstable nature of the pigments (von Elbe and Schwartz 1996; Wrolstad 2000). Moreover, if anthocyanins are successfully stabilized, their water-soluble nature doubles the obstacle to obtain red colored pears as most of the pigments will diffuse from the skin tissue into the solution.

The concept of this new value-added pear product is to first create a shelf-stable product using canning technology with a shelf-life up to 1.5-2 years, and then re-pack the product in clear glass or plastic containers so that the product has attractive appealing to consumers and a shelf-life up to another 6 months. The new packaging technology will combine convenience, freshness, and a colorful presentation. This concept takes efforts from a multi-disciplined team, including the Northwest regional pear industry, university researchers, and food processors for helping pear producers and processors meet the challenges now and in the future.

In an attempt to retain the red and green color of pears, this thesis research focused on known methods and mechanisms in stabilizing chlorophylls and anthocyanins in the literatures. The methods of retaining green pigments have been well developed for spinach, green beans, and peas (von Elbe and Schwartz 1996). Although the technologies using metal ions such as zinc have proved successful in vegetables, our initial attempts to directly apply the method in processed pears has not been proved to retain greenness of the product. Modification and further development are thus obviously necessary. For red anthocyanins, recent research results have revealed that anthocyanins can be stabilized by various mechanisms such as co-pigmentation, metallo-complexation, chemical transformation, and anthocyanin-embedment. However, no proven technology to retaining anthocyanins on the peels of any processed fruit and vegetable product.

The objectives of this thesis research on the chlorophylls were to develop methods and procedures to retain the green appearance of canned green pears through the formation of zinc-chlorophyll complexation and to investigate the nature of the created green pigments on the pear peels. The objectives of the study on anthocyanins were to develop a formula composed of metal ions, cross-linking agents, and phenolics and the processing procedures that are capable of retaining the red appearance of canned red pears as well as to investigate the mechanisms through which anthocyanin are stabilized on the peels of treated fruits. HPLC analysis and UV-Visible spectrophotmeteric methods as well as photographs were used to identify and report pigments and pear appearance changes in processed pear samples.

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CHAPTER 2

Literature review

2.1. Pigments and pear color

Color, flavor and texture are the primary attributes of food quality that determine consumers' purchase decision on the product. The most varieties of pears have a yellow skin (peels) at optimum ripeness. Some varieties have brown, green or red coloration, such as Bosc, D'Anjou, Starkrimson, respectively, which appeals very attractive to the consumers (Mazza and Miniati 1993). Pear peels may contain all three major families of natural plant pigments—carotenoids, chlorophylls, and anthocyanins. During ripening, degradation of chlorophylls a and b results in disappearance of the green color to yellow due to the presence of carotenoids (Gross 1984). Lutein is the major carotenoid in pear peals, with β-carotene being present at much lower concentrations (Gross 1984; Heinonen and others 1989). Some pear varieties are partially or fully red as a result of the presence of the anthocyanin pigments, cyanidin-3-galactoside and cyanidin-3-arabinoside (Mazza and Miniati 1993; Dussi and others 1995). The pattern of red pigment distribution in pear peels has been investigated by Dayton (1966), showed that the peels have a non-pigmented epidermis with two to seven additional layers containing anthocyanins lying above (Table 2.1**)**. An exception is Starkrimson pears where all epidermis cell layers contain anthocyanins. Pear peels are also rich in phenolic acids, flavonols, flavan 3-ols (-), and proanthocyanins, all of which may have an effect on pear peel color from their oxidative degradation, or through their interactions with anthocyanins.

2.2. Heating and pear color

Several industrial operations associated with heating can have significant impact on the color of fruits and vegetables or pears in this study. The produce is blanched, cooked, boiled, canned, and/or pasteurized before cooling down inside a packaging container.

Blanching is a short heat treatment in order to inactivate enzymes and to stabilize the fruits against deterioration during prolonged storage. Blanching is often used prior to freezing. In canning, blanching is normally conducted in water to evacuate the air inside the fruits to limit the amount of oxygen trapped in the cans.

Retorting by canning is generally conducted at 121 ^{o}C for 25 – 40 min to produce a sterile product. The target criterion is to destroy spores of *Clostridium botulinum*, the most heat-resistant food poisoning bacteria.

Pasteurization, normally at a temperature higher than 60 ^{o}C and lower than 100 ^{o}C is to target a destruction of spoilage and pathogenic microorganisms to stabilize the product during storage. In common practice, pasteurization is used in processing canned pears as the pH of the final product is below 4.5 at which *C. botulinum* spores can not grow.

As canned pears go through thermal processing, many physical and chemical changes occur, particularly in the pigments in the peels. The yellow color caused by the presence of carotenoids is relatively stable during processing and storage as observed for carotenoid-rich fruits and vegetables such as tomatoes and carrots. The green color from chlorophylls and the red color from anthocyanins, in the contrary, are extremely sensitive to physical and chemical changes encountered through food processing and storage. Since processed yellow-colored pears with good stability can be obtained with no major technical difficulties, the pear carotenoids will not be the focus of this research. The cause of green color loss of green produce during thermal process and the technologies to retain the green pigments will be reviewed in detail and the most promising method will be adapted to retain the greenness of thermally processed peels-on pears. Unlike chlorophylls and carotenoids which are water-

insoluble and for which chemistry information and stabilization methods have been well established and exploited, there have no pre-existing procedures and technologies in the literature for retention of water-soluble red anthocyanins on the peels or the surface of thermally-processed fruits. In the subject of anthocyanin stabilization, a variety of mechanisms revealed by researchers made on botanic items such as flower petal, or food products, such wine, or on non-food products, such as pigmented clay will be reviewed and used as a guidance to articulate/speculate the author's hypotheses to retain red pigments on processed red pear peels.

2.3. Chlorophylls and green pigment stabilization

2.3.1. Chemical structure

Chlorophylls are magnesium (Mg) complexes derived from porphin which is based on tetrapyrrole macrocyle linked by methene bridges forming a closed, conjugated loop (Ferruzzi and Schwarz 2005) (Figure 2.1).

Chlorophyll a and b are the major chlorophylls found in green plants in an approximate ratio of 3:1 (von Elbe and Schwartz 1996). They differ in the carbon C-7 substituent. Chlorophyll a contains a methyl group while chlorophyll b contains a formyl groups (Figure 2.1). Figure 2.1 shows that both chlorophylls a and b have a vinyl and an ethyl group at the C-3 and C-8 position, respectively, a carbomethoxy group at the C-13^2 position of the isocyclic rings, and a phytol group esterfied to propionate at the C-17 position. Phytol is a 20-carbon monounsaturated isoprenoid alcohol.

2.3.2. Location and physical characteristics.

As the major light-harvesting pigments in green plants, chlorophyll-rich cells accumulate in the exterior part of the plant leaves (Figure 2.2) or the skin of fruits. In the cell, chlorophylls are located in internal photosynthetic membrane (also known as chloroplast membrane or thylakoid) of intercellular organelles of green plants known as chloroplasts (Figure 2.3). They are water-insoluble and closely associated with

carotenoids (Figure 2.2), lipids, and lipoproteins (von Elbe and Schwartz 1996). Non covalent bonds form weak linkage between these molecules and are easily broken by maceration and solvent effects and hence chlorophylls can be easily extracted in organic solvents (von Elbe and Schwartz 1996). Polar solvent such as acetone, methanol, ethyl acetate, pyridine, and dimethylformamide are most effective for complete extraction of chlorophylls while nonpolar solvents such as hexane or petroleum ether are less effective (von Elbe and Schwartz 1996).

Chlorophylls absorb visual light at two separate bands between 600 and 700 nm (red regions) and between 400 and 500 nm (blue regions) (von Elbe and Schwartz 1996) (Figure 2.4).

2.3.3. Chlorophylls degradation by heat and acid

A number of studies have thoroughly reviewed the degradation of chlorophylls during food processing of vegetables and fruits (Heaton and Marangoni 1996; von Elbe and Schwartz 1996; Kidmose and others 2002). Presented here is a brief summary on the effects of high temperature and low-pH condition, which are reported to be the major causes of loss of green color in thermally-processed produce (von Elbe and Schwartz 1996). Few studies have been performed on green fruits.

Table 2.2 shows the quantity of various chlorophyll derivatives throughout a typical heat processing scheduled for retorting spinach. During the first 15 min heating, pheophytin increases rapidly while chlorophyll decreases rapidly. Further heating decreases pheophytin and increases pyropheophytin. These results suggested that the mechanism for chlorophyll decomposition during canning is a two-step process: displacement of Mg from the central position of green chlorophyll by proton H^+ resulting in olive-colored pheophytin and then replacement of the C-10 carbomethoxy group of pheophytin with a hydrogen atom resulting in olive-colored pyropheophytin (Schwartz and Elbe 1983; Weemaes and others 1999) (Figure 2.5):

Chlorophyll → Pheophytin → pyropheophytin

Chlorophyll degradation by heat follows a first-order reaction mechanism and a rise in temperature will result in an increase in degradation rate (Haisman and Clarke 1975; Schwartz and Elbe 1983; Steet and Tong 1996). At a lengthened heat process, total heat destruction of chlorophylls results generally in the formation of pheophytins and pyropheophytins (von Elbe and Schwartz 1996). The loss of magnesium from the porphyrin ring of chlorophyll causes a change in the absorption spectrum in that the peak at 428 nm moves to 408 nm with a small increase in extinction coefficient and the peak at 661 nm moves to 667 nm with a marked decrease in extinction coefficient (Houssier and Sauer 1970). The color of the treated produce thus changes from green-blue (chlorophyll) to dull olive (pheophytin, pyropheophytin) as commonly seen in canned spinach or beans.

Among the two chlorophylls a and b, the formation of pheophytin from chlorophyll *a* has been reported to be more rapidly than from the b form (Schwartz and Elbe 1983; Weemaes and others 1999). Besides pheophytinization, an isomerization of chlorophylls can also occur and there are as high as 5 – 10 % of chlorophyll a and b converted to a' and b' after heating for 10 min at 100 ^{o}C as observed in green leaves (Schwartz and others 1981).

Besides the temperature and the length of thermal treatment, chlorophyll degradation in heated vegetable tissues is closely affected by tissue acids. It is believed that the first effect of heat on the chlorophyll degradation is to increase the pass of hydrogen ions across cell membranes (Haisman and Clarke 1975) starting in the temperature range of 50 - 60 ^{o}C or higher at both atmospheric and high pressure conditions (Haisman and Clarke 1975; Weemaes and others 1999). Haisman and Clark (1975) studied the pheophytin formation in multiple plant tissues and reported that the pheophytin conversion in green beans was 12 times faster that in the leaves in spite of their similar pH value. This led them to conclude that the concentration and nature of the cellular acids as well as the degree of the association between chlorophyll and the materials in the chloroplast can largely influence the degradation rate. In a basic media, however, chlorophyll will be very heat stable (von Elbe and Schwartz 1996).

2.4. Green pigment stabilization

2.4.1. Attempts to retain green color for thermally processed produces

Various methods have been proposed to retain chlorophylls in processed vegetables. Most of them which were reported to be effective in limiting the degradation of chlorophylls during processing, unfortunately, did not successfully retain the green color during prolonged storage or caused a decrease in the overall quality of the products.

Acid neutralization to retain chlorophyll. Since chlorophyll is more stable in the basic environment than in acidic condition, neutralization of acids in the green vegetables by use of calcium oxide and sodium dihydrogen phosphate in blanching water has been proposed to retain the chlorophylls during processing (von Elbe and Schwartz 1996). The high pH condition, unfortunately, results in softening of the texture and alkaline flavor (von Elbe and Schwartz 1996).

High-temperature short-time (HTST) processing. Based on the finding that the amount of retained chlorophylls during thermal processing is closely related to the processing temperature and time, a procedure of HTST has thus been developed and showed success in processing spinach puree (Schwartz and Lorenzo 1991). However, this attempt controls only the loss of chlorophylls and do not stabilize the pigments which continue to degrade during the storage of packed product (Schwartz and Lorenzo 1991).

Enzymatic conversion. This method involves the use of chlorophyllases to convert chlorophyll to chlorophyllide which is believed to have a better thermal stability than the parent compounds. The color improvement by this approach was insignificant, thus insufficient to warrant its commercialization (von Elbe and Schwartz 1996).

Re-greening. Unlike other earlier described methods, a method invented by von Elbe team using zinc or cooper ions to retain green color has been shown to be a successful case (Canjura and others 1999). The technology is named re-greening or

Veri-Green procedure as the chlorophylls are replaced by green and more stable metallo-complexes of cholorophyll derivatives. There are yet not known efforts to apply this procedure to processed fruits.

Veri-Green™ was developed to improve the color of canned green vegetables by adding metal ions zinc to blanching solutions (von Elbe and Schwartz 1996). Elbe et al. (1986) 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 color of the products turned into greener when subjected to long storage period. For green vegetables such as spinach, the technology resulted in a spontaneous re-greening of the produce during processing (LaBorde and von Elbe 1994a). The zinc chlorophyll derivatives are more heat- and acid-stable than the parent compounds. Both the metallo-complexes contribute to the green color of the final products.

The production of Veri-Green processed peas, however, has not been successful because the concentration of Zn^{2+} ions required for a satisfactory green color after processing is above the Food and Drug Administration (FDA) limit of 75 ppm (in the final product) (LaBorde and von Elbe 1994a). A two-phase technology has then been developed to control the contact time between the product and the Zn solution. The idea is to blanch the vegetables in Zn solution prior canning step that is performed without any added Zn. An example is processing peas in 50 – 500mg/L of $ZnCl_2$ for 5 min at 83 ^{o}C, and then thermally processed without addition use of Zn in canning solution (traditional method) (Canjura and others 1999). Thermally processing fresh peas in Zn solutions has showed improved green color in contrast to traditionally processing (Canjura and others 1999).

2.4.2. Major parameters of regreening procedures using Zn

LaBorde and Elbe (1994) remarked that zinc complex formation in thermally processed green vegetables must be preceded by the degradation of chlorophyll to form pheophytin. With sufficient amount of zinc in the blanching solution, Mg-free chlorophyll derivatives which are newly formed by heat from the blanching solution

will come into contact with Zn to form Zn chlorophyll derivatives (Figure 2.6). The rate of zinc-chlorophyll-derivative formation is influenced by various factors such as zinc and chlorophyll concentration, pH, and the presence of other compounds such as divalent salt and surfactants.

Metal ions. Alike zinc, copper can form green metallo-complexes with the Mg-free chlorophyll derivatives and even has much higher an affinity to these derivatives than zinc (von Elbe and Schwartz 1996). The use of copper as technology aid in food processing, however, is not allowed by FDA. Other bi- or trivalent metal ions are either less effective than zinc and copper or ineffective (von Elbe and Schwartz 1996).

Metal ion concentration. Metallo-complex formation is reported to occur in the presence of 1-2 ppm of Cu although a range of amount from10 to 20 ppm is required for complete complex formulation with pheophytin in pea puree (Schanderl and others 1965). The formation of Zn complexes requires a minimum of 25 ppm to occur, and 100 ppm is required for their complete complexation (Schanderl and others 1965). In addition, not all of the metal ions absorbed into the plant tissue are available for reaction with chlorophyll derivatives since other components in the cells will interact with the metal ions and keep them from reacting with chlorophyll derivatives (Canjura and others 1999).

Derivatives of chlorophll a and b. There are differences in the reaction rate between different chlorophyll derivatives and Zn. Among the chlorophyll derivatives of the same form, pheophytin *a* does not react to form the complex as rapidly as pyropheophytin *a* does and their b forms are also less reactive than their a forms (Canjura and others 1999). Among the form a and b, derivatives of chlorophyll *a* form metallo-complexes more rapidly than does chlorophyll b (LaBorde and von Elbe 1994a). Pheophytin forms metallo-complexes more slowly than pyropheophytin and pheophorbide because of the presence of interfering substituent groups (Tonucci and Von Elbe 1992; LaBorde and von Elbe 1994a).

Concentration of chlorophylls. It is generally believed that the higher the concentration of chlorophylls, the higher the yield of zinc metallo-complexes.

LaBorde and von Elbe (1990) did a study on previously heated vegetable purees in which chlorophyll had initially been entirely converted to pyropheophytin. The authors found that spinach purees that had 12 times concentrations of chlorophyll derivatives than that of pea purees contained 40 times more zinc complexes after a identical heating.

*pH***.** In pea purees, zinc complex formation increased between pH 4.0 and 6.0 but decreased at pH 8.0 or greater. Chlorophyll *a* was retained in the purees with pH 8.0 or higher, suggesting that pH increase may decrease zinc complex formation by reducing the amount of chlorophyll derivatives available for the reaction (LaBorde and von Elbe 1994a). Hence, the optimum pH range to create the chlorophyll derivatives may not be optimal pH conditions to form the metallo-complex. When spinach puree is preheated and its Mg-free chlorophyll derivatives have been preformed, an increase in pH from 4.0 to 8.5 resulted in an 11-fold increase in zinc complex concentration after heating (LaBorde and Von Elbe 1990). Decreased zinc complex formation in pH 10 puree was attributed to the formation of insoluble $Zn(OH)_2$ (LaBorde and Von Elbe 1990).

Divalent salts. Divalent cations such as calcium (Ca) and barium (Ba) may negatively affect the Zn complex formation through their ability of lowering pH values and decreasing the permeability of zinc into cells (LaBorde and von Elbe 1994b). It has been reported that zinc complex formation in pea puree is significantly lower (c.a. 30 – 38 %) in pea purees containing added divalent cations Mg, Ca, Ba (0.1 M) than in the control samples (LaBorde and von Elbe 1994b).

Surfactants. Anionic surfactants such as sodium dodecylsulphate (SDS) has been reported to increase the formation of zinc pheophytin *a* 2.4 times compared to the control . A possible explanation is that the anionic surfactants increase the negative charge of chloroplast membrane surfaces, resulting in an accumulation of H^+ ions, and then an increase in pheophytin formation. Conversely, cationic surfactants such as polyoxyethylene sorbitan oleate (TW 80) and cetyl trimethyl ammonium bromide (CTAB) have been found to cause a decrease in the amount of Zn-Pheophytyl *a*

formed in pea puree by 49 and 56 %, respectively (LaBorde and von Elbe 1994b). It is believed that decreasing the negative surface charge of membrane surfaces repels H^+ ions and therefore decreases chlorophyll degradation. On the other hand, the repulsive electrostatic forces can cause a reduction in Zn^{+2} ion concentration at membrane surfaces, thus reducing Zn complex formation (LaBorde and von Elbe 1994b).

In Summary, during heat treatment, chlorophylls, the natural green pigments, are converted to chlorophyll derivatives causing product's loss of their natural green color. The regreening procedure of using Zn to react with heat-induced chlorophyll derivatives forms more stable green pigments than their parent compounds. The degree of regreening will depend on the materials (chlorophyll and acid contents, permeability toward Zn^{+2}), the condition of reactions (pH, types of present solutes, temperature), and procedures (preheating, treatment time). An important remark is that a common 20-min heat treatment of commercial sterilization (canning) is much shorter than the time needed to convert a major part of chlorophyll derivatives into Zn-derivatives complexes, which can be as long as 70-80 min in vegetables (LaBorde and von Elbe 1994a). Thus, one should expect to see a low yield of retained pigments. In general, formation of zinc complexes during heat processing will be mostly rapid in the vegetables containing the greatest amount of chlorophyll and processed with greater concentrations of zinc salts (LaBorde and Von Elbe 1990). However, the use of high concentration of zinc in fruit and vegetable processing can result in a high zinc residue in the finished product. In the U.S., this technology has been approved to produce market-test products only with a condition of less than 75 ppm in term of zinc residual. Thus, together with optimum reaction, there should be a careful process design to control the transfer of zinc into processed products. In addition, none of the investigations has studied the sensorial aspects of zinc-treated products.

2.5. Flavonoids and red pigments

Flavonoids belong to a larger group of natural organic plant compounds, the polyphenols (Brouillard and others 1997). Most polyphenols strongly absorb light in

the ultraviolet range (Brouillard and others 1997), but only one member of this family, anthocyanins, is visible to human eyes.

Anthocyanin pigments are commonly seen in flower petals and fruit epidermal tissues (Brouillard and others 1997) and are responsible for the red to blue colors in most plants. They are stored within the vacuoles of mature epidermal cells (Brouillard and others 1997). The natural physicochemical environment for the anthocyanins is well defined in the literature: slightly acidic aqueous solution (Brouillard and others 1997) kept at room temperature away from oxygen contact. The characteristic acidity of pear juice typically ranges from 3.5 to 4, while a broader range may be found for other fruit juices. *In vivo*, the color stability of fruits and flowers can normally last for hours, days, weeks, or may even be limited only by the lifetime of the fresh product (Brouillard and others 1997). In contrast, fruit juices and extracts of most naturally occurring anthocyanins tend to degrade quickly (Brouillard and others 1997). These anthocyanins, whether unaltered or in faded or chemically modified forms can subsequently play a major role in the production of many different colors in the complex environment of food systems rich in other phenolics and macromolecules. These degradative compounds can either enhance color stability, or accelerate anthocyanin degradation. Previous reviews on molecular interactions of phenolic compounds, with a special focus on the color and stabilization of anthocyanins, have been written by Timberlake (1980), Mazza and Brouillard (1987), Brouillard et al. (1997), Boulton (2003), Es-Safi 2004, and de Freitas 2006. This review will focus on the discussion on the potential natural stabilization mechanisms on pear peels and on the chemical and physical environmental conditions that would lead to anthocyanin stabilization.

2.5.1. Anthocyanins of fruits and vegetables – structure and characteristics

Anthocyanins are planar, aromatic phenolic $C_6C_3C_6$ nucleus bearing at least one sugar residue (Wrolstad 2000b). In addition, aliphatic or aromatic organic acids can be linked to the sugar moiety through an ester bond.

The pigments are water soluble, and have greater solubility in ethanol or acetone which is frequently used for extraction of the pigments (Wrolstad 2000b). Structural variations in the B-ring result in six major groups of aglycones (Figure 2.7). Hydrogen substitution with OH and/or OCH_3 group shifts the wavelength of maximum absorption to longer values (Wrolstad 2000b). This shift toward higher wavelengths is referred to as a bathochromic shift. These structural changes affect color with changes in hue from orange-red to red to purple (Wrolstad 2000b). Those anthocyanins with *o*-substituted hydroxyl groups, such as cyanidin and delphinidin, can complex with metal ions (Asen and others 1969; Wrolstad 2000b).

Anthocyanins reversibly undergo structural transformation with a change in pH (Wrolstad 2000b) (Table 2.3); the total amount of pigment in aqueous medium is shared between colorless ultra-violet-absorbing forms (hemiketals, chalcone) and colored chromophores (flavylium and quinonoidal) (Brouillard and others 1997). Essentially, 100% of the pigment will be in the colored flavylium form at pH 1.0, while nearly all of the pigment exists as the colorless hemiketal form at pH 4.5 (Wrolstad 2000b). Quinoidal forms of the anthocyanins predominate at pH of 7.0 and above (Wrolstad 2000b). The positive charge of the oxonium salt of the anthocyanin increases the polarity and water solubility of anthocyanins (Wrolstad 2000b). Anthocyanins such as cyanidin glycosides are stable in the flavylium form at pH under 3 and significantly less stable at pH above 3 when being stored in pure solutions without the presence of other phenolics (Fossen and others 1998; Cabrita and others 1999). The molecules become unstable and enter into different paths of degradation via the first step of cleavage of sugar moieties (Sadilova and others 2006). High temperatures in the order of 100 ^{o}C combined with a pH above 3 as the case in canning fruits, will have disastrous effects on the fate of anthocyanins.

These earlier described reactions are characteristic of the aglycone moiety. In nature, anthocyanins are always present as glycosides, never as an un-substituted aglycone (Wrolstad 2000b). Glycosidic substitution in the 3 and/or 5 positions with mono-, di- and tri-saccharides accounts for most of the structural variation found in

nature, explaining for the presence of over 500 anthocyanins that have been identified in nature (Andersen and Jordheim 2006). The sugar also increases the water solubility of the pigments. Acylation (esterification) of sugar substituents with organic acids creates an additional possible variation in the structure of anthocyanins (Wrolstad 2000b). Common acylating acids are the cinnamic acids (p-coumaric, caffeic and ferulic) and aliphatic acids (acetic, malonic and succinic acids) (Wrolstad 2000b). Acylation can have a marked positive effect on pigment stability (Giusti and Wrolstad 2003).

The pKa of the transformation between flavylium and hemi-ketal is in the range of 3.0 - 3.1 for non-acylated cyanidin 3-glycoside (Stintzing and others 2002) and cyanidin 3-galactoside which is the major pigment in pear and apple peels (Dussi and others 1995). Pears have a pH of 3.5, and therefore have over 50 % of its pigments in the colorless forms. The hemiketal and chalcone forms not only result in reduced absorbance intensity, but are also less stable and more sensitive to irreversible degradation reactions (Wrolstad 2000b).

While anthocyanins *in vivo* are well stabilized and highly colored, in food products they commonly exhibit poor coloration and undergo a number of degradative reactions during processing and storage that may result in color deterioration (Skrede and others 1992; Garzon and Wrolstad 2002; Rein and Heinonen 2004). Processing plant products results in tissue damage that destroys the protective natural supramolecular edifices of the cell (Brouillard and others 1997), thus exposing anthocyanins to various conditions favoring pigment destruction (dilution by water, exposure to pH change, reaction with oxygen, light, and enzymes (Wrolstad 2000a), and high temperature conditions (Sadilova and others 2006)). Some phenolics that are present along with anthocyanins may show even higher instability than anthocyanins themselves. Phenolic compounds have been characterized as potential causes of instability as they are involved in the formation of undesirable yellow and brown pigments (Spanos and Wrolstad 1992). A prime example is the case of enzymatic browning. Since some anthocyanins, such as cyanidin glycosides, contain *o*-

substituted phenolic groups, they can theoretically serve directly as substrates for polyphenoloxidase (PPO). When cell walls are disrupted during processing operations, PPO comes in contact with oxygen and phenols (Wrolstad 2000b). Although anthocyanins are rapidly destroyed in fruits with high PPO activity, such as plums (Siddiq and others 1994) or cherries (Pifferi and Cultrera 1974), compounds such as chlorogenic acid or catechin will be, in most cases, preferential substrates for PPO as compared to anthocyanins (Wrolstad 2000b). The oxidized quinones will subsequently condense with anthocyanins resulting in pigment degradation. Hence, the color instability of anthocyanin-containing foods may be caused by the interactions of anthocyanins with other components rather than inherent instability of the anthocyanin pigment itself (Maccarone and others 1985; Wrolstad 2000a). Garzon (1998) reported that anthocyanin degradation followed first-order reaction kinetics with half-lives of only 4-9 days in strawberry juice, but more than 6 months in model systems with purified strawberry anthocyanins. Thus, when investigating the color instability of anthocyanins-containing food products, one should consider the contribution of other added or endogenous compounds in the environment as well as the stability of the anthocyanins.

Thermal degradation of anthocyanins starts with the hydrolysis of sugar moieties with further degradation by scission into phloroglucinaldehyde (cyanidin, pelargonidin), 4-hydroxybenzoic acid (pelargonidin), and protocatechuic acid (cyanidin), the residuals of the A- and B-rings, respectively (Sadilova and others 2006).

2.6. Principles and mechanisms for stabilizing red pigments

Several mechanisms and strategies have been proposed and/or studied for helping retain red pigments during food processing and storage. These include copigmentation of anthocyanins, formation of metallo-anthocyanins complexes, chemical transformation of anthocyanins, and stabilization of anthocyanins by embedment, and are discussed in the following sections.

2.6.1. Intramolecular and intermolecular copigmentation – effects of concentration, structure, pH, medium, and temperature

Copigmentation is a phenomenon in which a cofactor having no color itself stacks with anthocyanins (Figure 2.8) and intensifies or modifies the initial color of the anthocyanin solution. The color enhancement effect of copigmentation has been known theoretically as early as 1916 and 1931. Since then, almost all phenols have been shown to have cofactor potential (Brouillard and others 1997). In addition to phenolics, compounds belonging to any chemical family that posses a planar, π-electron rich ring structure such as purines and alkaloids (Asen and others 1972) can be effective cofactors.

2.6.1.1. Effects of type and concentration of anthocyanins

The sugar free portion of the anthocyanin molecule can be interspersed between two copigment molecules in a 1:2 ratio complexation, which is called a sandwich type configuration (Dangles and Elhajji 1994). However, in the case of anthocyanin glycosides, possibly because of steric hindrance from sugar substituents, the copigments will have access to only one side of the planar aromatic faces of the colored anthocyanin moiety and thus will associate 1:1 with anthocyanins (Brouillard and others 1997).

The copigment effect can be measurable only when anthocyanin content reaches a certain concentration, known to be approximately 10^{-6}-10^{-5} M (Asen and others 1972; Brouillard and others 1997; da Silva and others 2005). The required cofactor-to-anthocyanin molar ratio is normally in the range from 10 to 100 for simple phenolic acids and (+)-catechin (Asen and others 1972; Brouillard and Dangles 1994). In the case of more effective cofactors such as rutin, copigmentation may be expressed at a level as low as 1:1 (Brouillard and Dangles 1994).

Scheffeld and Hzadrina (1978) investigated copigmentation between malvidin derivatives (malvidin-monoglucoside, diglucoside, and acylated-malvidin glycosides)

and rutin, and reported that malvidin 3,5-diglucoside had a stronger copigmentation with rutin than its 3-monoglucoside form (Asen and others 1972). Thus, the difference in number of position of sugar on the anthocyanin was the main reason of the increased copigmentation while the nature of the sugar did not play a significant role on the phenomena (Asen and others 1972; Chen and Hrazdina 1981). Eiro and Heinonein (2002) have also echoed that the increased number of position of sugar on the molecule will increase the copigmentation strength.

In regards to the anthocyanidin moiety, it has been shown that the number of hydroxyl and methoxyl groups, which varies among the six major anthocyanidins (Figure 2.7), influences the electron-donating capacity of the molecule, thus the extent of copigmentation (da Silva and others 2005).

2.6.1.2. Cofactors

Color stabilization and variation is believed to occur to a different extent with different types of cofactors (Brouillard and others 1997). The most efficient cofactors with anthocyanins such as cyanidin glycosides that have been reported in the literature are glycosides of flavonols (rutin and quercetin) (Mazza and Brouillard 1987). The next most effective groups are the phenolic acids (Mazza and Brouillard 1990; Mazza and Miniati 1993). Flavanols such as catechin show less strong compigmentation effects (Mazza and Miniati 1993). Since pear peels contain flavonols (quercetin glycosides (Spanos and Wrolstad 1992), isorhamnetin (Schieber and others 2001), phenolic acids (chlorogenic and p-coumaric acid, coumarylquinic, caffeic acid) (Spanos and Wrolstad 1992), and flavanols (catechin, epicatechin) (Spanos and Wrolstad 1992), some degrees of copigmentation are expected to occur between cyanidins and those copigments in its peels as has been found on apple peels by Lancaster et al. (1994).

2.6.1.3. pH

Change in pH can cause transformation in the structure not only of anthocyanins, but also of other phenolic compounds, and possibly the copigment complex. The pH may predetermine the molecular structures and affinity of the partners involved in the copigmentation (Houbiers and others 1998; da Silva and others 2005).

At lower pH, most of the anthocyanins are in the colored flavylium form and their complex with cofactors shows only a slightly observed hyperchromic shift along with the typical bathochromic shift (Brouillard and others 1997; da Silva and others 2005).

At higher pH, a large proportion of the anthocyanins are in the colorless hemiketal form that transforms into the colored flavylium cation/quinonoidal base system by complexing with the cofactors, and yielding significant hyperchromic and bathochromic shifts (Brouillard and others 1997; da Silva and others 2005). Copigmentation establishes another equilibration between colorless and colored anthocyanins at higher pH in favor of the colored form. The form of cofactors such as phenolic acids also changes with pH change according to their dissociation pKa, thus showing various manifestation of copigmentation due to the actual dissociated forms of the cofactor at different pH conditions (Rein and Heinonen 2004; da Silva and others 2005). In addition, the colorless form of anthocyanin molecule such as Z-chalcone can also complex with cofactors (Dangles and Elhajji 1994) or associate with the flavylium colored form at high anthocyanin concentration (Houbiers and others 1998). At relatively high pigment/cofactor molar ratios, flavylium–cofactor will be favored as the complex has high binding forces (Dangles and Elhajji 1994). The Z-chalcone – cofactor is easier to form at a low ratio of pigment/cofactor, but the complexation is not strong enough to cause loss of color in the colored forms (Dangles and Elhajji 1994).

2.6.1.4. Various molecular-stacking types

Wigand and others (1992) investigated molecular association by use of ^{1}H NMR spectroscopy between gallic acid and a flavylium chloride, and found that all anthocyanin protons are shifted up-field in the presence of gallic acid. This observation proved that anthocyanins experience the magnetic ring-current effects of gallic acid, and is evidence for the vertical stacking of pigment and cofactor (Brouillard and others 1997). Some dimer or oligomer cofactor esters of gallate, such as galloyl ester of D-glucose (tannic acid or ellagictanin) (Mistry and others 1991) or dimer procyanidin B2-3”-O-gallate (Berke and de Freitas 2005) also have an ability to form a pocket that contains anthocyanins (Figure 2.9), which enhances its effectiveness of copigmentation.

Unlike intermolecular copigmentation, which occurs between anthocyanin and other different molecules, intramolecular copigmentation can only be found for those acylated anthocyanins possessing at least one cofactor residual in their structure, commonly a cinnamic acid (Figure 2.10) (Giusti and Wrolstad 2003). Yet, at the molecular level, the characteristics of both phenomena are similar (Jackman and Smith 1996). Acylated anthocyanins are found mostly in flowers and some vegetables such as red radish (Giusti and others 1998), red cabbage, black carrot (Stintzing and others 2002), red-fleshed potato (Rodriguez-Saona and others 1999), and purple yam (Yoshida and others 1991), but not in fruits; an exception is that acylated anthocyanins, in comparatively low concentrations, are found in some berries such as grapes. No artificial acylated anthocyanins have been known to be created during processing. Thus, in spite of their potential use as colorants (Giusti and Wrolstad 2003), further discussion on this type of anthocyanins with respect to pear fruit will not be done here. At high milli-Molar concentrations, anthocyanins can vertically stack together in a manner similar to copigmentation, which is called self-association (Hoshino and others 1982) and protects the anthocyanin from hydration. The phenomenon is likely to happen only *in vivo* as the pigments are likely to be diluted during processing.

2.6.1.5. Attractive forces

Although the effectiveness of a co-pigment is believed to be related to its planarity and the potential surface area available for “pi-pi” stacking (Figure 2.11), other attractive forces between different pi systems are important (Mistry and others 1991). Multiple attractive interactions between pi-systems have been rationalized for the existence of molecular association, and the question of what the major attractive interactions influence complex stability has been a controversial issue. The association in anthocyanin copigmentation is commonly believed to be initially driven by hydrophobic effects in aqueous solution (Brouillard and others 1991; Mistry and others 1991). Water molecules solvating the aromatic surface have a higher energy than bulk water, resulting in face-to-face stacking (Brouillard and others 1997) of the aromatic surfaces, which reduces the total surface area exposed to the solvent (Hoeben and others 2005). However, the hydrophobicity of the anthocyanin cation and/or the cofactor could not be explained from the equilibrium constants of complex formation and the resulting copigmentation effect (da Silva and others 2005). Hunter and Sanders (1990) developed a practical model for π-π interactions, which is entirely electrostatic. Recently, Sinnokrot and Sherrill (2004) studied the effects of various substitutions of aromatic ring on molecular association and indicated that electrostatic, dispersion, induction, and exchange-repulsion contributions should all be significant to the overall binding energies in π-π interactions; all but induction are important in determining relative energies. Da Silva and others (2005) studied charge transfer from the cofactor to the anthocyanin and found that the difference between the electron affinity of the anthocyanin and the ionization potential of the cofactor is useful to predict the copigmentation strength. Nevertheless, Hunter and Sanders (1990) stated that charge-transfer transitions observed for such complexes are a consequence, not a cause of the π-π interaction. The involved interactions are believed to be weak and exothermic, which explains the loss of the copigmentation effects found at high temperature (Brouillard and others 1989; Bakowska and others 2003).

2.6.1.6. Effect of temperature on copigmentation in retaining anthocyanin during thermal process

In cases where processing is done with no or minimal use of heat, such as winery operations, copigmentation is believed to favor pigment extraction by modifying the equilibrium between different forms of anthocyanins and stabilizing the pigments. Copigmentation can be responsible for a third of the color of aged wine (Darias-Martin and others 2007). The color of berry juices with added phenolic acids were found to be enhanced during storage compared with the control with no cofactor fortification (Rein and Heinonen 2004). New compounds can also form during storage, as has been shown for both juice (Rein and Heinonen 2004) and wine. In the latter case, results have shown that anthocyanins enter in chemical transformation (de Freitas and Mateus 2006) and copigmentation can provide an initial step in the formation of new stable pigments (Brouillard and Dangles 1994). High temperature, however, is known to diminish the copigmentation effects (Brouillard and others 1989) and displace colored forms with unstable forms that are prone to degradation under high temperature conditions. Thus, copigmentation by itself would not be expected to be effective in retaining anthocyanin pigments during thermal processing such as in canning fruits.

2.6.2. Metallo-anthocyanins complexation

It has been known that metal ions can interact with anthocyanins bearing *o*-substituted hydroxyl groups. With the presence of cofactors, metal ions are reported to strengthen copigmentation (Dangles and others 1994) and, in some cases, the copigmentation may only occur in the presence of metal ions (Jurd and Asen 1966). Since not much work has been done on the influence of metal ions on the color of fruits, many of the phenomena are mostly interpreted based on the observation made on flower pigments. In protocyanin, a stable blue complex pigment from the blue cornflower, Fe^{+2}, Mg^{+2}, and Ca^{+2} ions located in the center of the molecule and surrounded by two other components (cyanidin and flavone) which are self-associated

and stacked in a intermolecular interaction (Figure 2.12) (Shiono and others 2005; Takeda and others 2005). Changes in the concentration of calcium in solution can affect the yield of formed protocyanin (Takeda 2006). In another example, the formation of commenilin, a pigment from the blue flowers of *Commelina communis*, requires a delphinidin, a flavonoid, and Mg^{+2} and K^{+}. Even though the Mg ion is replaceable by various metal ions such as Mn^{+2}, Co^{+2}, Ni^{+2}, Zn^{+2}, or Cd^{+2} (Takeda 1977), the stability of the pigment in acidic environment will be modified (Takeda 2006). On the formation of metallo-anthocyanin, very fine and strict molecular recognition, including chiral molecular recognition, occurs (Ellestad 2006). The length and nature of the sugar moiety are critical factors for the formation of the metallo-anthocyanin (Takeda 2006), while the metal coordination sites in the center of its structure serves as a template for the assembly of the pigment (Ellestad 2006). Thus, unlike copigmentation where the nature of sugar attached to the molecule is not important and hydrogen bonding is often negligible, the chelation of anthocyanin and cofactors, molecular stacking between the π-π systems, and hydrogen bonding between the chiral sugars are all important factors in this type of pigmentation (Ellestad 2006). In these flower pigments, anthocyanins are believed to be in the colored quinonoidal form (Takeda 2006) and strongly bound to metal cations (Brouillard and others 1997).

Elhabiri et al. (1997) studied the complexation of aluminum with anthocyanin and reported that at a low pH of 2-3, the anthocyanin is mostly in the flavylium form and little metallo-complexation occurs. At higher pH, aluminum chelates with anthocyanin which is now in quinonoidal form (Figure 2.13) (Elhabiri and others 1997). The complexation is in competition with hydration. In the presence of phenolic acids, Al^{+3} is believed to conjugate with the dihydroxyl on the anthocyanin and the hydroxyl group of the quinic acid moiety, with the result that the complex is stabilized in a manner similar to that of acylated anthocyanins in hydrangea flowers (Takeda and others 1990).

In actual food systems such as raspberry juice, complexation is believed to occur between metal ion, anthocyanin and another unknown molecule, not with anthocyanin alone (Coffey and others 1981). In most investigations on fruit skin discoloration, metallic effects are believed to be a cause of discoloration (Cheng and Crisosto 1994). This "inking" effect may be from interactions between metal ions and various phenols (Cheng and Crisosto 1997). In strawberry juice with its low cyanidin glycoside content, the red color generated from metal ions may be from reaction of certain metal ions with unidentified phenols, which are believed unrelated to anthocyanin (Wrolstad and Erlandson 1973). Since studies on the impact of metallic ions on fruit color have not been extensively investigated, two key unanswered questions have been raised by Brouillard et al. (1997): Which metal ions are capable of increasing the cofactor effects? What are the compounds accompanying the anthocyanin that can stabilize the pigment?

2.6.3. Chemically transformed anthocyanin in the presence of other phenolics and reactive compounds

Another way to preserve pigmented anthocyanins is to chemically transform the pigments into more stable chromophores (Fulcrand and others 2006). As the pH of fruit products is normally in the acidic range, multi acid-catalyzed reactions between electrophile and nucleophile can occur when phenolics from the cells are mixed with each other as well as with those compounds formed during processing or added to the system.

2.6.3.1. Electrophilic and nucleophilic reactivity of fruit phenolics

A hydroxyl group on the aromatic system directs the electrons to *o*- and *p*-substituted positions (Figure 2.14) which can lead to electrophilic aromatic substitutions found in phenols. The presence of a second or third hydroxyl activates even more phenol reactivity such as in the case for resorcinol and phloroglucinol (Figure 2.15). Gallic acid possesses an enhanced reactivity toward electrophilic

aromatic substitution compared to phenol (Garro-Galvez and Riedl 1997). The A-ring shared by pear flavonoids is a phloroglucinol type structure possessing two electron-rich sites that can participate in electrophilic substitution reactions. Anthocyanins have several reactive sites that can participate in both electrophilic and nucleophilic substitutions. Anthocyanins can undergo nucleophilic attack on C-2 and C-4 of the flavylium form, or react with electrophilic groups at C-6 and C-8 of the hydrated form (de Freitas and Mateus 2006) (Figure 2.16) to be transformed into pigmented and non-pigmented higher molecular weight polyphenols.

2.6.3.2. Case studies in wine and berry juice

The formation of these compounds has first and most thoroughly been studied in wines (de Freitas and Mateus 2006). The fermentation process in wine making promotes the formation of covalent bonds between anthocyanins and phenolics, which are thought to be brought to a proximity thanks to copigmentation (Brouillard and Dangles 1994). Acetaldehyde bridging can accelerate this reaction. In a recent study, Rein et al. (Rein and others 2005) added phenolic acids to berry juices and identified stable anthocyanin derivatives that were formed during storage. Reactions of this type were reported to take place over a significant time period (Morata and others 2006). There are bathochromic or hypsochromic effects depending on the structural change of the chromophore moiety (de Freitas and Mateus 2006). These compounds form condensation products together with oxidation enzymatic processes may explain the changes in color and anthocyanin reactivity during aging of red wine (Brouillard and Dangles 1994; Monagas and others 2005; Fulcrand and others 2006).

In model wine solutions there are at least six identified types of reaction involving the interaction of anthocyanins with phenols (Timberlake and Bridle 1976).

1) Reactions between anthocyanins and phenolics/tannins
2) Transformations of phenolics alone
3) Degradations of anthocyanins alone
4) Reactions between anthocyanins and acetaldehyde

5) Reactions between anthocyanins, acetaldehyde and phenolics/tannins

6) Reactions between phenolics and acetaldehyde.

Catalyzed by the fruit's acidity, the nucleophiles and electrophiles react to form a multitude of compounds. It is likely that all of these reactions occur to some extent in fruit processing such as in winemaking. The resulting pigments have been revealed to be formed either from anthocyanins and small reactive compounds, or between anthocyanins and flavanols in the presence or absence of aldehydes (de Freitas and Mateus 2006) or between anthocyanins and anthocyanins (Atanasova and others 2002).

Anthocyanins (A) could react both as nucleophiles and electrophiles in wine to form direct covalent bonds between themselves or with flavanols (F) (Fulcrand and others 2004; Duenas and others 2006) (Figure 2.17). The direct reactions between a flavanol as an electrophile (F-) and an anthoycanin as a nucleophile (A+) are strongly related to pH as F-A+ is obtained at pH 2.0 and A+-F at pH 3.8 (Fulcrand and others 2006). The reactions between anthocyanins and smaller molecular weight molecules (mono-, oligomer) predominate more than those with larger molecules (proanthocyanidin) (Remy and others 2000). Temperature is another determining factor of the reaction (Fulcrand and others 2006) as when flavanol di-, tri-, or tetramers react with anthocyanins at pH 3 and at high temperature, only A+-F was found (Malien-Aubert and others 2002). Anthocyanin-tannin compounds are known to be unstable in acidic condition, and anthocyanin can be released (Kennedy and Hayasaka 2004).

Pyranoanthocyanins resistant to bisulfite bleaching can be formed by reaction between anthocyanins and small compounds such as pyruvic acid and acetaldehyde (Morata and others 2006), or phenolic acids such as ferulic acid and sinapic acid (Rein and others 2005) (Figure 2.18).

Reactions between anthocyanins and flavanols can also be mediated by aldehydes, giving rise to various types of pigments such as anthocyanin-alkyl-flavanol pigments (Pissarra and others 2004) (Figure 2.19), pyranoanthcyanin-flavanol

pigments (de Freitas and Mateus 2004) (Figure 2.18), vinyl-pyranoanthocyanin-flavanol pigments, or portisins (Figure 2.20). Reactions between anthocyanins and aldehydes such as acetaldehyde in wine model solutions can also yield dimeranthocyanins in the absence of flavanols (Figure 2.21). It has been known that an aldehyde such as acetaldehyde produced from alcohol during wine fermentation, can act as a strong electrophile in acidic condition and combine with tannin. The resulting alcohol product can undergo dehydration, forming a vinyl compound that combines with an anthocyanin to create a secondary brick-red pigment. By mediation of aldehyde, the condensation between phenolics proceeds at a faster rate than the direct condensation (Monagas and others 2005). However, ethyl bridged anthocyanin-proanthocyanidin products are believed to be unstable and readily converted to other pigmented materials (Kennedy and Hayasaka 2004). The reaction is pH dependent and a lower pH will lead to the formation of higher amounts of new pigments in a shorter time (Pissarra and others 2003). In comparing various aldehydes, researchers found that steric hindrance should be the major factor influencing the reactivity of the different aldehydes with anthocyanin and catechol (Pissarra and others 2003). In conditions of excess aldehyde, the amount of pigmented polymer formation is believed to be much higher than when aldehyde is equimolar with anthocyanin and catechin (Baranowski and Nagel 1983).

Compared to direct condensation, the condensation reaction mediated by aldehyde proceeds at a higher rate and causes a high degree of polymerization that will eventually precipitate (Monagas and others 2005). Nevertheless, all reactions proceed slowly over a time span of weeks, months, or even years to yield a significant amount of reaction products under normal processing and storage conditions (pH and temperature).

In general, reactions are expected to be pH dependent, because the electrophilic and nucleophilic characters of the anthocyanins and phenolics, such as flavanols, are strongly related to pH (Duenas and others 2006). Formation of pyranoanthocyanins and flavanyl-pyranoanthocyanins are facilitated by lower pH

(Fulcrand and others 2004). Transformed anthocyanins have higher stability toward pH change than anthocyanins (de Freitas and Mateus 2006) as the structure of pyranoanthocyanin protects them from hydration of the C-ring. This later compound is also resistant to bisulfite bleaching since the C4 position is no longer available for bisulfite attack (Figure 2.18).

The interactions between phenolics and tannins can result in polymerization and large macromolecules that are able to form a structure to host and stabilize anthocyanins through an embedment mechanism that is presented in a separate section.

2.6.4. Phenolic condensation of tannins, and phenolics mediated by aldehyde and catalyzed by metal ions

Aldehydes have been known to crosslink phenolic monomers, and the repeated condensations should produce large polymers or even a resin. Tannins, including condensed tannins and hydrolysable tannins which are phenolic in nature, should undergo a similar catalytic reaction with formaldehyde and smaller phenols (Pizzi 1979b), which leads to the formation of polymers that are well studied in non-food areas such as wood adhesive science.

2.6.4.1. Phenolic condensation

The mechanism of the reaction is a Friedel-Crafts-type reaction (Fulcrand and others 2006) in which formaldehyde or other aldehyde under acid conditions will first be protonated and thus become a strong electrophile. In the presence of nucleophilic phenolics, the substitution will proceed slowly. In the case of small phenolics and flavonol monomer, aldehyde can thus mediate the formation of various oligomers as presented in Figure 2.23. As seen with the low stability of the products of reaction between anthocyanin and flavanols mediated by aldehyde, products first formed from the aldehyde-mediated flavanol-flavanol interactions underwent decomposition and

recombination, giving various bridged oligomers (Es-Safi and others 1999). Repeated reactions will form high-molecular-weight polymers that precipitate.

2.6.4.2. Three dimension structures

For large molecules such as hydrolysable (gallotannin) or condensed tannins (proanthocyanin), protonated aldehyde can initiate cross-linking and condensation of the phenolics depending on the reaction conditions. In case of gallotannin, formaldehyde reaction at the *o*- position of a sufficiently large number of galloylated rings of tannin, would form a three dimensional structure (crosslinking) (Özacar and others 2006). For condensed tannins, however, because of their large size and shape, the tannin molecules become immobile at a low level of condensation with formaldehyde (Pizzi 1979b). Without the presence of medium size reactive phenolic molecules that can join these large size molecules, they are too far apart for further methylene bridge formation. The polymerization of condensed tannins is thus incomplete and weak (Pizzi 1979b). Fortification or copolymerization of the tannins with various phenolics is a method of obtaining longer cross linking agents to overcome the problem (Pizzi 1983).

2.6.4.3. Roles of alcohols, metal ions, and acids

The process of phenolic condensation mediated by aldehydes can be controlled by addition of alcohols to the system. Under these conditions, some of the formaldehyde is stabilized by the formation of hemiacetals if methanol is used (Figure 2.22). This ensures that less formaldehyde is volatilized when the reactants reach curing temperature (Pizzi 1983).

Many authors have emphasized the role of acidic pH conditions in promoting the condensation reactions. Pizzi (1979b), however, reported the use of metal ions to both increase the crosslinking, hence an increase in the size of the polymers and the rate of the reaction. The author has stated that while the B-rings of flavanoids do not normally participate in the reaction with formaldehyde except at high pH values, the

addition of metal ions to the reaction mixture induces the B-ring to react with formaldehyde at low pH. All di- and tri- valent metals can act to different degrees, as accelerators of the reactions (Pizzi 1979b). Chelating is the most likely mechanism that involves the primary formation of a metal/formaldehyde complex in the presence of a phenol (Figure 2.23) (Pizzi 1979a). The metals do not change their valence state and catalyze the reactions in a manner similar to hydrogen ions except for the fact that the effect is much stronger than that of hydrogen ions because of their higher charge and greater covalence (Pizzi 1979a). In the case of tannins, Pizzi (1979b) found that the quantities of metal ions and formaldehyde presence would dictate the magnitude of cross-linking.

2.6.5. Stabilization of anthocyanins by embedment/incorporation

Anthocyanins have been reported to be stabilized by inclusion with host materials that are able to present an acidic environment (Kohno and others 2007). These hosts can be aggregates of alginate and pectin (Hubbermann and others 2006), amphiphilic molecules such as negative micelles of sodium dodecylsulphate (SDS) (Lima and others 2002), or a solid matrix of clays as ion exchange materials (Kohno and others 2007). Until today, only a few studies have been done to verify the ability of micellar systems to stabilize anthocyanins (Mulinacci and others 2001).

Alginate and pectin are believed to have the ability to electrostatically interact with the anthocyanin flavylium cations through their dissociated carboxylic groups (Hubbermann and others 2006). Due to this association anthocyanins may be protected from water attack, which leads in turn to color stabilization (Hubbermann and others 2006). In fact, based on studies made on SDS aggregates, Lima and others (year) have proved that the incorporation of anthocyanins on negative micelles of SDS resulted in a preferential stabilization of the cationic form of anthocyanin with respect to the neutral base, causing changes in the two key equilibria that affect color, deprotonation and hydration (Lima and others 2002). The number of available negative charges and the presence of an organized distribution of the negative charges on the micellar

surface appear to be necessary conditions to achieve the anthocyanins' stability and color retention (Mulinacci and others 2001).

Corn starch has been reported to have some positive effects on anthocyanin stability (Hubbermann and others 2006), but the mechanism has not been revealed. Dangles et al. (1992), however, described the fading effects of cyclodextrins which are known to have the ability to produce inclusion complexes with anthocyanins. The starch was proposed to catalyze the slow chalcone E-Z isomerization and to include the compound while desolvating it.

In summary, as anthocyanins exist as various structures (glycosides, acylated glycosides, etc.) and in different forms (flavylium, hemiketals, chalcones) together with other organic compounds (e.g. monomers, dimers, and polymers of flavonoids, polysaccharidess, proteins) and possibly with metal ions as well in food systems and in nature, the color of natural products and anthocyanin-rich foods involves different mechanisms that stabilize the original anthocyanins or modify the pigments to make them more stable. In red pear peels, the native anthocyanins do not possess an acylated structure, thus are unstable when exposed to processing, especially thermal processing conditions. The possible mechanisms to protect the anthocyanins in processed pear peels from degradation are as follows.

Firstly, the pigments may be stabilized through non-covalent interactions to form supra-molecular pigments through self-association of anthocyanins, association of anthocyanins with other simple phenolics, or metallo-anthocyanins complexation such as commelinin. These association and complexation phenomena, however, will be nullified at high processing temperatures. Secondly, anthoycanins can be incorporated in aggregates of amphiphilic molecules. This much less well known stabilization strategy is similar to the previous strategy which relies on the formation of molecular complexes capable of inhibiting or hindering the nucleophilic attack of water on the flavylium cation, thereby stabilizing the pigments. There are no such effective structures found in the natural pear peels and there have been no existing

methods developed for processed fruits. Thirdly, effective stabilization might be obtained by structural changes of anthocyanins through formation of covalent bonding between anthocyanins and other phenolics or organic compounds. This chemical transformation of anthocyanins has been extensively studied over the last century in order to understand wine color evolution. However, the process is known to be very slow, and the use of catalysts such as those described in resin formation in wood science literature should be considered. A fourth possibility is to apply metallo-complexation to precipitate and stabilize color in a similar manner to that which occurs with flower pigments. Alternatively, the solution to color stabilization of pear anthocyanins may lie in various combinations of the above four mechanisms.

2.7. References

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Table 2.1 - Distribution of cell layers containing anthocyanins in red pear varieties

Variety	Nonpigmented cell layers	Cell layers containing anthocyanins	Estimated % of surface with red color	Estimated intensity
Seckel	1-2	4-7	20-40	Medium-high
Anjou	1-2	1-4	10-20	Low
Max-Red Bartlett	2-3	2-7	60-80	Medium-high
Rosi-Red Bartlett	1-2	3-4	70-80	Low-medium
Red Bartlett, P.I. 258948	3	3-4	70-80	Medium-high
Starkrimson	-	All epidermal cells	99	Very high

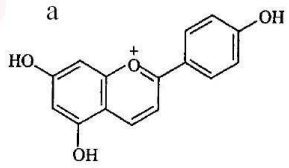
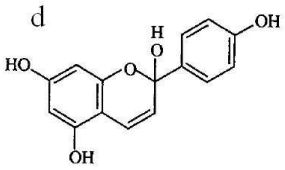
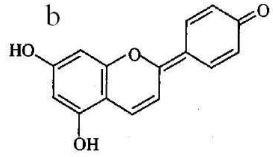
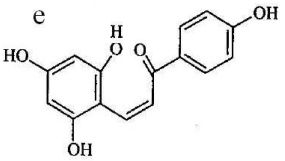
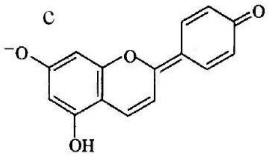
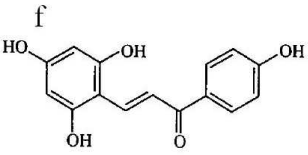
Modified from Dayton (1966)

Table 2.2 - Concentration of Chlorophylls a, b, Pheophytins a, b and Pyropheophytins a, b in fresh, blanched and heated spinach processed at 121 ^{o}C for various times.

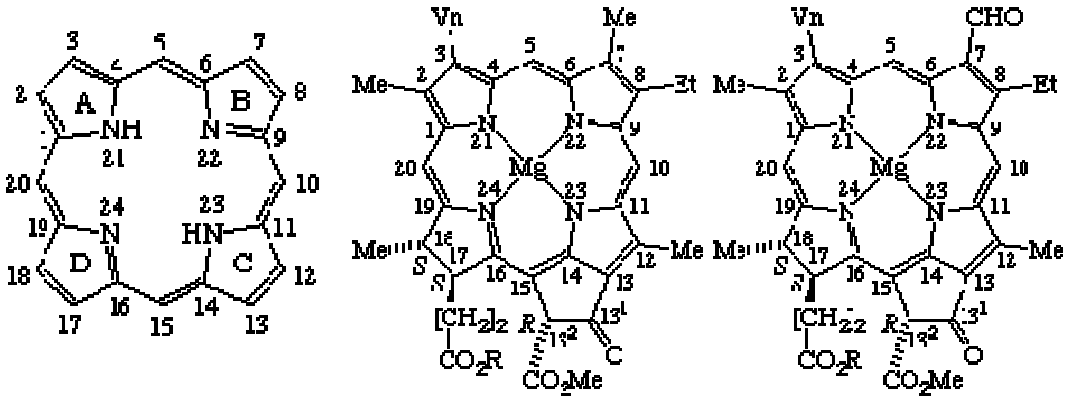
(mg/g dry weight)	Chloro-phyll a	Chloro-phyll b	Pheophy-tin b	Pheophy-tin b	Pyrophe-ophytin b	Pyrophe-ophytin a
Fresh	6.98	2.49				
Blanched	6.78	2.49				
Processed						
2 min	5.72	2.46	1.36	0.13		
4	4.59	2.21	2.20	0.29	0.12	
30		0.24	2.45	0.66	1.74	0.57
60			1.01	0.32	3.62	1.24

Modified from Von Elbe and Schwartz (1990)

Table 2.3 - Structures of apigeninidin at pH values of 7 or lower

Structure	pH range	Structure	pH range
a 	**< 3**	d 	**2-7**
b 	**3-6**	e 	**2-7**
c 	**6-9**	f 	**2-7**

a) Flavylium cation AH^+ b) Neutral quinonoidal bases A; c) ionized quinonoidal bases A^-; d) Hemiactal e) Open neutral species d) retrochalcone C_E f) retrochalcone C_Z. Modified from Brouillard (1994)



Porphin Chlorophyll a Chlorophyll b

Figure 2.1 - Chemical structure of porphin and chlorophylls.
Modified from von Elbe and Schwartz (1996)

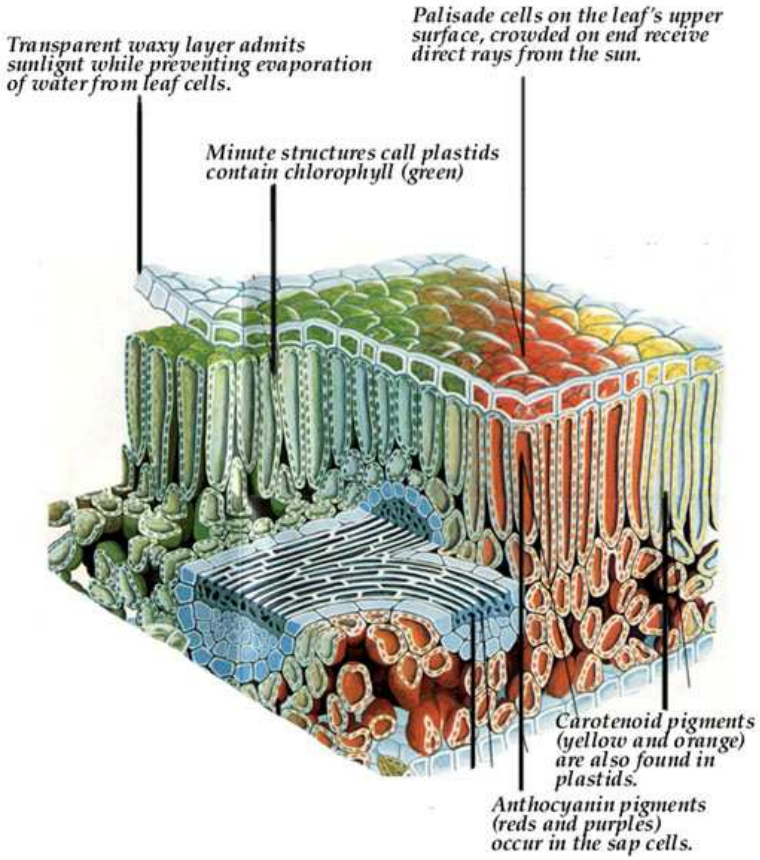


Figure 2.2 - Cross-section of the exterior part of a leaf showing the distribution of the three major pigments in a plant.
Modified from the internet source http://www.vtfpr.org/resource/foliage/chgcolor.htm

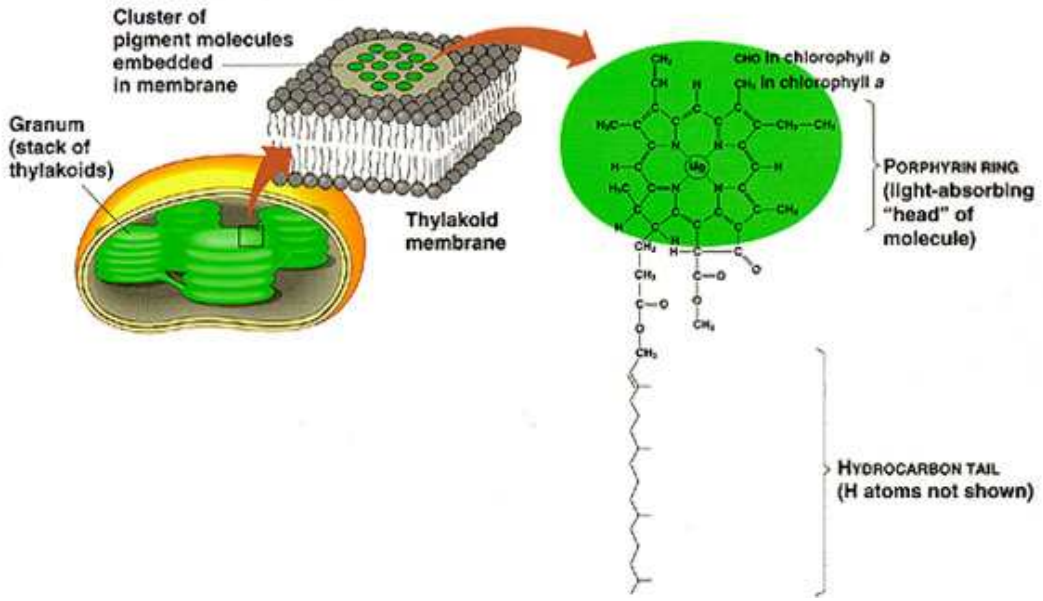


Figure 2.3 - Chlorophylls are embedded in the cell chloroplast system
(From http://www.ualr.edu/botany/chlorophyll.jpg)

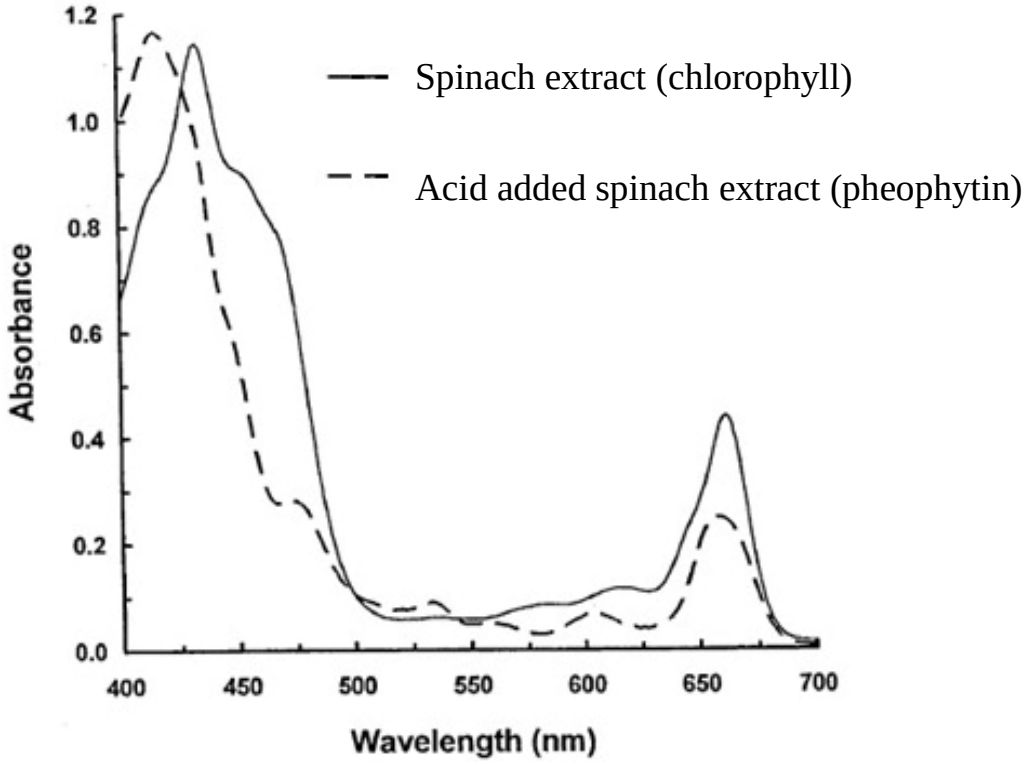


Figure 2.4 - Visual light absorption spectra of spinach extract of chlorophyll a and b and pheophytin a and b.
Modified from Wrolstad (2000b)

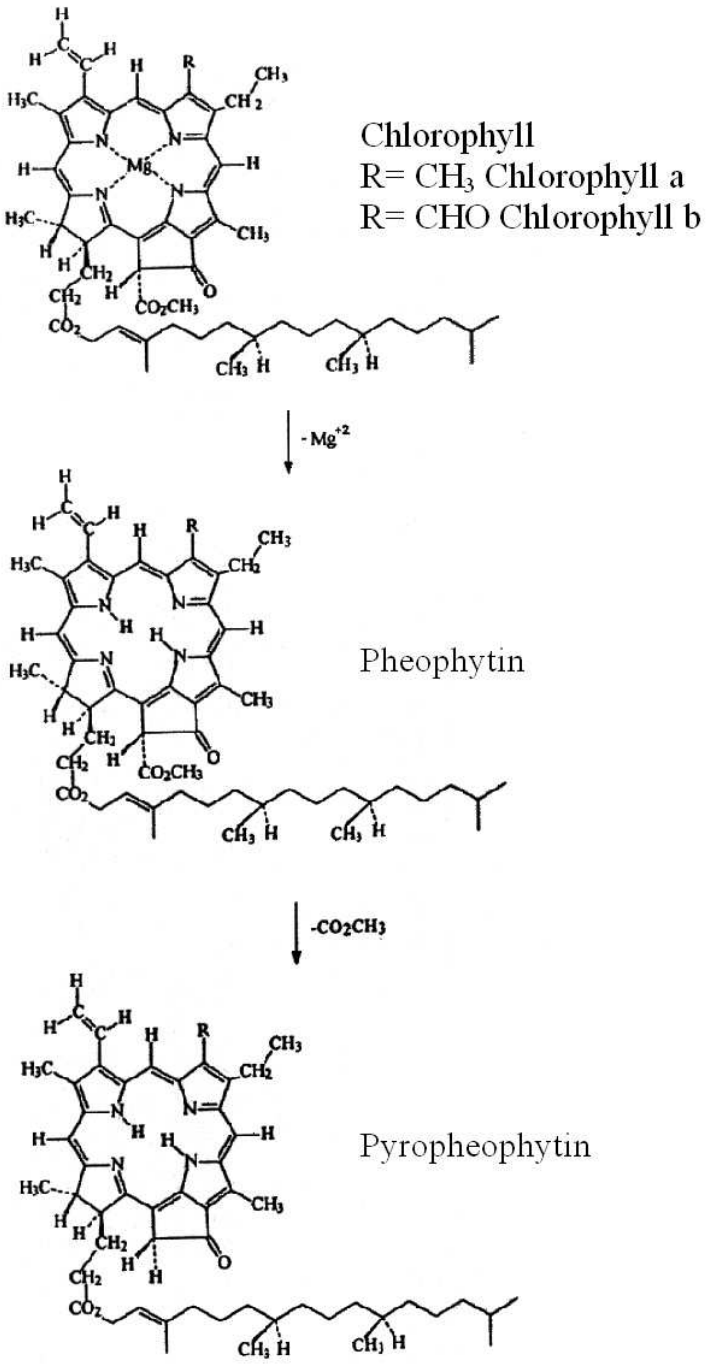


Figure 2.5 - Consecutive degradation of chlorophylls by heat
Modified from von Elbe and Schwartz (1996)

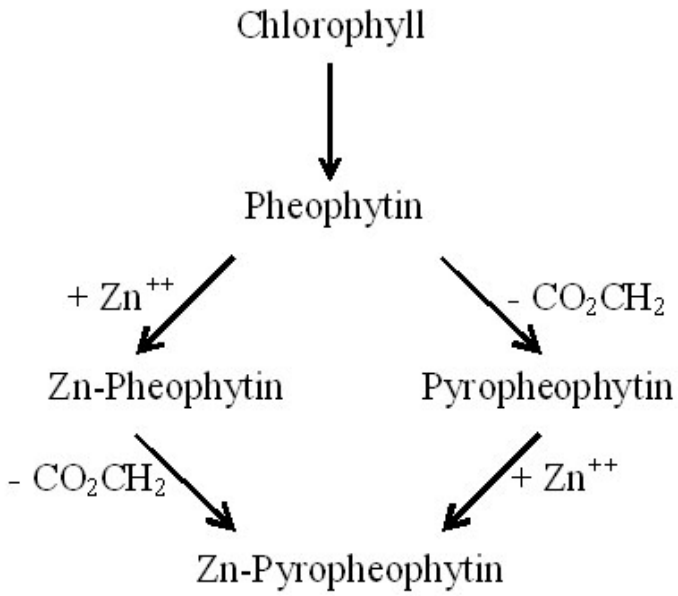
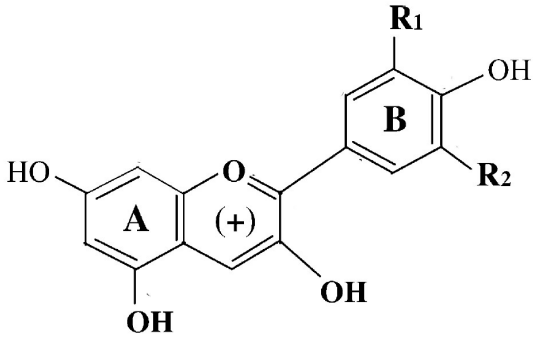


Figure 2.6 - Degradation of chlorophyll and formation of Zn derivatives during heat process.
Modified from von Elbe and Schwartz (1996)



Aglycon	R_1	R_2	Color
Pelargonidin	H	H	Orange
Cyanidin	OH	H	Orange-Red
Peonidin	OMe	H	Orange-Red
Delphinidin	OH	OH	Red
Petunidin	OMe	OH	Red
Malvidin	OMe	OMe	Bluish-red

Figure 2.7 - Major anthocyanins in fruits and vegetables
Modified from Giusti and Wrolstad (2003)

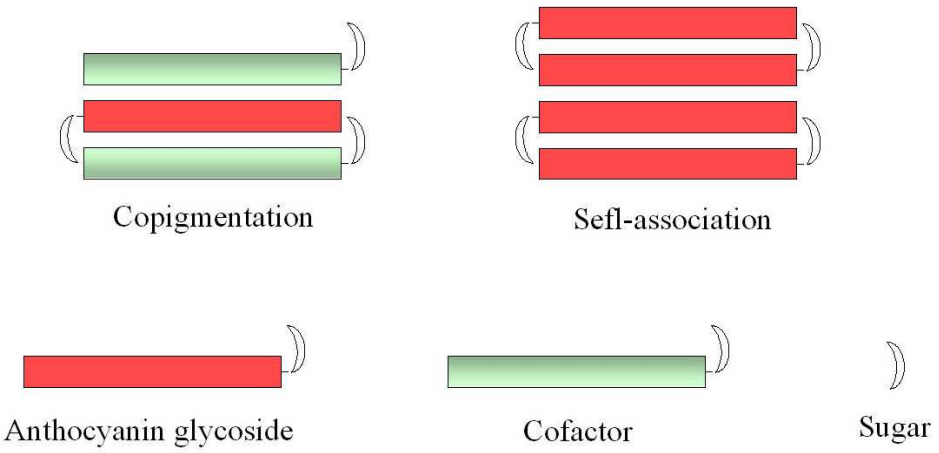


Figure 2.8 - Structural schemes of anthocyanin-phenolic and anthocyanin-anthocyanin association
Modified from Jackman and Smith (1996)

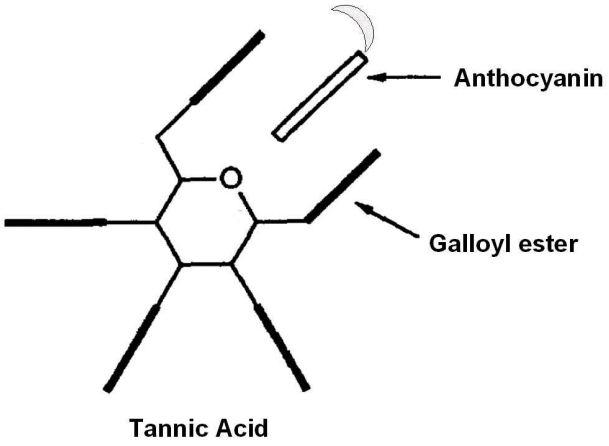


Figure 2.9 - Graphic description of a pocket formed by tannic acid enclosing anthocyanin
Modified from Mistry et al. (1991)

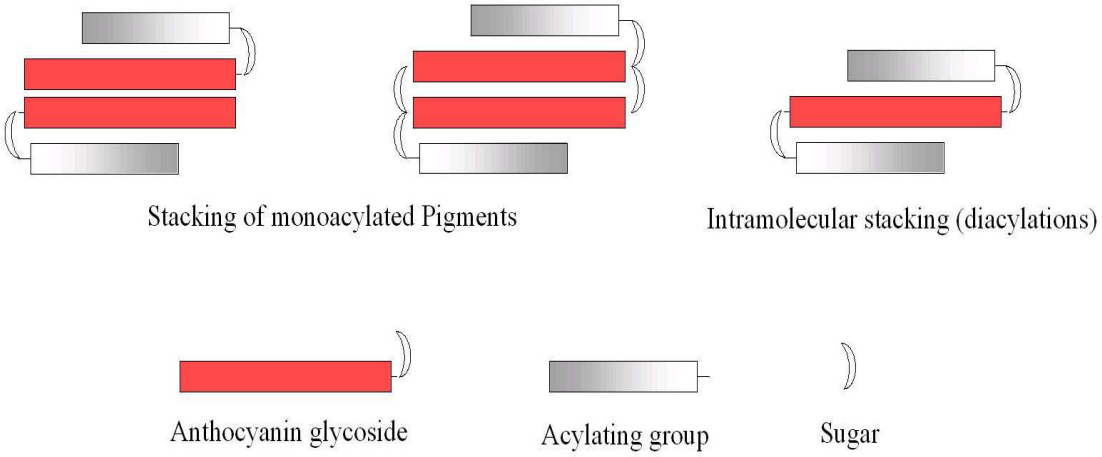


Figure 2.10 - Configuration and stacking of acylated anthocyanins
Modified from Giusti and Wrolstad (2003)

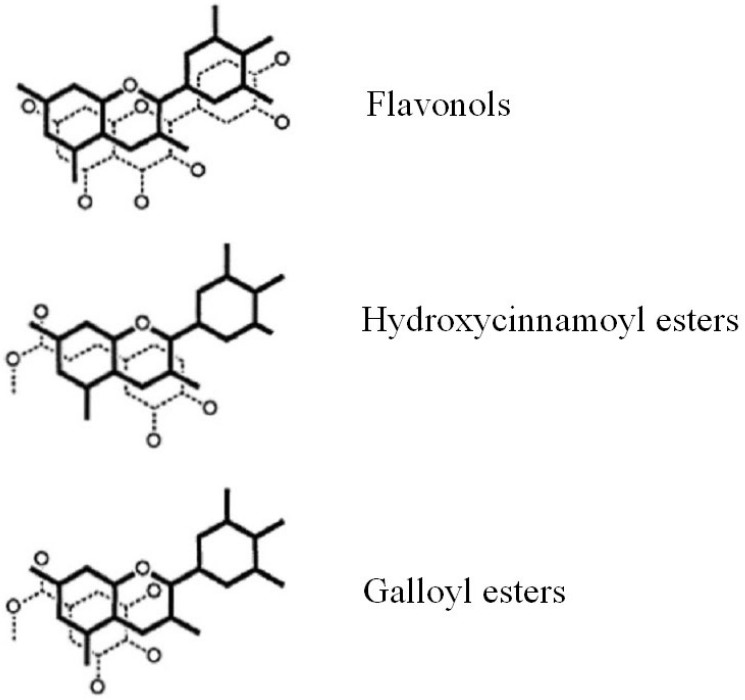


Figure 2.11 - Overlap π-π of anthocyanin with flavanol and phenolic acids.
Modified from Mistry et al. (1991)

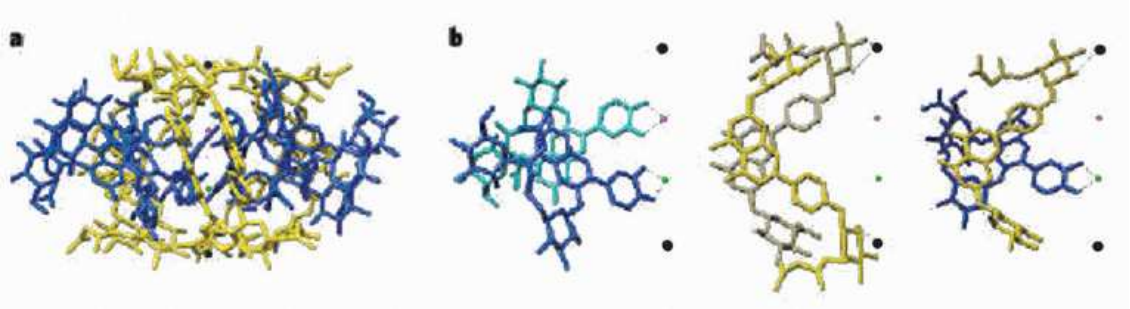


Figure 2.12 - Crystal structure of protocyanin in blue flower. a, side view of the whole structure. Blue, anthocyanin; yellow: flavone glycoside; red spheres: Fe^{3+}; green spheres: Mg^{2+}; black spheres: Ca^{2+}. b, side views of stacking pigments pairs. Left: one anthocyanin molecule binds Fe^{3+} while the other binds Mg^{2+}; centre: two flavones each bind to one Ca^{2+}; right: flavone and anthocyanin molecules bind to Ca^{2+} and Mg^{2+}, respectively. Source: Shiono et al. (2005)

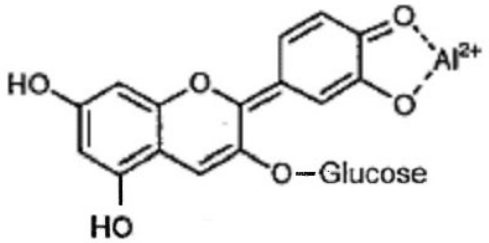


Figure 2.13 - Aluminium complexation with cyanidin 3-glucoside.
Modified from Danlges et al. (1994)

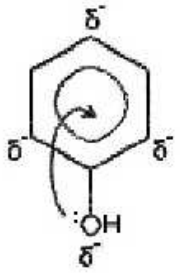


Figure 2.14 - Activating effects in phenol.
Modified from Fulcrand et al. (2006)

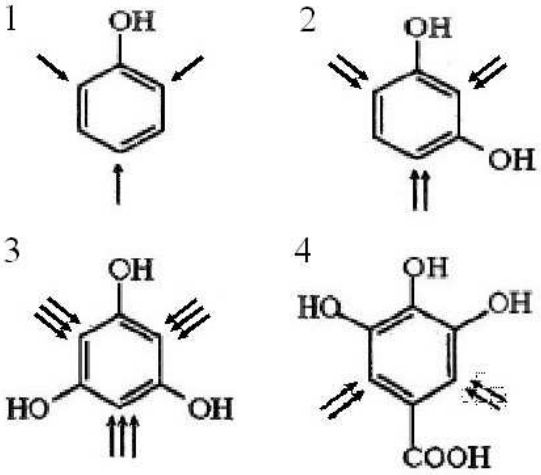


Figure 2.15 - Activating effects in 1. phenol, 2. resorcinol, 3. phloroglucinol, and 4. gallic acid under the electron displacement effects of the phenol group.
Modified from Garrol et al. (1997)

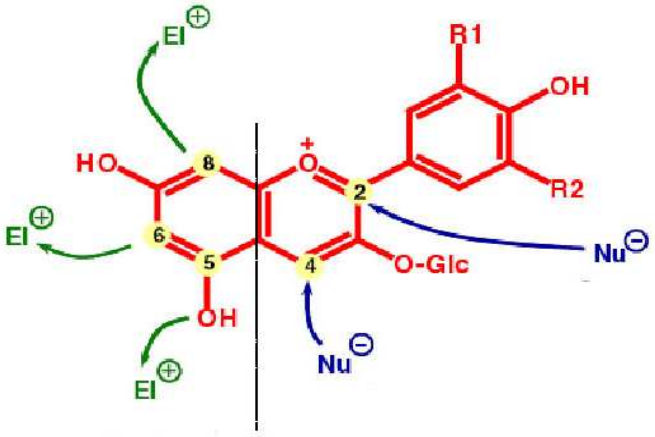


Figure 2.16 - Main reaction positions on anthocyanin. Nu^-: sites can be under nucleophilic attack (the anthocyanin is in its flavylium form) ; El^+: sites can be under electrophilic attack (the anthocyanin is in its hemiacetal form).
Modified from Freitas et al. (2006)

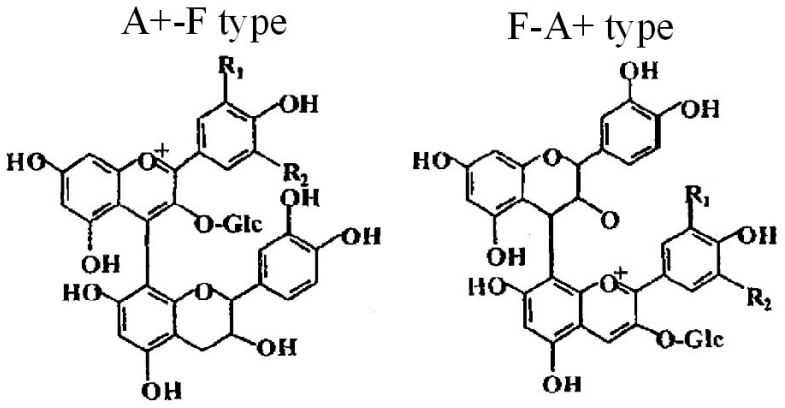


Figure 2.17 - Transformed anthocyanins from direct reactions between Anthocyanin (A+) and Flavanol (F) to form A+-F or F-A+ type pigments.
Modified from Fulcrand et al. (2004)

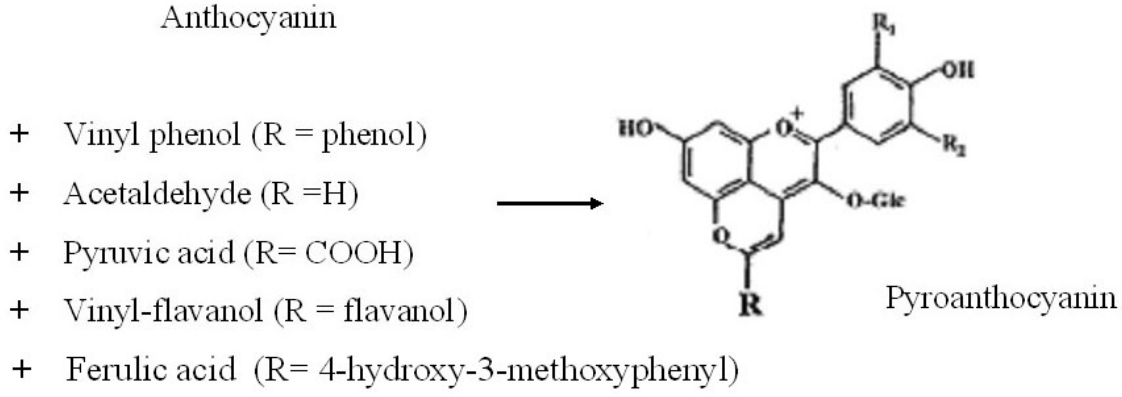


Figure 2.18 – Transformed-anthocyanin forms from reactions with small compounds.
Modified from Fulcrand et al. (2004)

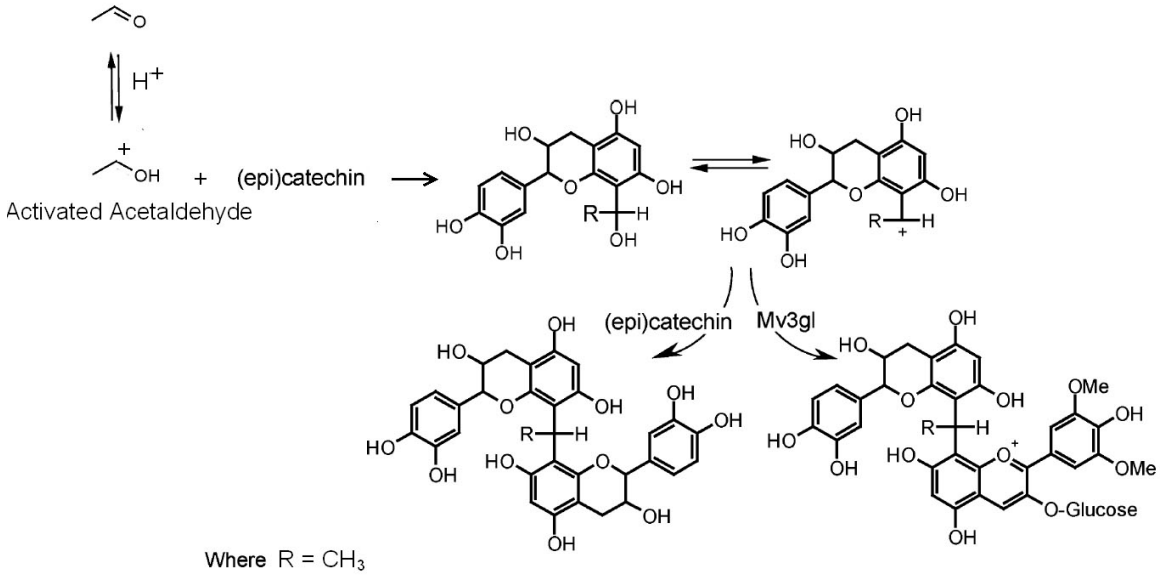


Figure 2.19 - Reaction of glyceraldehydes with catechin and epicatechin and malvidin in acidic media.
Modified from Laurie and Waterhouse (2006)

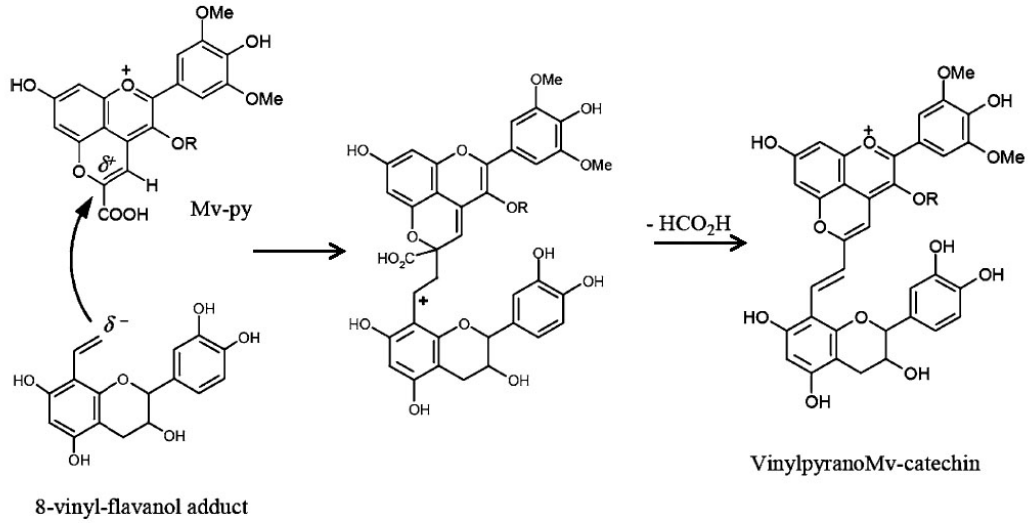


Figure 2.20 - Formation of pyroanthocyanin (portisin or vinylpyranoMv-catechin) from the reaction between anthocyanin (Malvidin)-pyruvic acid adducts and flavanols mediated by acetaldehyde.
Modified from De Freitas and Mateus (2006)

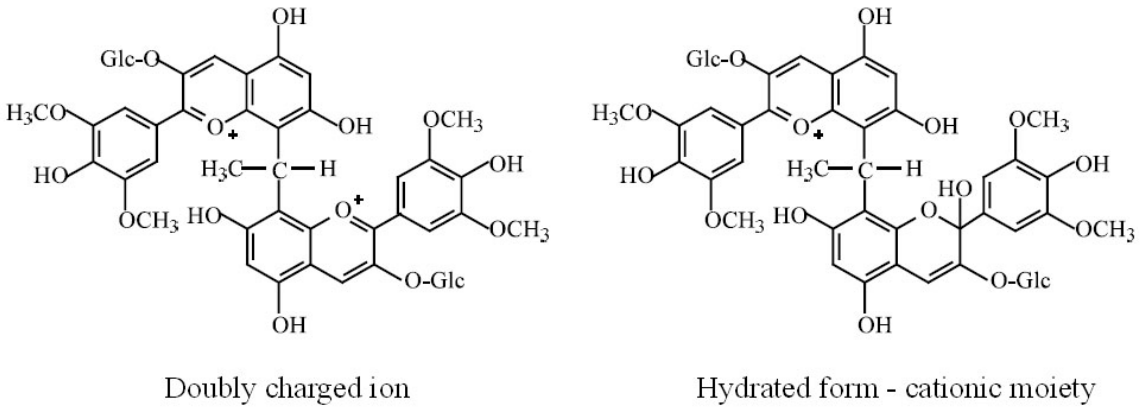


Figure 2.21 –Structure of methyl methane-linked anthocyanin (Malvidin) dimers.
Modified from Atanasova2002

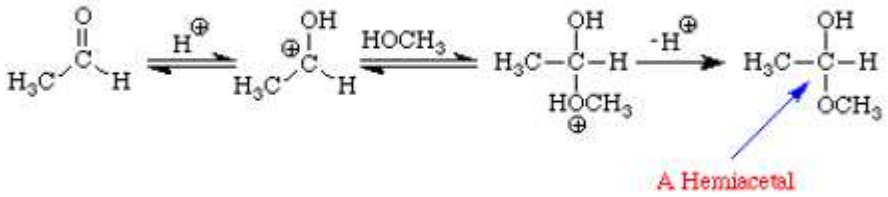


Figure 2.22 – Formation of a hemiacetal from the addition of a methanol to a formaldehyde.
Modified from Pizzi (1983)

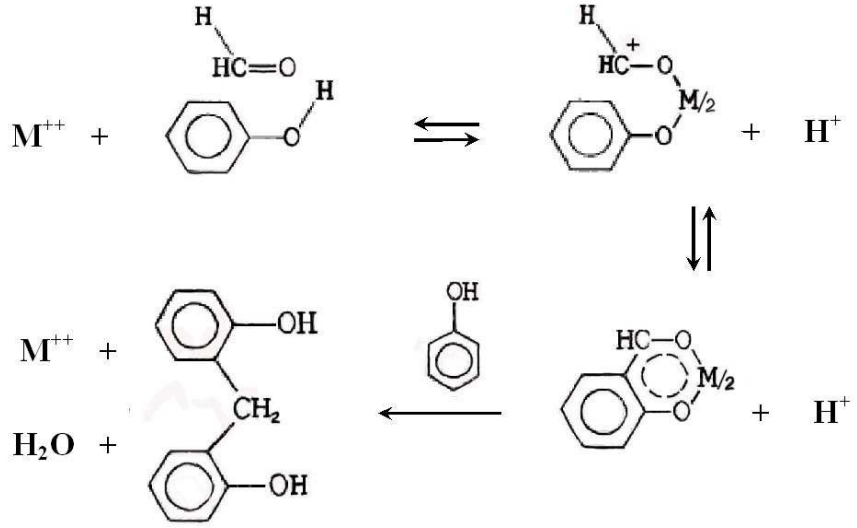


Figure 2.23 - Electrophillic substitution reaction mediated by aldehyde and divalent metal ion.
Modified from Pizzi (1979a)

CHAPTER 3

Retaining Green Pigments on Thermally Processed Peels-on Green Pears

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Publication in Journal of Food Science
Volume 70 Issue 9 Page C568-C574, November 2005

3.1. Abstract

The interest in developing peels-on thermally processed (canned) green pears has prompted the investigation of retaining green pigments during thermal processes. Use of zinc ions as a processing aid for the retention of green pigment and surface pretreatment to remove waxy layer and a part of the top cuticle layer of the peels for enhancing reactions of zinc ions with chlorophylls in peel tissues were studied. Whole pears or pear chunks were subjected to zinc treatment during presoaking in 5,200 ppm Zn^{2+} solutions for 60 min, or blanching at 94 ^{o}C for 6, 12, and 18 min in a 1,300, 2,600, or 5,200 ppm Zn^{2+} solution for identifying the most appropriate procedures for retaining green peel pigments. Pears were then thermally processed in glass jars at 94 ^{o}C for 20 min following commercial canning practices. Canned pears were subjected to a shelf-life test in rooms set at 10, 21, and 38 ^{o}C under fluorescent light. CIELAB L*, a*, and b* color values of pear samples were monitored during storage up to 35 weeks. Results showed that surface pretreatment is essential for ensuring the reactions of zinc ions with green pigments, thus retaining green pigment during thermal treatment. Blanching pears in zinc solution containing about 1,300 ppm Zn^{2+} was effective in obtaining attractive green colored canned pears. Hue angle and -a* (greenness) values of canned pears were significantly ($P<0.05$) associated with blanching time and zinc concentration in blanching solutions, and remained stable over 19 weeks of storage under intensive illumination at all three temperatures. The technology used zinc ions as a processing aid, thus significantly minimizing the zinc content in the final processed pears.

3.2. Introduction

Thermal processing of green fruits and vegetables often results in a shift of attractive green color to yellow olive due to sequential and irreversible degradation of chlorophylls in aqueous solution (von Elbe and Schwartz 1996). Initially, the magnesium atom in the chlorophyll is displaced by hydrogen ions, forming pheophytins. Prolonged heating in commercial sterilization causes subsequent decarbomethoxylation of pheophytins to form pyropheophytins (LaBorde and von Elbe 1994a; von Elbe and Schwartz 1996; Weemaes and others 1999). The olive-brown pheophytins and pyropheophytins are the pigments usually shown on heated green fruits and vegetables (von Elbe and Schwartz 1996).

Several techniques have been investigated and/or applied for helping retain green pigments in thermally processed (canned) fruits and vegetables. These include using high temperature and short time, adding alkalizing agents in canning solutions, and blanching prior to the canning process (von Elbe and Schwartz 1996). Formation of green derivatives of chlorophyll, such as chlorophyllides, through enzymatic conversion has also been proposed (von Elbe and Schwartz 1996). While these methods have been reported to retain green color immediately after treatment or within a short storage time, the green pigments are unstable during long periods of storage (LaBorde and von Elbe 1994a; von Elbe and Schwartz 1996). Moreover, some treatments cause tissue softening and flavor change in the product (von Elbe and Schwartz 1996).

Application of Zn^{2+} or Cu^{2+} during thermal processing of green vegetables, such as green beans, peas, and spinach, has helped preserve green pigments throughout processing and storage (Leake and others 1992; von Elbe and Schwartz 1996; Theuer and Richard 2001). The principle of this method is based on the reactions between Mg-free chlorophyll derivatives, such as pheophytin or pyropheophytins, and zinc or copper ions. It has been found that the two hydrogen atoms within the tetrapyrrole nucleus of these chlorophyll derivatives are easily displaced by zinc or copper ions. Further heating increases the zinc pyropheophytin

concentration at the expense of a decrease in zinc pheophytin (von Elbe and Schwartz 1996). The formation of the metallo chlorophyll derivative complexes depends on Zn^{2+} concentration, chlorophyll concentration, and pH value of product (von Elbe and Schwartz 1996). For example, in spinach purees, zinc complex formation does not occur in purees containing less than 25 ppm Zn^{2+} (von Elbe and Schwartz 1996). The yielded zinc complex concentration is also proportional to the chlorophyll concentration in the vegetables (LaBorde and von Elbe 1990). In respect to pH, zinc complex formation increases in purees with a pH between 4.0 and 6.0, but decreases at pH value of 8.0 or greater (LaBorde and von Elbe 1994 a and b). The newly formed pigments are very similar to chlorophylls in color, but much more thermally resistant and stable in a low-pH environment than the chlorophylls (Tonucci and von Elbe 1992; von Elbe and Schwartz 1996). LaBorde and von Elbe (1996) proposed a technology to improve color quality of green beans by blanching the vegetables with an aqueous solution containing zinc ions and then packing them into a container with an aqueous packing solution. This technology was reported to precisely control the amount of zinc present in the final products.

For nutritional and aesthetic reasons, it is desirable to prepare color-stabilized thermally processed peels-on green pears. Unfortunately, the heat in thermal processing results in many changes to the physical characteristic of the pears. The chlorophyll found in the skin of green pears turns brown when heated. The resultant olive color is much less desirable than the bright green of fresh pears. As a consequence, pears are normally peeled prior to thermal processing leaving the familiar yellow or tan or white product usually associated with canned or bottled pears. Many nutritional constituents such as phenolics are concentrated in the peels, thus retaining fruit with peels-on is nutritionally more beneficial to consumers than consumption of peeled fruit.

There has been no published research or patents on the application of zinc ions to retain green color of thermally processed green fruit. Due to different nature and surface characteristics of pears from green peas, beans, or other green vegetables, the

current technology that works for green vegetables may not simply directly applied to green pears. This study was driven by the need of developing a new value-added pear product: ready-to-eat, thermally processed “peels-on” color-stabilized green pears packed in clear glass or plastic packaging to generate higher consumer appeal. It was hypothesized that infusing zinc ions into the peels of green pears would help to retain green pigments in the peels during thermal processing. Specific objectives of this study were to investigate the use of zinc ions as a processing aid for retaining green pigments on the peels of thermally processed green pears and to evaluate the color stability of processed pears during storage using an accelerated shelf-life test. This study is expected to help promote production and marketing of packed peels-on green pears, distinctly different from traditional canned pears.

3.3. Materials and Methods

3.3.1. Materials

Three varieties of green pears (*Pyrus communis*, L. Rosaceae), Bartlett, D’Anjou, and Comice were evaluated in this study. They were either provided by a local pear grower, the Diamond Fruit Growers, Inc. (Odell, OR, U.S.A.), or purchased from a local grocery store. Zinc lactate dehydrated salt (PURAMEX ZN) was donated by PURAC America (Lincolnshire, IL, U.S.A.). Ascorbic acid (99.8% pure) was purchased from Mallinckrodt Baker, Inc (Paris, KY, U.S.A.). Granulated cane sugar was manufactured by the C&H Sugar Company, Inc. (Crockett, CA, U.S.A.). Surfactants were Tween 20 (Aldrich Chemical Company, Milwaukee, WI, U.S.A.) and Ajax dishwashing liquid (Colgate-Palmolive Company, NY, U.S.A.).

3.3.2. Sample preparation and surface dewaxing treatment

Whole pears or pear chunks with peels-on were used in this study. To make chunks, pears were first cut in half lengthwise, and then carefully cut crosswise into slices. To prevent browning discoloration of pears during preparation, the pears were

immersed in 1% ascorbic acid solution for coring and dicing into chunks with a dimension of about 2x2 cm^2 in peel area and 1.5 cm in flesh thickness.

For zinc ions to retain green pigments on the peels, it is essential for zinc ions to be able to directly react with chlorophylls in the peel tissues. For achieving this, our preliminary studies found that the surface wax and a part of the top cuticle layer of the peels need to be removed. Attempts to increase the permeability of the peels by removing the waxy layer through washing or brushing were tested. Washing was performed by rinsing whole pears under warm tap water (60-65 ^oC) followed by washing in Tween 20 or Ajax dishwashing liquid solutions before rinsing again with water.

Mechanical means of manually rubbing pear surfaces by knife or spraying whole pears with a beam of sugar grains were also applied. When using a knife, pears were gently brushed with the edge of a knife in the vertical direction until the wax and a part of skin cuticle were removed and the bare green cell underneath was revealed. Pretreatment by spraying with sugar beam was done by shooting a pear surface with a continuous beam of sugar grains generated by a spray gun with sucking air pressure of 1atm (Anderson and Zhao 2005). Treated fruit was afterwards immediately immersed in 1% ascorbic acid solution and diced into chunks of 2x2x1.5 cm^3

3.3.3. Zinc treatment

One of the important goals of this study was to identify the optimal procedures to retain green pigments by infusing zinc ions into peel tissues of green pears, while minimizing zinc content in final products. Zinc application was tested at different processing stages, including presoaking, blanching, hot filling, and canning in solutions containing 0 to 5,200 ppm Zn^{2+}. In addition, presoaking was evaluated at atmospheric pressure or under vacuum (vacuum impregnation, VI). In the VI treatment, samples were immersed in Zn^{2+} solution in a glass jar inside a sealed desiccator subjected to 100 mm Hg by use of a vacuum pump (Model 0211 P204; Gast Mfg. Corporation, Benton Harbor, MI, U.S.A.) for 20 min following the method

described by Xie and Zhao (2003). In atmospheric pressure presoaking, fruits were immersed in Zn^{2+} solution at room temperature for 60 min.

Our preliminary studies showed that the most critical stage of using zinc for retaining green pigments in pear peels is during the blanching process. By properly controlling Zn^{2+} concentration in blanching solution and controlling blanching time, the green pigments can be successfully retained at the end of the canning process. In this case, zinc ions do not need to be added in canning solution, but as a processing aid during blanching, thus minimizing zinc content in the final canned pears.

A two-way completed randomly factorial design with 3 replications was conducted to investigate the effect of Zn^{2+} concentration (1,300, 2,600, and 5,200 ppm) and blanching time (6, 12, and 18 min) on color retention of canned pears. Surface dewaxed peels-on pear chunks (green Bartlett) were placed in blanching solution (94^{o}C) containing Zn^{2+} (1,300, 2,600, and 5,200 ppm) in wide-mouth 1.9-L Mason jars (Alltrista Corporation, Muncie, IN, U.S.A.). A 1:2.5 fruit to Zn^{2+} solution ratio (weight base) was applied. The jars were sealed and put inside a 20-L lab scale retort (Model 25X; All American, Manitowoc, WI, U.S.A.) filled with 13 L boiling water, and heated for 6, 12, or 18 min. The retort has a heater coil at the bottom in contact with the water to maintain water temperature around 94 to 98^{o}C. A mercury thermometer was used to read water temperature inside the retort. After blanching, the jars were cooled under tap water for 30 min to room temperature. Blanched fruit was then placed inside 235-ml Mason jars (Alltrista Corporation, Muncie, IN, U.S.A.) previously filled with distilled water at 100 ^{o}C (1:1 fruit:water ratio, weight base), sealed, and heated at 94 ^{o}C for 20 min in a Precision water bath (SN 601071309; Jouan Incorporation, Winchester, VA, U.S.A.). After heating, the jars were immediately cooled under tap water till reaching room temperature. Color of the peels of thermally processed pears was then measured to determine the effect of zinc concentration and blanching time.

3.3.4. Zinc measurement

For measuring zinc content in the final products, canned pears were pureed by use of a blender and dried in a mechanical convection oven at 80 ºC for 4 days and then grinded into powders. About 0.2 g of dried pear powder was put into a fluorocarbon microwave vessel with 2 ml H_2O_2 (30%) and 2 ml HNO_3 (70%). The digestion was performed with the vessel capped and heated using microwave heating in a discreet flow automated microwave sample preparation system (MDS-2000, CEM Corporation, Matthews, NC, U.S.A.) for 60 min. After cooling, the vessel contents were adjusted to 10 ml for analysis by Inductively Coupled Plasma Optical Emission Spectrometer (Perkin-Elmer dual view, model 3000; Shelton, CT, U.S.A.), which measured characteristic emission spectra by optical spectrometry according to Method 6010B in SW-846 (EPA 2005).

3.3.5. Evaluation of color stability

An accelerated shelf-life study (Labuza 1982) was applied to evaluate color stability of the peels-on thermally processed pears. The peels-on pear chunks (green Bartlett) were first dewaxed by gentle manual friction using a knife, blanched in a 2,600 ppm Zn^{2+} solution for 13 min, and consecutively thermally processed using the same conditions as described above. A randomized block design with 5 sub-samplings at each sampling time and storage temperature and 2 replications (as blocks) was adopted. Six randomly selected pear chunks were packed in each glass jar and stored at temperatures of 10 ± 3, 21± 2, or 38 ± 2 ^{o}C in rooms with fluorescent light for 35 weeks. The surface color of the peels was measured at intervals of 4 weeks. The fluorescent light was used to mimic the effects of lights used in grocery stores. The storage rooms were lit with two 610-mm fluorescent lights (F20T12/Sun GE lighting; General Electric Company, Nela Park, Cleveland, OH, U.S.A.) set up at 50 mm above the samples.

3.3.6. Color measurement

Objective color measurement was conducted using a Hunter Lab spectrometer (Lab Scan II; Hunter Associates Laboratory, Reston, VA, U.S.A.). The instrument parameters used were a mode of 0/45, a 10^{o} Observer, D65 sodium aluminates, a port size of 0.635 cm, and an area view of 0.635 cm diameter. A pear chunk with its peel facing the light beam was placed on the opening of the sample port above the light source and covered with a black box. Color values were recorded in terms of CIELAB L*, a*, and b*. Sample color measurement was performed on 3 to 5 chunks of canned pears per jar. For each measurement, two readings were consecutively performed and mean values were reported. Chroma (C^{*}) and hue angle (h^{o}) values were calculated by use of the formula: $C^{*} = (a^{*2} + b^{*2})^{1/2}$ and $h^{o} = \arctan b^{*}/a^{*}$.

3.3.7. Data analysis

The general linear model (GLM) procedure was applied in testing differences among different treatments and possible interactions of factors using the SAS (Statistical Analysis System Institute Inc., Cary, NC, U.S.A.). Duncan's multiple-range test was used for the multiple means comparison. A 95% confidence level was applied for all statistical analyses.

3.4. Results and Discussion

3.4.1. Pretreatment

A processing scheme that successfully retained green pigment on thermally processed peels-on pears was developed and is described in Figure 3.1. Surface dewaxing treatment and blanching in zinc ion solution are the most critical steps to retain green pigment in canned green pears. Hence, the process consists of surface dewaxing, blanching in zinc ion solution, cooling, and canning (commercial sterilization). Pretreatment to remove the surface waxy layer and a part of the cuticle layer on the peels of the pears is essential for ensuring the reaction of zinc ions with chlorophylls in peel tissues for retaining the green pigment during the thermal process. Figure 3.2 shows canned pears presoaked (vacuum-impregnated) in 5,200 ppm Zn^{2+}

solution with or without surface dewaxing treatment before thermal processing. The pears without surface dewaxing ("1" in Figure 3.2) turned brown after canning, while the dewaxed sample canned in water ("2" in Figure 3.2) retained some degree of green. In contrast, the number "3" pear, canned in 2,600 ppm Zn^{2+} solution has an attractive green color, confirming an effective diffusion of zinc ions into the peels once the surface waxy layer was removed. It is believed that the outer waxy and cuticle layers on the surface are not only the major barriers preventing zinc ions from penetrating into and locating on the peel tissues (Schönherr 2001; Schreiber 2005), but also limited oxygen escape from the pores of the fruit. With plenty of oxygen present under the peels, oxidation reactions were favored during thermal treatment, further enhancing the browning discoloration on the pear peels ("1" in Figure 3.2).

In this study, surface washing of the pears with surfactant or detergent (Tween 20 or dishwashing detergent) did not effectively remove the surface waxy layer, thus yielding brown canned pears (results not shown). Mechanical use of knives or a sugar-spraying system both successfully removed the surface waxy layer of the pears, ensuring the interactions of zinc ions with chlorophyll. The sugar-spray system developed in this study was much more efficient than manual scraping with knives (Anderson and Zhao 2005). A whole pear was uniformly dewaxed in 5-6 s compared with about 10 min when using a knife (Anderson and Zhao 2005), thus it is suitable for commercial applications (patent pending).

3.4.2. Effect of zinc treatment during blanching

The CIELAB L^*, a^*, and b^* and calculated chroma (C^*) and hue angle (h^o) values of fresh and processed green pears (Bartlett) are reported in Table 3.1. Green pigments of control samples (dewaxed and blanched in water without the addition of zinc ions) were mostly destroyed after 12 min heating at 94 ^oC. There were no significant ($P>0.05$) differences in L^* (lightness), b^*(yellowness), and C^* (color intensity) values between treated (dewaxed and blanched in zinc ion solution) and control samples, but significant ($P<0.05$) differences in the $-a^*$ (greenness) and h^o

values, suggesting that the zinc ion blanching treatment significantly ($P<0.05$) retained the green pigment of pears after the thermal process. These results were consistent with those reported by von Elbe and others (1986) on green beans.

Statistical analysis indicated that blanching time and zinc ion concentration are significant factors affecting the $-a^*$ and h^o values of canned pears, and there is also a significant interaction between blanching time and zinc concentration on the L^* values (Table 3.2). Figure 3.3 shows an increase in hue angle of canned pears when increasing blanching time or zinc ion concentration ($P<0.05$). Hue angle has been shown to be a reasonable predictor of sensory perception of green color (Gnanasekharan and others 1992; Lau and others 2000). An angle of 90^o represents a yellow hue. The higher the hue angle, the greener the sample and an increase in hue angle corresponds to a decrease in yellowness (Little 1975). Treated samples at all tested zinc concentrations showed an increase in the hue angle over blanching time of 6 to 12 min ($P<0.05$). These results substantiated previous indications for green peas that increased holding time of zinc treatment resulted in an increase in sample greenness (Canjura and others 1999). However, blanching in 1,300 and 5,200 ppm Zn^{2+} solutions for a longer time (18 min) did not show significant improvement in the hue angle of canned pears compared to treatment for a shorter time (12 min) ($P>0.05$) (Table 3.1), indicating that 12 min blanching in zinc solution is sufficient to retain green pigment on pears. The hue angle of pear peels may be adjusted through the control of blanching time and zinc concentration (Figure 3.3) to achieve different degrees of greenness in the final products.

In this study, the zinc concentration needed for blanching pears to retain green pigments was higher than the amount proposed for vegetables (LaBorde and von Elbe 1990; Canjura and others 1999). Our preliminary tests using lower Zn^{2+} concentrations (50 to 500 ppm) in blanching solution yielded unattractive green pear color (results not shown), suggesting that zinc complex formation in pears does not occur under the condition suitable for vegetables. This might be due to the low concentration of chlorophylls in the fruit peels compared to that of chlorophylls in vegetables, as well

as the slow formation of metallo complexes of zinc ions in pears (von Elbe and Schwartz 1996). The reaction might also be affected by other pear constituents capable of binding metal ions, such as pectin (Gallardo-Guerrero and others 2002), thus not all the added zinc ions were available for reactions with chlorophyll derivatives (Canjura and others 1999).

Thermal treatment is essential when using zinc for retaining green pigments, as heat is needed to dislodge Mg^{2+} and improve the diffusion of Zn^{2+} into pear peel tissues so that zinc complexes can be formed. This explained why pear presoaked in zinc solution at room temperature was not as green as that blanched (heated) in zinc solutions (sample number "2" vs. "3" in Figure 3.2). Samples heated in zinc ion solutions had significantly ($P<0.05$) higher $-a^*$ and h^o values, but lower L^*, C^*, and b^* values than those of dewaxed pears without thermal treatment (Table 3.1). The decrease in the lightness of thermally processed samples might be due to the removal of air in the fine porous structure between the epidermal cells that led to a change in the surface reflecting properties (Woolfe 1979) as generally found in heat-treated plant materials (Cantwell and others 2003). In addition, the pH value of blanching solution changed, from about 6.2 before blanching to 4.3 after blanching. These values fell within the range of pH favoring zinc complex formation suggested by LaBorde and von Elbe (1990). Organic acids released from fruit cells during thermal heating (LaBorde and von Elbe 1994a) would be the main explanation for this decrease in pH value of blanching solutions.

The technology developed in this study uses zinc as a processing aid during blanching, thus it is unnecessary to add it directly into canning solution. This agrees with the remarks made by LaBorde and von Elbe (1996) for green vegetables. In this way, it significantly minimizes zinc level in the final products. Table 3.3 reports the zinc content in the final processed peel-on pears. Depending on the zinc levels used in the blanching solutions, the final zinc content (residual) is 74.57 ± 3.51 and 117.93 ± 7.58 ppm, respectively when 1,300 and 2,600 ppm zinc were used in blanching

solutions, respectively. In both conditions, bright green color was retained in the final products.

Zinc is an essential trace mineral element in the human body. It plays an important role in body growth and development, and helps to maintain a healthy immune system. The World Health Organization (WHO) estimated that zinc deficiency affects around one-third of the world's population, particularly infants, pregnant and lactating women and the elderly. The current DRI (Dietary Reference Index) value for zinc is 15 mg/day for adults. Zinc salt used in this study is zinc lactate (PURAMEX ZN), self-affirmed GRAS (Generally Recognized As Safe) as nutrient supplement in foods and as dietary ingredient in dietary supplements, and has been used in many zinc fortified products including sport drinks, juices, cereals, and baby foods (www.purac.com). Hence, zinc lactate used in the thermally processed pears not only helps retain green color, but also provides additional health benefits.

One concern about the use of some types of zinc salt in food products is developed astringency, a mouth-feeling. The PURAMEX ZN used in this study has a neutral taste and mild flavor, and has been used in many fortified foods. In addition, syrup or juice based canning solutions may help cover the undesirable mouth-feeling. An in-house sensory study was conducted; the product was well received in respect to its sensory quality (data not shown). However, formal sensory study needs to be done to further evaluate the consumer acceptance of this product.

This study did not further investigate the nature of formed green pigment, chromophores. As previously reported by von Elbe and others (1986), zinc pheophytin *a* and zinc pyropheophytin *a* are expected to be the major compounds responsible for the green appearance on zinc-treated samples. More studies need to be conducted to fully investigate the exact compounds contributing to the green pigment as a result of reactions between zinc ions with chlorophylls in green pears.

3.4.3. Color stability of thermally processed pears during storage

Table 3.4 reports the color values of thermally processed peels-on pear chunks during storage at 10, 21, and 38 ^{o}C, respectively. The hue angle of the pear samples did not change ($P>0.05$) during 35 weeks of storage under the fluorescent light at all storage temperatures, giving values in the range between 104 and 106, representing bright green color of canned pears. Similarly, there was no significant change in $-a^{*}$ values at any given temperature during the storage ($P>0.05$). While the samples stored at 10 and 21 ^{o}C showed a slight increase in L^{*} values (lightness) during storage ($P<0.05$), there was no difference in samples stored at 38 ^{o}C ($P>0.05$). Color intensity, as expressed by C* values, decreased along with increased storage time at 21 and 38 ^{o}C (Figure 3.4). The decrease in color intensity represents the fading of sample greenness. It was more pronounced and mostly significant in the first 19 weeks of storage ($P<0.05$). Samples stored at 10 ^{o}C showed no significant changes in color intensity during 35 weeks of storage ($P>0.05$).

There are no published data on the stability of zinc-chlorophyll derivative complexes during product storage. However, it is plausible that observed color changes in the pear peels during storage are related to the degradation of the chromophores due to light. Metallo chlorophyll derivatives upon intense illumination can absorb visible radiant energy from the fluorescent light and become an unstable, excited, singlet-state molecule (Min and Boff 2002). The excited molecule may undergo an intersystem crossing, changing into an excited triplet-state molecule. The triplet state molecule may react directly with an electron acceptor, such as quinones, phenol compounds, and change into free-radical ions (Min and Boff 2002; Berezin and others 2004). Their further conversions might eventually lead to destruction of the porphyrins, causing color loss (von Elbe and Schwartz 1996). The changes in peel color might also be due to the reaction of other compounds present in the fruit materials.

3.5. Conclusion

A procedure for peels-on thermally processed (canned) green pears was developed by appropriate zinc treatment during blanching to retain attractive green pigments in the final product. Surface pretreatment before zinc application to remove wax and part of the cuticle layer is essential for the reactions of zinc ions with chlorophyll in the peel tissues. In the developed procedure, zinc ion was used as a processing aid, thus significantly minimizing zinc content in the final products. The degree of green pigment retention depends on zinc concentration in blanching solution and also on blanching time. The green pigments formed were highly stable during storage, not changing for 19 weeks at 38 ^{o}C under intensive illumination or for at least 35 weeks at 10 ^{o}C.

3.6. Acknowledgement

The authors sincerely appreciate the financial support from the Winter Tree Fruit Research Commission for part of this research and the technical assistance from Mr. Dennis Anderson at Green & Green, Inc., Corvallis, OR.

3.7. References

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Table 3.1 - Effect of blanching time and zinc concentration on the color of thermally processed peels-on pears (green Bartlett)+

Zinc concentration[a] (ppm)	Blanching time (min)	L*	a*	b*	h[ob]	C*[c]
1300	6	49.55 ± 2.47abc	-2.26 ± 1.15d	37.99 ± 1.80b	93.22 ± 1.74e	38.07 ± 1.80bc
	12	48.30 ± 1.91bc	-3.87 ± 1.12cd	36.86 ± 1.36b	95.79 ± 1.74cd	37.08 ± 1.36c
	18	49.42 ± 3.32abc	-4.52 ± 1.87bc	38.73 ± 3.67ab	96.51 ± 2.94bcd	39.04 ± 3.62bc
2600	6	51.47 ± 2.04a	-3.64 ± 0.81cd	38.63 ± 2.24ab	95.24 ± 1.43ed	38.81 ± 2.18bc
	12	50.49 ±2.52ab	-5.85 ± 1.15b	37.83 ± 3.15b	98.66 ± 2.07b	38.30 ± 3.07bc
	18	47.28 ± 2.44c	-7.77 ± 1.28a	38.42 ± 1.91ab	101.24 ± 1.89a	39.22 ± 1.90abc
5200	6	48.06 ± 1.56bc	-5.66 ± 1.68b	39.40 ± 1.84ab	97.97 ± 2.37bc	39.84 ± 1.85ab
	12	48.16 ± 3.12bc	-8.80 ± 1.50a	40.79 ± 1.91a	101.96 ± 1.87a	41.75 ± 1.99a
	18	50.46 ± 2.43ab	-9.41 ± 1.36a	39.16 ± 2.51ab	103.29 ± 1.77a	40.29 ± 2.57ab
Control[d]		49.17 ± 2.48abc	0.32 ± 1.16e	40.51 ± 2.01ab	89.34 ± 1.96e	40.53 ± 1.99ab

Note: + Thermal processing is under commercial canning condition: heating at 94 ^{o}C for 20 min. Reported means (± standard deviations) derived from 3 replications with 3 samples per replication. Means within a same column followed by the same letter were not significantly different ($P<0.05$).

[a] Zn^{2+} concentration in blanching solutions.

[b] h^{o}: hue angle = $\arctan b^{*}/a^{*}$.

[c] C*: chroma = $(a^{*2} + b^{*2})^{1/2}$.

[d] Control samples received the same blanching treatment in water without the use of zinc ions.

Table 3.2 - Selected results from ANOVA tables (p=0.05) for statistical significance and interactions between treatment factors

Source of variation	L*			- a*			b*			h^{o}[2]			C^{*}[3]		
	df[1]	F	P	df	F	P	df	F	P	Df	F	P	df	F	P
Linear terms															
Blanching time (BT)	2	0.67	0.517	2	43.72	<0.0001	2	0.10	0.909	2	40.90	<0.0001	2	0.49	0.612
Zinc concentration (ZC)	2	0.87	0.425	2	70.48	<0.0001	2	4.88	0.010	2	57.53	<0.0001	2	8.49	0.005
Interaction term															
BT x ZC	4	4.81	0.002	4	1.75	0.149	4	1.42	0.237	4	1.25	0.229	4	1.50	0.210
Model	8	2.79	0.009	8	29.55	<0.0001	8	1.95	0.065	8	25.23	<0.0001	8	3.00	0.006
Error	72			72			72			72			72		
Corrected total	80			80			80			80			80		

[1] df = Degree of freedom.
[2] h^{o}: hue angle = arctan b^{*}/a^{*}.
[3] C*: chroma = $(a^{*2} + b^{*2})^{1/2}$.

Table 3.3 - Zinc content in the thermally processed peels-on pears (green Bartlett)+

Processing conditions	Zinc content in pears (ppm)
Blanching in 1,300 ppm zinc solution prior to canning	74.57 ± 3.51
Blanching in 2,600 ppm zinc solution prior to canning	117.93 ± 7.58
0 ppm zinc (control)	1.31 ±0.59

+ Pears were processed following same procedures as described in Figure 3.1 with a blanching time of 13 min in zinc solution. Fruit and canning solution ratio is 1:1 (wt). Zinc contents of the pears were analyzed after one week of processing.

Table 3.4 - Color of the peels of thermally processed peels-on pears (green Bartlett) during storage at different temperatures+

Storage temperature (^oC)	Storage time (weeks)	L*	a*	b*	h^{o1}	C^{*2}
10 ± 3	0	46.16 ± 1.38a	-8.45 ± 0.73a	34.11 ± 1.49ab	103.72 ± 1.41a	35.15 ± 1.41a
	3	47.70 ± 1.51ab	-9.53 ± 0.92a	36.69 ± 2.49a	104.38 ± 1.55ac	37.92 ± 2.46b
	7	47.65 ± 3.12ab	-9.00 ± 1.14a	32.43 ± 2.79b	105.30 ± 1.58abc	33.66 ± 2.87a
	11	48.11 ± 1.41b	-8.88 ± 1.13a	33.09 ± 3.38b	104.84 ± 1.54ac	34.28 ± 3.44a
	15	49.00 ± 2.02bc	-9.22 ± 0.71a	33.70 ± 3.11b	105.17 ± 1.53abc	34.95 ± 3.05a
	19	49.26 ± 1.00bc	-9.02 ± 0.52a	32.27 ± 2.54b	105.47 ± 1.26ab	33.52 ± 2.49a
	31	50.42 ± 1.76c	-8.70 ± 1.22a	28.96 ± 3.96b	106.46 ± 2.54b	30.33 ± 3.95c
	35	49.85 ± 1.75c	-9.17 ± 0.75a	31.79 ± 2.91b	105.97 ± 1.74ab	33.11 ± 2.83a
21 ± 2	0	47.18 ± 2.52a	-9.26 ± 0.70ac	36.37 ± 2.03a	104.11 ± 1.35ac	37.54 ± 1.94ab
	3	46.79 ± 2.48a	-9.26 ± 1.36ac	34.77 ± 2.57bc	104.67 ± 1.71abc	36.00 ± 2.72abc
	7	46.44 ± 2.29a	-9.63 ± 0.96a	37.22 ± 2.18a	104.30 ± 1.27abc	38.46 ± 2.23a
	11	47.74 ± 2.41abc	-9.37 ± 0.94ac	34.40 ± 3.89bcd	105.19 ± 2.28abc	35.68 ± 3.72bc
	15	47.61 ± 2.73a	-9.25 ± 0.70ac	33.42 ± 2.29cde	105.33 ± 1.75abc	34.69 ± 2.14dc
	19	48.84 ± 2.65bc	-7.60 ± 0.80b	32.23 ± 2.66def	103.12 ± 1.69a	33.13 ± 2.60de
	23	48.29 ± 2.47abc	-8.85 ± 0.95abc	31.60 ± 1.93ef	105.46 ± 1.76abc	32.83 ± 1.90de
	27	48.44 ± 1.09abc	-9.11 ± 0.74ac	30.26 ± 2.56fg	106.62 ± 1.67b	31.62 ± 2.48e
	31	49.61 ± 1.78c	-8.09 ± 1.08bc	31.05 ± 3.13efg	104.50 ± 2.30abc	32.12 ± 3.04e

	35	49.36 ± 2.51c	-8.66 ± 0.81abc	29.29 ± 3.17g	106.02 ± 1.49bc	30.55 ± 3.47e
38 ± 2	0	46.62 ± 1.29a	-9.13 ± 0.77a	36.57 ± 1.89a	103.82 ± 1.27ab	37.70 ± 1.86a
	3	47.70 ± 2.10a	-9.24 ± 0.61a	35.81 ± 2.28a	104.29 ± 1.13ab	36.99 ± 2.24a
	7	46.48 ± 1.52a	-8.73 ± 0.70ac	34.99 ± 3.21a	103.84 ± 1.03ab	36.07 ± 3.22ab
	11	47.11 ± 1.20a	-8.78 ± 0.92ac	33.08 ± 2.10ab	104.66 ± 1.40ab	34.23 ± 2.13abc
	15	47.78 ± 2.32a	-8.01 ± 1.06abc	31.55 ± 3.71bc	104.09 ± 1.81ab	32.56 ± 3.74bcd
	19	48.24 ± 0.93a	-7.73 ± 0.46abc	29.27 ± 2.08c	104.61 ± 0.86ab	30.27 ± 2.08d
	23	48.02 ± 1.19a	-7.93 ± 0.98abc	30.77 ± 1.86bc	104.26 ± 1.76ab	31.79 ± 1.86cd
	27	46.64 ± 2.54a	-8.28 ± 1.15ac	30.38 ± 3.41bc	105.09 ± 1.81a	31.50 ± 3.45cd
	31	47.68 ± 1.51a	-7.75 ± 0.77abc	29.98 ± 1.85bc	104.29 ± 1.34ab	30.97 ± 1.86d
	35	47.68 ± 2.14a	-7.43 ± 0.89b	28.51 ± 2.72c	104.10 ± 1.57b	29.79 ± 2.71d

+ Pears were dewaxed, blanched at 2,600 ppm Zn^{2+} solution for 13 min, and then thermally processed at 94 ^{o}C for 20 min.
Means (± standard deviations) derived from 2 replications with 5 samples per replication. Means within the same column at same storage temperature followed by the same letter were not significantly different ($P<0.05$).
[1] h^{o}: hue angle = $\arctan b^{*}/a^{*}$.
[2] C*: chroma = $(a^{*2} + b^{*2})^{1/2}$.

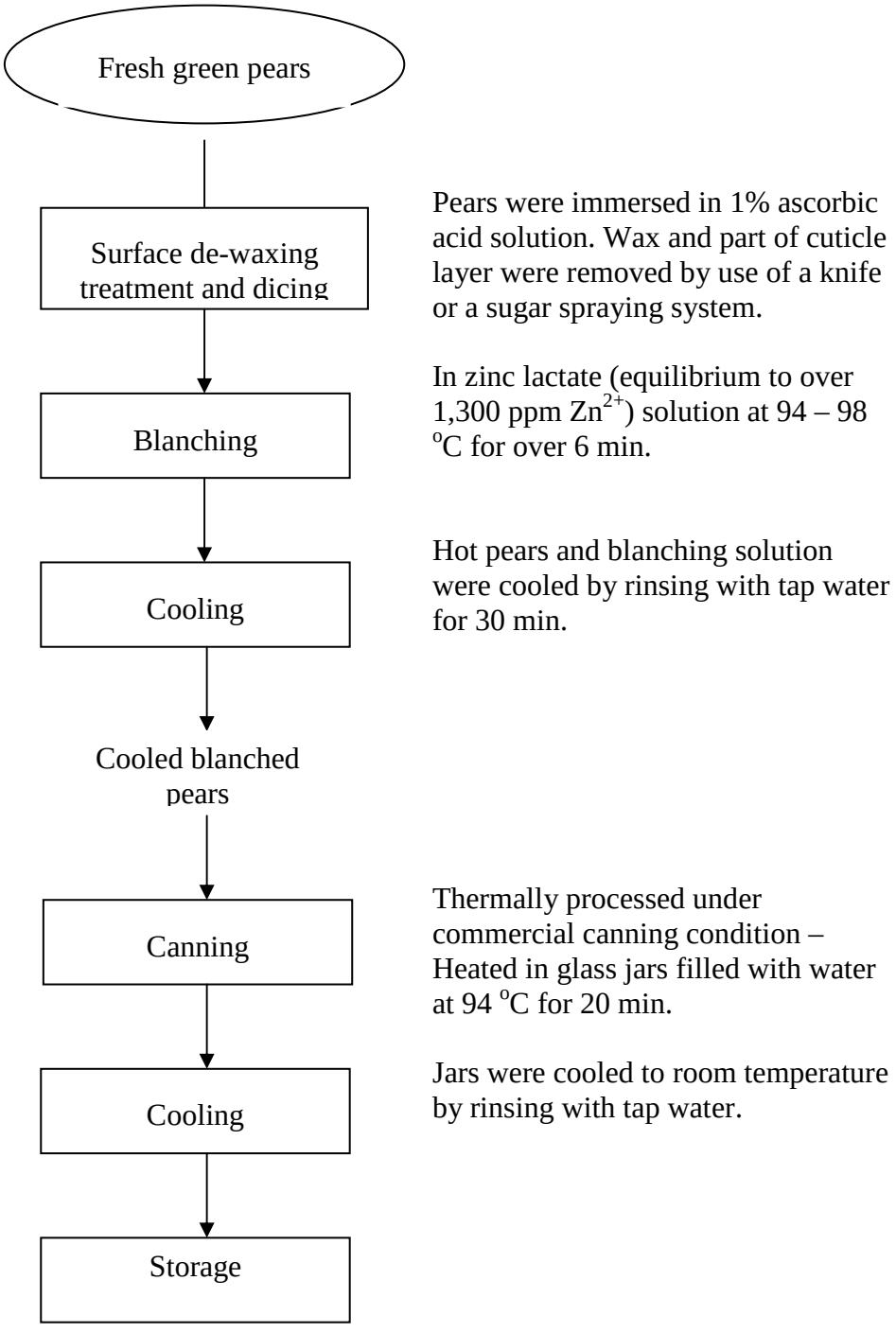

Figure 3.1 - Processing scheme developed for retaining green pigment on thermally processed peels-on green pears

Figure 3.2 - Color of Comice pears thermally processed at 94 ^{o}C for 20 min. 1 - Pear without surface dewaxing treatment; 2 - Pear with surface dewaxing treatment, but canned in water; 3 – Pear with surface dewaxing treatment and canned in 2,600 ppm Zn^{2+} solution. Prior to the thermal process all pears were vacuum-impregnated (VI) in 5,200 ppm Zn^{2+} solution under vacuum at 100 mm Hg for 20 min followed by atmospheric restoration for 40 min.

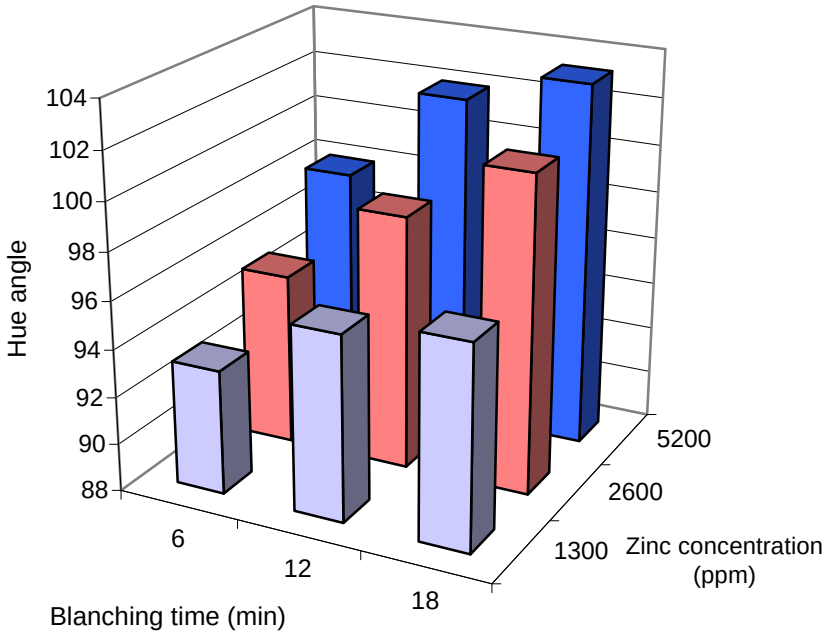

Figure 3.3 – Effect of blanching time and zinc concentration on the hue angle values of thermally processed pear peels (green Bartlett, processed at 94 ^{o}C for 20 min after blanching treatment)

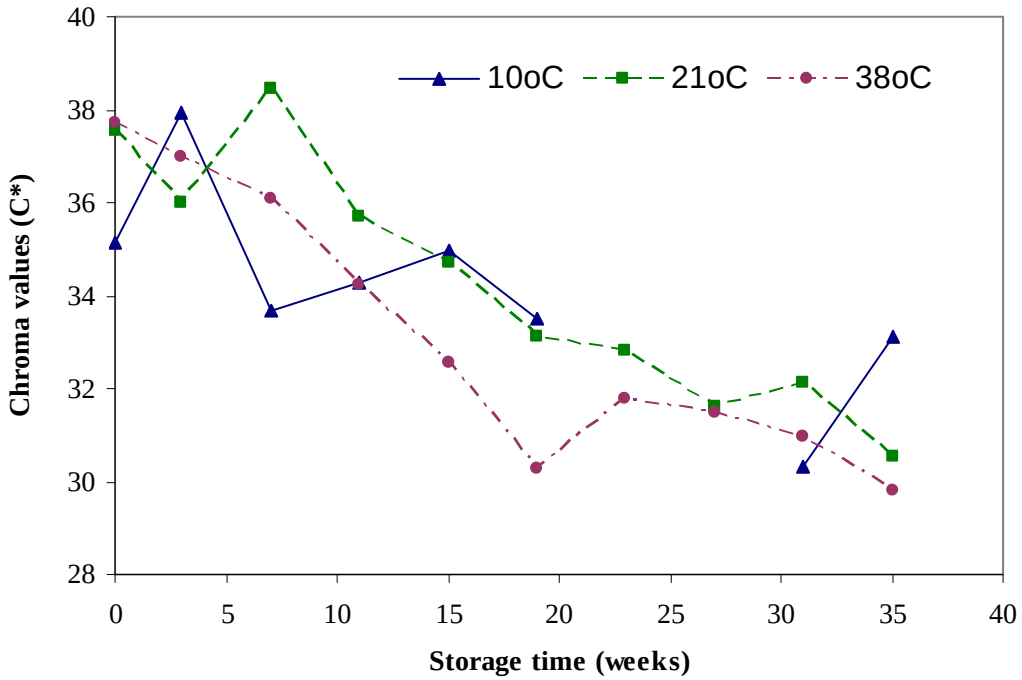

Figure 3.4 - Change in chroma (C*) values on the peels of thermally processed peels-on pear chunks (green Bartlett, processed at 94 ^{o}C for 20 min after blanching at 94 ^{o}C for 13 min) during 35 weeks of storage at 10, 21, and 38 ^{o}C with fluorescent light on.

CHAPTER 4

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

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Publication in Journal of Food Science
Volume 72 Issue 7 Page C397-C404, September 2007

4.1. 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 (1,300, 2,600, and 0 ppm) at 94 ^{o}C for 6, 12, or 18 min and then canning at 94 ^{o}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 by use of reverse-phase HPLC . Furthermore, pears with or without the peels were blanched in 2,600 ppm of zinc solution for 12 min following the canning process in 10 ^{o}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 in pear peels, where pheophytins were found to be the major degraded compounds while trace or small amounts of pyropheophytins were also formed. In zinc blanched peels, Zn pheophytin a was the dominant green compound, and its amount increased about 100% and 144.4% in peels blanched in 1,300 ppm zinc solution for 6 and 12 min, respectively. When blanching peels in 2,600 ppm zinc solution for 6 and 12 min, the pigment increased about 118% and 242%, respectively. Significant reductions in TA and TPC were found in the peels of zinc treated pears, but the overall TA and TPC of whole fruits were not significantly affected by the treatments.

4.2. Introduction

Chlorophyll a and b, two 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 ^{o}C or higher (Haisman and Clark 1975; Weemaes and others 1999) as a result of increased permeability of hydrogen ions across cell membranes (Haisman and Clark 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 re-greening the products (von Elbe and Schwartz 1996).

Recently, a technology for retaining green pigments in thermally processed green pears by use of 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 spinach with zinc reported that the pigment retention was attributed to the formation of metallo-chlorophyll 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 are 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 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 and have greater reactivity than the b forms (Jones 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 that have 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 non-polar solvents, such as diethyl ether (Lichtenthaler and Buschmann 2005b; Hagerthey and others 2006).

Since pear peels contain many times more phenolics 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 process 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.

4.3. Materials and Methods

4.3.1. 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 from C&H Sugar Company (Crockett, Calif., U.S.A.). Zinc chloride and anhydrous sodium sulfate were obtained from EM Science (EM Industries, Inc. NJ., U.S.A.) and zinc lactate from Purac America, Inc. (Lincolnshire, Ill., U.S.A.). Acetone and ascorbic acid was from J.T. Baker (Malinckrodt Baker, Inc. Phillipsburg, NJ., U.S.A.), diethyl

ether, ethyl acetate, and methanol from EMD Chemicals Inc. (Gibbstown, NJ., 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 from TCI America (Portland, Oreg., U.S.A.).

4.3.2. Preparation of pears

Pears were de-waxed by use of 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. Fruits were then drained and blotted with paper towel. The green area of the pear peels were manually peeled using a dual peeler, immediately frozen in liquid nitrogen, and stored in sealed 3-L glass jars at -22 ^{o}C for 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 ^{o}C.

4.3.3. Thermal process of pear peels in zinc solutions

Thirty grm 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, 1,300, or 2,600 ppm of zinc ions prepared from zinc lactate. For providing the chlorophylls in the peels a reaction condition close to what actually occur 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 2,600 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). Jars were sealed and immersed in 94 ^{o}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 with boiling water, then immediately hot filled in glass jars with boiling water and thermally treated at 94 ^{o}C for 20 min. The thermally processed peels were collected,

immediately frozen by liquid nitrogen, and stored at -22 ^{o}C for less than 4 hr before freeze-drying under a 100 mmHg vacuum pressure for 48 hr by use of a Consol 4.5 (The Virtis Company, Inc., Gardiner, NY., U.S.A.).

4.3.4. Extraction of green pigments from pear peels

Freeze-dried peels were ground into powders in liquid nitrogen by use of a blender (Waring Products, Model 34B97, Dynamics Corporation of America, New Hartford, Conn., U.S.A.). About 1.5 g of powder was 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 ^{o}C until analysis. Approximately 2 g of the peel powder of each sample was dried until reaching constant weight in a vacuum dryer (Model 58401, National Appliance Company, Portland, Oreg., U.S.A.) at 70 ^{o}C to determine the dry weight of the material.

4.3.5. Visual spectroscopic properties of peels extracts

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

4.3.6. 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 mL of ether peel extract was evaporated to dryness, followed by addition of 0.2 mL of acetone. Duplicate 20-µL injections of acetone extract were made onto an Altex Ultrasphere^{TM} reverse phase column (ODS, 5µ, 4.6 mm x 150 mm) (Altex-Beckman, Berkeley, Calif., U.S.A.). All pigments were monitored at 656 nm and 436 nm on a Shimazu dual-wavelength

detector. The HPLC apparatus consisted of a injection valve of 20-µL sample loop (Model 7725i, Rheodyne LLC, Rohnert Park, Calif., U.S.A.), two 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, TX., 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 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 by use of Reversed Phase HPLC has been 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, CA, 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^{TM} column was used.

4.3.7. 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 mL of acetone containing 15 µL of hydrochloric acid was added into 30 mL of ether extract. The

pheophytins were formed within 2 min and the acid was removed by washing 3 times with water.

To prepare pyropheophytins, 50 g of frozen powders of spinach leaves was filled in 125 mL of near-boiling water contained in a 125 mL glass jar. The jar was loosely sealed, heated at 98 ^{o}C in a Precision water bath (Jouan Incorporation, Winchester, Va., U.S.A.) to inactivate chlorophyllases before thermally processed at 121 ^{o}C for 1 hr in a Tomy Digital Autoclaves (Model SS-325, Peninsula Laboratories Inc., Belmont, Calif., U.S.A.). The powders in water were then filtered, drained off 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 mL of pheophytins or pyropheophytins in ether was dried under a stream of nitrogen gas. Twenty mL of acetone containing 3 g of zinc chloride was then added. After a reaction time of 30 min 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 were removed by addition of anhydrous sodium sulfate before storage at -22 ^{o}C.

4.3.8. 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 ^{o}C for 20 min. Two de-waxed peels-on whole pears were blanched in 2,600 ppm zinc solution for 12 min following with canning process in 10 ^{o}Brix sugar solution at 94 ^{o}C for 20 min. The 10 ^{o}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 processing and storage. Jars of processed pears were immediately cooled to room temperature under running tap water before being set aside for 24 hr 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 by use of a blender, while the peels were freeze-dried before powdered. Approximate 5 g of flesh powders or about 1.4 g of dried peels was extracted three times for phenolic content by use of 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 ^{o}C for determination of their moisture content. Total phenolic content and antioxidant activity were determined by use of FC and DPPH assay, respectively, as described in the previous study by Ngo and others (2007). The experiment was replicated four times. The peels from two different replicates were mixed together to give a larger quantity sufficient for powdering.

4.3.9. Experimental design

A completely randomized design (CRD) with two factors (zinc concentration of 0, 1,300, and 2,600 ppm and blanching time of 6, 12, and 18 min) was duplicated. A CRD design with one 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 (Statistical Analysis System Inst. Inc., Cary, N.C., U.S.A.) was applied in testing differences among different treatments. Least-significant-difference (LSD)'s multiple-range test was used for the multiple mean comparisons.

4.4. Results and Discussion

4.4.1. Spectral properties of processed pear peels

Figure 4.1 and Table 4.1 represent three typical visual spectral properties of ether extracts from fresh peels, peels blanched and canned without use of zinc, and

peels blanched with 2,600 ppm zinc ions and then canned. Both blanching (18 min) and canning (20 min) processes were performed at 94 ^{o}C. Figure 4.1 shows that three 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 4.1 shows a coincidence between the absorption maxima (abs max) of the three peel extracts and those of chlorophyll a derivatives reported in the literature. Absorption maxima of fresh peels were found at 660.5 nm and 430 nm, respectively, which corresponds fairly well with those of chlorophyll a at 660.5 nm 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 nm and 409 nm. For peels blanched in 2,600 ppm zinc solution, their extracts showed absorption maxima at 655.5 nm and 422 nm, respectively, corresponding well with those of 655 nm and 423 nm reported for Zn pheophytin a (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.

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

The analysis of the pear peel extracts revealed that chlorophyll a was present in the fresh peels (Figure 4.2a) and zinc-pheophytin a and pheophytin a (Figure 4.2b and 2c) were found in the zinc treated samples.

Figure 4.3 shows the three 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 4.3a corresponds to the fresh peel extracts, in which chlorophyll a (peak 2) and b (peak 1) are present. Pheophytin a (peak 5 in Figure 4.3a) 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 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 4.3b, 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 4.3b) 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 5-10% of chlorophyll a and b into a' and b' after 10 min heating at 100 ^{o}C (von Elbe and Schwartz 1996). In this study, peak area of pheophytin a' at 656 nm was approximately 20% of that of pheophytin a (Figure 4.3b).

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 4.3c). 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 4.3b and Figure 4.3c). 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 4.1 and Figure 4.1). 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 early, 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 ^{o}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 ^{o}C. In this study, pheophytins were formed in large amount compared to the trace amount of pyropheophytins when no zinc ion pretreatment was applied. The abundance of pheophytins favored the formation of Zn complexes, Zn pheophytins, when peels entered into 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 are the sole compounds responsible for the green color of spinach leaves processed at 100 ^{o}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 mention here concerns the amount of the chlorophyll reacting with zinc, i.e., 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 about this limitation.

4.4.3. Evolution of major heat-generated pigments in treated pear peels

The kinetic data collected for canned peels previously blanched in different zinc solutions (0, 1,300, or 2,600 ppm) for 6, 12, or 18 min are presented in Figure 4.4. Note that the concentrations of Zn pheophytin a in heated peels blanched in 1,300 ppm Zn solution for 6 min were set as 100%. For pheophytins a, b and a', their concentration in the heated peels blanched in water for 6 min was 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.

Figures 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 (1,300 ppm), both pheophytins a and a' followed the same trend of reduction (Figures 4a and 4b). Figure 4.4c 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).

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

Table 4.2 shows the antioxidant activity and 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, i.e., no blanching, but hot filling with 10 ^{o}Brix syrup solution.

No significant difference in 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 4.2), about twice as high as 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, maturity, environment factors, etc. 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 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 total phenolics of pears were not affected by the processing, regardless there was an extra step of blanching for retaining the green pigments.

4.5. Conclusion

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 1,300 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.

4.6. Acknowledgement

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, OR, USA.

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Table 4.1 - Spectral properties of ether extracts of fresh and processed pear peel pigments vs. chlorophyll a and its derivatives.

Sample/Compound	Absorption maxima (nm)		Visual color
	"Red" Region	"Blue" Region	
Fresh pear peels	660.5	430	Green
Pear peels canned (94 ^{o}C) without use of zinc ions	666	410	Brown Yellow
Pear peels blanched with 2,600 ppm zinc ions at 94 ^{o}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.

Table 4.2 - Antioxidant activity and total phenolic content of whole pear and pear peels of fresh and canned pears (based on dried weight of samples) [*]

Samples	Pears	Antioxidant capacity mg EAA/g[++++]	Phenolics mg EGA/g[+++++]
Whole pears	Fresh pears	2.1 ± 0.4a	4.63 ± 0.28a
	Canned peeled pears[+]	2.3 ± 0.4a	4.98 ± 0.45a
	Canned with peels-on[++]	2.8 ± 0.8a	5.58 ± 0.46a
	Canned with peels-on and treated with Zn[+++]	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

[*] 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, $P<0.05$).

[+] Pears were peeled.

[++] Pears were de-waxed with peels-on.

[+++] Pears were de-waxed and blanched at 94 ^{o}C for 12 min in 2,600 ppm of zinc solution.

[++++] Antioxidant activity was expressed as mg of equivalent ascorbic acid/g of dried sample.

[+++++] Phenolic content was expressed as mg equivalent gallic acid/g of dried sample.

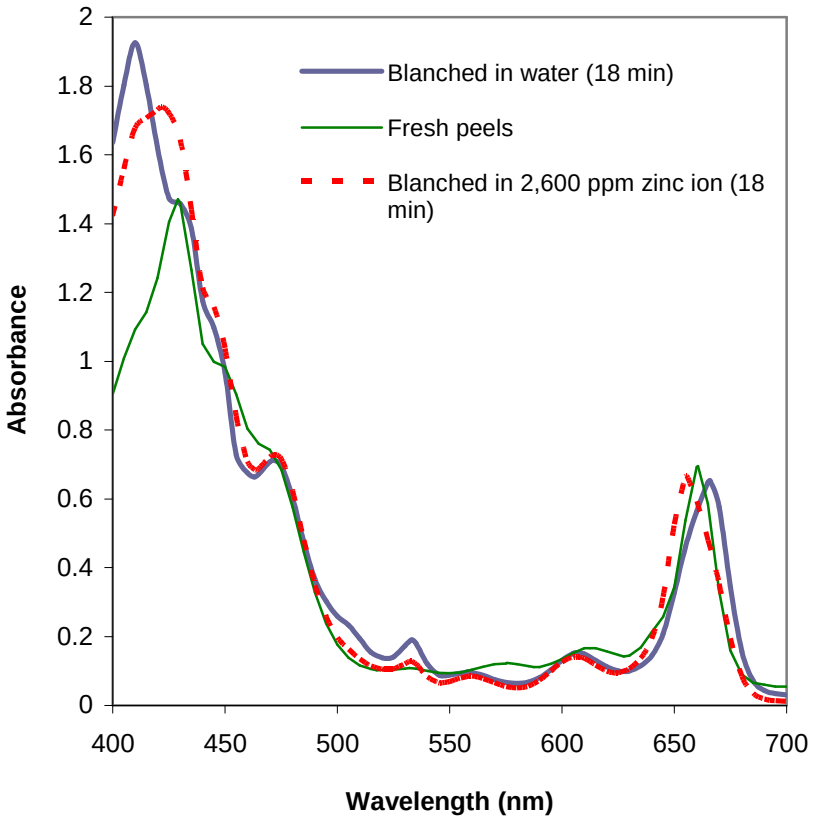

Figure 4.1 - Absorbance spectra of ether extracts of fresh pear peels and canned pear peels with or without zinc blanching pretreatment (blanching in 2,600 ppm of zinc ion solution at 94 ^{o}C for 18 min and canning at same temperature for 20 min).

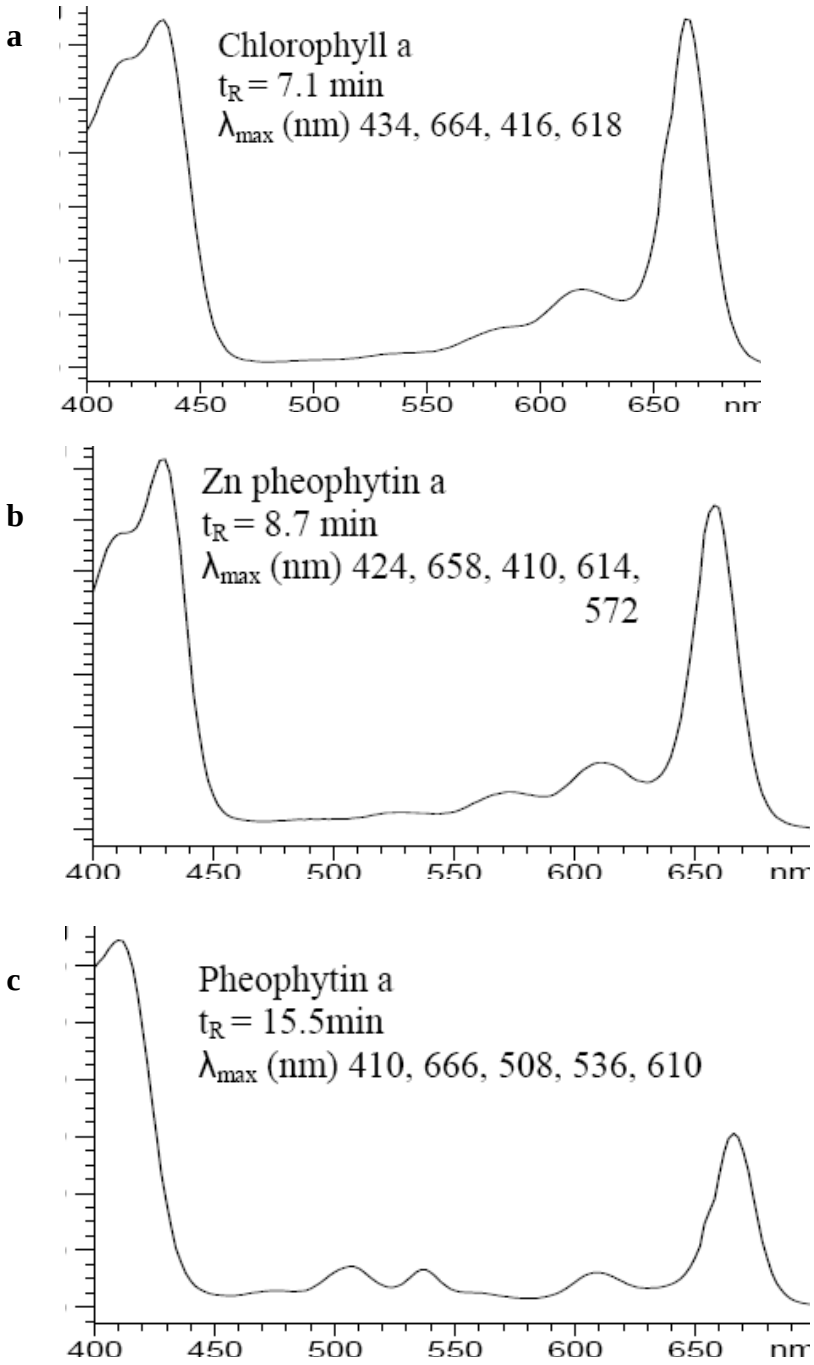

Figure 4.2 - Visual spectra of major pigments in fresh and zinc-treated pear peel extracts eluted from an Altex Ultrasphere™ reverse phase column (ODS, 5µ, 4.6 mm x 150 mm) and detected by a diod array detector couple with a HPLC system using an isocratic mobile phase of ethyl acetate/methanol/water (40:54:10 v/v/v).

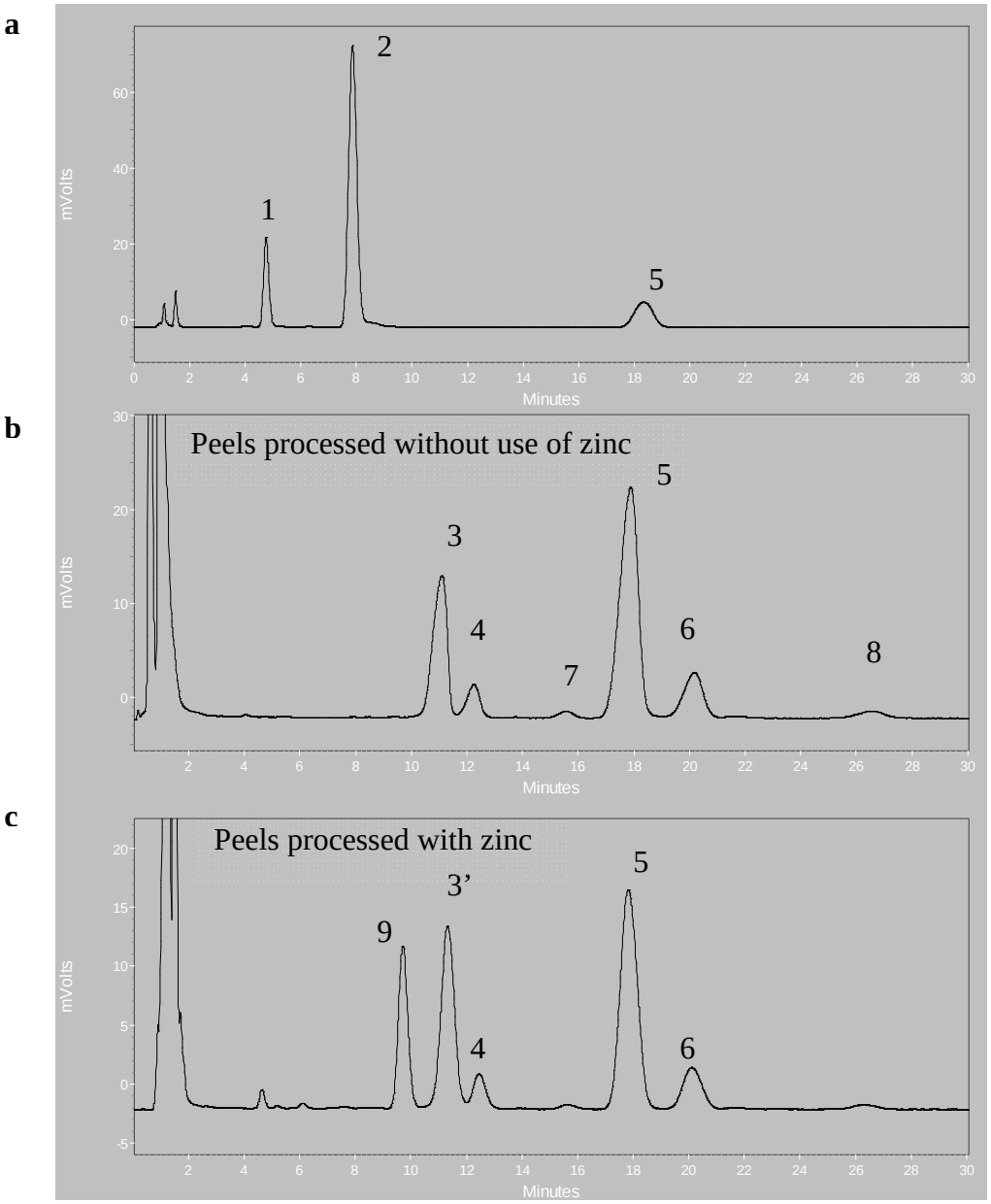

Figure 4.3 - Typical reversed-phase HPLC chromatograms detected at 656 nm on pear peels. (a) Fresh peels; (b) Thermally processed peels (blanched in water at 94 ^{o}C for 6 min and canned at same temperature for 20 min); (c) Peels processed with zinc blanching (blanched in 2,600 ppm of zinc solution 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.

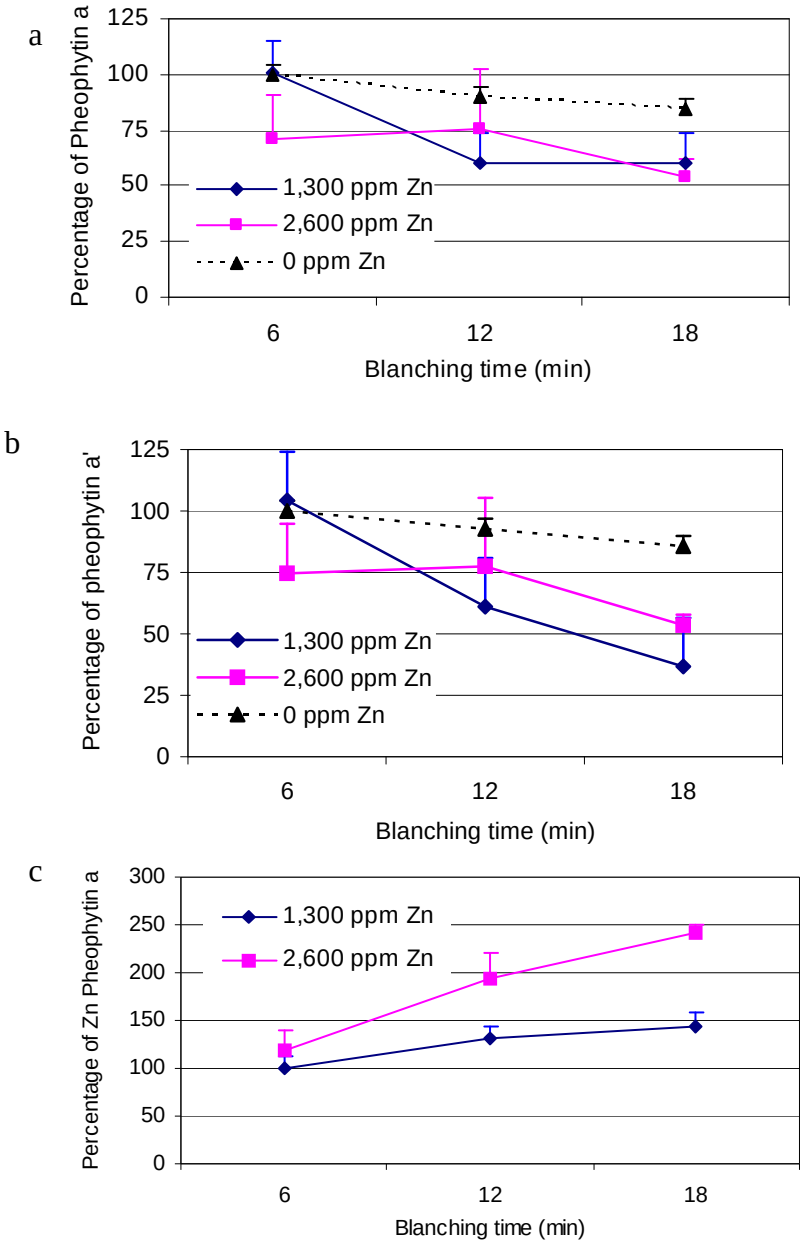

Figure 4.4 - Evolution of pheophytin a (a) and a' (b) and formation of Zn pheophytin a (c) (relative percentage of 100% as in canned pear peels previously blanched in water for 6 min) in pear peels previously blanched in 0, 1,300, or 2,600 ppm zinc solution at 94 ^{o}C for different blanching times. 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.

CHAPTER 5

Stabilization of Red Pigments on Thermally Processed Red D'Anjou Pears

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5.1 Abstract

Thermal-labile anthocyanins were stabilized in thermally processed peel-on red D'Anjou pears through complexation with metal ions and covalent bonding with co-pigments. Red D'Anjou pears reacted with 11 individual metallic ions in aqueous solutions for pigment complexation with anthocyanins. A formula containing metallic ion, hydrochloric acid, formalin, and tannic acid was then developed as a pretreatment for stabilizing anthocyanins on the peels of red pears during thermal process. Treated pears were subjected to a water solubility test for evaluating CIEL*C*h color values of the fruits and browning and anthocyanin content of the solution by use of spectrophotometers. Among tested metallic ions, stannous yielded promising red-purple stable pigment on the peels, thus used in the developed formula. Pears treated in the developed formula retained attractive red color after thermal treatment and showed high water insolubility. The nature of the new pigments are believed to be the complexes of stannous and phenolics, including anthocyanins. This study revealed a new patentable technology for developing processed colorful fruit products containing anthocyanins.

5.2. Introduction

Anthocyanins are responsible for most of the red, purple, and blue pigmentation of flowers, fruits and vegetables. Because of their highly reactive nature, anthocyanins degrade readily to form colorless or brown colored compounds (Jackman and others 1987), particularly under high temperature condition (von Elbe and Schartz 1996).

A certain degree of anthocyanin stabilization may be reached through acylation with various organic acids, self-associate ion, or co-pigmentation (Jackman and others 1987). Anthocyanin glycosides may associate with their co-pigments, such as certain phenolic acids, flavonoids, or derivatives of flavonol and flavones, in which the pigments are intensified and protected from oxidation (Boulton 2001). This molecular complexation process is instantaneous and expected to occur in nature (Figueiredo and others 1996). In food models, the co-pigmentation is believed to be the early steps to form covalent linkages between the molecules through chemical reactions (Brouillard and Dangles 1994). The new compounds formed between anthocyanins and other phenolics can result in higher stability of the pigments (Fossen and others 2004; McDougall and others 2005). Malvidin-3-glucoside was shown to be very reactive toward catechin in the presence of aldehydes, rendering anthocyanin-aryl/alkyl-flavanol pigments (Francia-Aricha and others 1997; Pissarra and others 2003). Although the new pigments show higher stability with regard to water attack and bleaching by SO_2 (Escribano-Bailon and others 2001), their formation caused bluing effects on the color of samples (Pissarra and others 2003). Meanwhile, the reactions can be catalyzed by acids, such as hydrogen chloride (HCl), or base. Direct anthocyanin-catechin coupling can also occur but with a much lower rate than through aldehyde-mediated reactions (Escribano-Bailon and others 1996).

Unlike polyphenolic co-pigments, metal ions that could be present in the anthocyanin natural media seem more rarely involved in color stabilization. In the presence of some metal ions, such as niken (Ni^{+2}), magesium (Mg^{+2}), and calcium (Ca^{+2}), anthocyanin and flavone glycoside can form stable co-pigment-metal-anthocyanin coordination complexes (Shiono and others 2005). Aluminum (Al^{+3}) and

gallium (Ga^{+2}) have been reported to strengthen the pigment and co-pigment interactions leading to color changes from red to deep purple (Elhabiri and others 1997). In food models, Wrolstad and Erlandson (1973) and Coffey and others (1981) found that stannous (Sn) formed pink complexes with procyanidins. As the results of the dark blue color of the metallo-phenolic complexation on damaged fruit peels, metal ions, particularly iron (Fe) and copper (Cu), are most often regarded as the discoloration contaminants (Cheng and Crisosto 1994). For canned pears (peeled), Sn-anthocyanin complex has been found to yield insoluble stable pink pigment of the solid fraction (Chandler and Clegg 1970). Several attempts have made use of metal ions (Starr and Francis 1974; Kallio and others 1986) and co-pigments (Maccarone and others 1985; Malien-Aubert and others 2001; Rein and Heinonen 2004) in stabilization of anthocyanins in fruit juice and puree. However, no previous work has been reported for retaining red pigments on the peels of processed fruits. The real challenges for creating stable pigments on the anthocyanin-contained peels of processed fruits include rendering the pigments water-insoluble and heat-stable while retaining the attractive natural color.

This study used red pears as a food model. The fruit has anthocyanins cyanidin-3-glycosides as the major red pigment in its peels (Mazza and Miniati 1993; Sanchez and others 2003). Specific objectives of this study were to investigate the interactions of metallic ions with red pigments in the peels of red pears, and to evaluate a developed stannous-based formula for improving water solubility and heat stability of developed pigments on the peels of red pears during thermal processing. The results would reveal a new technology for developing processed colorful fruit products containing anthocyanins.

5.3. Materials and Methods

5.3.1. Materials

Eleven different types of metallic ions from different salts were tested in this study. Aluminum chloride and magnesium chloride were from EMD Chemicals (Gibbstown, NJ, U.S.A.). Ferric chloride, zinc chloride, stannous chloride, and

sodium hydroxyl were from Em Science (Cherry Hill, NJ, U.S.A.). Nikelous chloride, ferrous chloride, calcium chloride, cobalt sulfate, cupric chloride, hydrochloric acid (36.5 - 38.0 %), formaldehyde solution (36 %), and ascorbic acid were purchased from Mallinckrodt Baker (Phillipsburg, NJ). Manganese chloride was from Mallinckrodt Baker (Paris, KY, U.S.A.). Cadmium sulfate was from Mallinckrodt (St. Louis, MO, U.S.A.). Monobasic potassium phosphate was from Sigma (St. Louis, MO, U.S.A.). Tannic acid was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Cane Sugar was from Western Family Foods, Inc. (Portland, OR, U.S.A.).

5.3.2. Fruits

Red D'Anjou pears harvested in the 2005 season in Northwest of U.S.A. were purchased in March and April 2006 from local groceries in Corvallis, OR, U.S.A. Fruits were carefully selected for uniform size and maturity.

5.3.3. De-waxing pears

For promoting the interactions between the natural compounds in the pear peels and metallic ions, it is essential to remove the wax and partially cuticle layer on the fruit surface (Ngo and Zhao 2005). This de-waxing process developed in the authors' laboratory was done by a continuous beam of sugar grains generated by a spry gun with sucking air of 1 atm (Ngo and Zhao 2005).

5.3.4. Pigment reactions with metallic ions

The treatment solutions of aluminum (Al), calcium (Ca), cupric (Cu), ferrous (Fe^{+2}), ferric (Fe^{+3}), magnesium (Mg^{+2}), manganese (Mn^{+2}), nikelous (Ni^{+2}), stannous (Sn^{+2}), zinc (Zn^{+2}) chloride, cobalt (Co^{+2}), and cadmium (Cd^{+2}) sulfate salts were prepared in final ion concentrations of 0.01 and 0.001 M in a monobasic potassium phosphate solution (0.1 M, pH of 4.5) (Table 5.1). A control solution was made solely from the phosphate buffer solution. The metallic ions were selected based on their possible interactions with anthocyanins reported in the literature (Takeda and others 1984; Cheng and Crisosto 1994). Fifty-two fruits were selected from two cases of

pears with uniform red color, size, and maturity. Each de-waxed fruit was randomly assigned to a solution of 300 mL and immersed for 15 min before being drained in an air flow of about 30 m/min for about 5 min. Color values of the samples were then measured. A completely randomized design with 2 replications was implemented.

5.3.5. Development of stannous chloride based formula

Based on the results from the above described metallic ion tests, Sn resulted in promising purple-red pigments on treated pears. However, the purple-red pigment of Sn treated pears was not well retained, and turned into brown after heat treatment in water, although no pigments leaching was observed. In addition, the resulting pigment is more purplish than red, showing difference from the natural red appearance of pears. Hence, further efforts were made to improve the thermal stability of the pigment and shifting the pigment toward natural red pear color. Pizzi (1994) described a formulation of resin adhesive by use of phenol-formaldehyde condensation for mending cellulose structures together, such as wood. Our initial assays used tannic acid as a source of phenols to react with formaldehyde and hydrochloric acid to catalyze the reactions. Preliminary results suggested that treating pears in a tannic acid – formaldehyde – hydrochloric acid solution with the addition of Sn ions helped to create red-purple water insoluble pigments on red pear peels that were not depleted or destroyed after a mild thermal treatment. Further efforts were then made to refine the formulation and to determine the preparation procedures and reagent concentrations for best retaining the red pigments. The formulation developed for sample preparation has been filed for a patent application (Zhao and others 2007) and is described as: To make 525 ml of the formula, 400 ml of 1% tannic acid solution, 100 mL of formaldehyde, and 25 mL of HCl was mixed together. Tannic acid solution was previously prepared by adding 10 g of tannic acid in 1 L of deionized water and stirring until homogeneity. Based on the observation, at least 4 h is necessary for the reactions of phenols from tannic acid with formaldehyde at room temperature before accumulating resulted products. Thus, the mixture was set for about 4 h 30 min at room temperature, and stannous chloride was then added into the

formula to make a final Sn ion concentration of 0.0016 M (~200 ppm). The mixture was let stand for another 1 h before use.

For treating the pears, individual pear (~120 g) was immersed in the formula (~ 400 mL) for 4 min. The same solution was consecutively used for twelve de-waxed pears. After treatment, pears were set on a rack to drain and dry by flowed an air flow rate of about 30 m/min for about 10 min, and stored in plastic bag for 2 hours in a refrigerator. The pears were then washed in cold water and drained again. The middle 4 treated pears were used later for the investigation of water solubility of the pigments.

5.3.6. Water solubility test

To investigate the water solubility of developed co-pigments, individual pears previously treated in the formula solution was packed and sealed in 1 L of glass jar (Olshen's Bottle Supply, Co., Portland, OR, U.S.A.) filled with deionized water at 5 ^{o}C for 24 h. One of the 4 treated pears was randomly selected after storage for 3, 6, 9, or 24 h for color measurement and the solutions were used for the determination of soluble anthocyanins and brown compounds. For preparing the water for the test, distilled water was filled into glass jars, and sealed jars were heated to boiling in a steam kettle for removing oxygen presented in the water. The jars were then cooled to room temperature and kept chilled in iced water over night. Immediately before water test solubility, ascorbic acid was added into water to make an ascorbic acid concentration of about 0.5%, approximately pH value of 3.1. The purpose of this pH adjustment is to prevent browning reaction and microbial growth of pears during the test. Non-treated pears were used as controls, in which de-waxed pears were immediately packed in a 0.5% ascorbic acid solution, stored at 3 ^{o}C, and sampled within 24 h as done with treated pears. A split-plot completely randomized design with sampling hours as subplots was applied. The water solubility test was replicated 4 times with treated samples and twice with the control.

5.3.7. Thermal stability test

After treatment in formula solution, 12 treated pears were drained, washed with distilled water, and drained again. To evaluate the thermal stability of developed co-pigments in treated pears, 6 pears were used, in which 2 were randomly selected from the first 4 of 12 treated pears (Early) or the next 4 (Middle) or the last 4 (Late). Different stages of treatment were investigated to understand whether the developed formula can be reused, and if so, how many times. Individual pears were thermally processed at 73 ±4 ^{o}C for 7 min in a cylindrical chamber (20 cm diameter x 30 cm length) designed in our laboratory with steam pertinently flowing-in through the bottom and out through a bell-shaped lid. Based on our preliminary study, a temperature of higher than 80 ^{o}C under the steam would damage the peels of treated pears, thus 74 ^{o}C for used in this test. The temperature inside the chamber was maintained by changing the flow rate of the steam input. After steam-heating, pears were transferred to another chamber under vacuum pressure of 150 mmHg (Model 0211 P204; Gast Mfg. Corp., Benton Harbor, MI, U.S.A.) and cooled to room temperature in about 40 min. This procedure is necessary to control oxidation of the peels of hot pears. Pears were then washed by water and drained before further assessment for color. Control samples were prepared following the same procedures except for treatment in a 5% HCl solution instead of using the formula described above, and an immersing time of 1 min. HCl was used in control samples for controlling enzymatic browning reaction and creating a pH condition alike that of treatment solutions. A split plot completely randomized design with the stage of immersion solution (Early, Mid, Late) as subplots was applied. The thermal treatment was conducted 4 times for treated samples and controls.

After steam-heating, individual pears were hot-filled into glass jar filled with 10 ^{o}Brix cane sugar solution and subjected to heat processing at 94 ^{o}C for 7 min (a simulation of commercial canning process of pears) for observing color stability of pears during storage at ambient condition.

5.3.8. Color measurement

A Hunter LabScan II spectrophotometer (43.2 mm diameter view area, 0/45 geometry, D65 light source, 10^{o} view angle) was used to measure the color of fruit samples. For color reading, fruit was placed inside a glass cell (130 mm in diameter x 50 mm in height) with the reddest area facing the light source. Sample was covered by a black box to prevent light leakage. Sample color was reported in CLEL*C*h color values.

5.3.9. Analysis of soluble anthocyanin and browning index

The anthocyanin content and browning index of tested water solutions at different sampling times during pigment water solubility test was determined using the pH-differential method at maximum peak of 512 nm and absorbance at 420 nm of bisulfited-treated solution, respectively (Giusti and Wrolstad 2005). Results were expressed as absorbance per mL of water. Sample spectral absorbance was performed on a Shimazu UV160U spectrometer (Shimazu Corp., Kyoto, Japan) with 1-cm disposable cells.

5.3.10. Experimental design and statistical analysis

Specific experiment design for each experiment was described above. Statistical analysis was performed using the SAS (Statistical Analysis System Institute Inc., Cary, NC, U.S.A.). Multifactor analysis of variance and differences between means were tested for significance by using GLM procedure with Duncan test, using a level of significance of $P < 0.05$. For comparing the effects of metallic ion with controls, a least square difference (LSD) one-way multiple mean comparison was performed by use of GLM procedure.

5.4. Results and Discussion

5.4.1. Effects of metallic ions

The results of color measurement for metallic-ion treated samples and the control are presented in Figure 5.1. L* values of treated samples, control, and fresh pears were found not significantly different ($P>0.05$) (in a range of 25 to 30), thus

were not reported here. Hue angle values of the samples reacted with Sn^{+2}, Fe^{+2}, or Fe^{+3} were significantly different from that of the control and fresh pear ($P< 0.05$) (Figure1a), showing a yellowish color (Figure 5.2a), suggesting interactions between these metallic ions and phenolics in pear peels. Chroma values of the samples treated with Fe^{+3} were close to zero (Figure 5.1b), representing a black color of the samples (Figure 5.2a, b). These findings support the results reported by Cheng and Crisosto (1994) on peach and nectarine peels that ferrous was a very effective discoloration agent. This "inking" effect was not only restricted to the pear peels which were originally dominated by red pigments, but also on the whole de-waxed fruit surface, indicating that ferrous reacts with other phenolics in the peels beside anthoycanins as described in the study by Cheng and Crisosto (1997) on evaluating iron-polyphenol complex formation in fruit model. Although samples treated at high level of Al^{+3}, Fe^{+3}, and Zn^{+2} (0.01 M) showed hue values as low as c.a. 25.8, 21.4, and 24.4, respectively, representing a promising red (Figure 5.1a, Figure 5.2b), the pigments did not stay on the peels, but quickly leached into the solution during a water-test as described below. Pears treated with Cd^{+2}, Ca^{+2}, Co^{+2}, Mg^{+2}, Mn^{+2}, or Ni^{+2} did not show changes in any L*C*h color values compared to the control (Figure 5.1). Treatment with Cu did not change hue angle value but yielded a significant lower color intensity as reflected in the color diagram (Figure 5.2a), indicating significant loss of anthocyanins. Previous studies reported that the presence of Cu^{+2} can accelerate the formation of H_2O_2 from ascorbic acid available in fruits, this latter then reacts with anthocyanins resulting in their destruction (Shrikhande and Francis 1974; Jackman and Smith 1996). Between the 2 levels of tested metallic ion concentrations, the higher level (0.01M) of most tested metal ions did not yield significant difference in pear color ($P>0.05$) (Figure 5.1), except for Al^{+3}, Cd^{+2}, Fe^{+2}, Fe^{+3}, and Zn^{+2}, in which samples treated with high concentration retained a lower hue angle value than those in low concentration (Figure 5.1b). Based on this result, the low concentration of approximately 0.001M metallic ion was used in the final formula development as described in the materials and methods.

After reactions with Sn^{+2}, the surface area of the pear peels turned into purple color where the peel areas were originally dominated by red anthocyanins. This purple pigment did not leached out during long hours of water test. Moreover, the white flesh pear tissues did not show any color change as appeared on samples reacted with some other metallic ions. All these suggest that anthocyanins on the red pear peels form water insoluble co-pigments with stannous. Hence, stannous can be a potential reagent to stabilize anthocyanins on pear peels, thus selected to formulate the pretreatment solution for red pigment retention.

5.4.2. Water solubility of developed co-pigments on pear peels

After pretreatment in developed formula, pears were washed and subjected to water test for evaluating water solubility of developed co-pigments on the pear peels. During the water test, the control showed significant increases in L*, C*, and h^o values, reaching 37, 29, and 58, respectively at the end of 24 hr (Figure 5.3). Changes of these color values represented decrease in redness and increase in yellowness. Compared to the dark red color of de-waxed fresh pears (L*= 32, C*= 26.8, h^o = 28.7), the bright yellow color of the control at the end of 24 hr water test indicated the complete loss of red pigments from the peels. Unlike the control, treated pears did not show significant changes in color values during the 24 h of water test (Figure 5.3), where the hue angle value of treated samples was about 20, representing a red color at the end of 24 h water test.

The tested water was initially colorless, but turned red after 24 hr of immersion of control pears as showing in the spectral absorption curve with an absorption maximum at ~510 nm (Figure 5.4a), coinciding with that of cyanidin-glycoside reported by Jurd and Asen (1966). The anthocyanin contents in tested water were 0.039 and 0.245 (Abs unit/ mL) for treated and control samples, respectively (Figure 5.4b), indicating that leach of anthocyanins from treated samples is significantly lower than that of control. In addition, the amount of anthocyanin detected in water solution from treated samples did not change significantly during the last 21 h of the test

(Figure 5.4a). These results coincide well with unchanged red color detected by measured color values of treated samples during the water test (Figure 5.3).

The browning index of the water test solution with treated and control samples did not change significantly during the water test (Figure 5.4c). This may be partially attributed to the low pH value (3.1) of the solution and the use of ascorbic acid that successfully controlled the browning reactions on the fruit. In addition, it is well known that the available oxygen level determines the anthocyanin stability (Kallio and others 1986; von Elbe and Schwartz 1996). Using boiled water as well as complete filling of the bottles helped to limit the presence of oxygen, contributing effectively to delaying the degradation of anthocyanin.

Compared to the purple water-insoluble complex by treatment with stannous alone (0.01 and 0.001 M) (Figures 1 and 2), the developed formula composed of stannous (0.0016 M), hydrochloric acid (5% in volume), tannic acid (1% in weight), and formaldehyde (20% in volume) was effective in creating water-insoluble red pigments on pear peels (Figure 5.3). Multiple tests without using stannous in the formula failed to create a water-insoluble form of pigments. Hence, stannous is one of the key elements to stabilize red anthocyanins on pear peels. However, the exact compounds in the formed co-pigments are unknown. Additional studies have been conducted by the authors to understand in more depth the chemical reactions and to quantify the formed compounds.

5.4.3. Thermal stability of developed red pigment on pear peels

Figure 5.5 shows the results of color measurement for treated pears and control before and after steam-heating. After heating, lightness and hue angle values of the control (c.a. 32.5 and 26, respectively) were significantly increased (c.a. 36 and 37.5, respectively), representing the loss of red color and development of dominant yellow color on pear surface (Figure 5.5a, b). The loss of red color can be attributed to the thermal lability and oxidation of anthocyanins during heating process (*2*). Unlike the control, the lightness and hue angle values of early- and middle-formulated pears did not change significantly after heating (Figure 5.5a, b) although the chroma values

were significantly reduced from c.a. 26 before heating to less than 20 after heating (Figure 5.5c). These results support the visual observation that treated pears retain attractive red color after heating process. However, the late-treated pears showed a significant increase in hue angle value from c.a. 18 to 27 after heating, suggesting a color change from red toward yellow and a considerable loss of red pigments compared to that on early- and middle-treated pears. This indicates that the developed formula can't be reused for too many times without a loss in the effectiveness of the treatment. It was also found that control of oxygen during steam-heating is critical for retaining the pigments (data not shown) as oxygen can easily oxidize the pigments.

Figure 5.6 shows the pear after further thermally processing at 94 ^{o}C for 7 min in glass jars filled with 10 ^{o}Brix sugar solution. Treated pears retained red color even after 6 month of water storage at ambient condition. However, samples that omitted steam-heating showed a yellow appearance (results not shown), indicating that a mild heat treatment before canning process may be a necessary step for stabilizing the pigments.

This study used red pears as a model food system to evaluate a developed formula as a pretreatment to retain red pigments on the surface of thermally processed fruits. Two major challenges associated with retaining anthocyanin red pigments were overcome: water solubility and heat stability. Results demonstrated that the developed formula and procedures successfully created a chromophore that can stay on the surface of pears during thermal process in aqueous solution, thus providing great potential for retaining red anthocyanins on processed red fruits, such as cherries, plums, and peaches.

The developed formula and procedures are used as a processing aid and pretreatment, respectively, thus the functional ingredients/reagents in the formula used in the pretreatment are not included in the finial product processing stages. All the ingredients used are food graded except formaldehyde. Hence, the residual of formaldehyde may be a concern for commercial application of this technology. The inhalation of formaldehyde has been shown to have carcinogenic and probably neurotoxic impacts (NIOSH 1981; Pitten and others 2000). However, the weight of

evidence indicates that formaldehyde is not carcinogenic by the oral route (FPTCDW 2003). Formaldehyde has been shown to cause no appreciable health risk from consumption of cheeses made using formaldehyde at low level (Restani and others 1992). The European Food Standards Authority recently reported that the presence of low-level formaldehyde in gelling agents like carrageenans and alginates does not pose a threat to human health (Scientific-Panel-AFC 2006). It was estimated that exposure to gelling additives containing residual formaldehyde at the levels of 50 mg/kg of additive would be of no safety concern (Scientific-Panel-AFC 2006). An ingestion of formaldehyde in drinking water for two years caused only stomach irritation in rats in several studies, particularly at higher doses (FPTCDW 2003). Tolerable Daily Intake (TDI) value of 150 micrograms/kg body weight is set by the World Health Organization (WHO) for drinking water. However, under certain conditions of exposure, potential carcinogenic hazard associated with the ingestion of formaldehyde can not be eliminated (Liteplo and others 2002). Another possible safety concern is the presence of Sn in the finished product. Stannous chloride, commonly known as tin (II), is normally found in canned foods due to dissolution of the tin coating or tin plate used in tin plated lacquered or un-lacquered containers (Howe and Watts 2005). The U.S. Food and Drug Administration (FDA) has temporarily amended the standard of identity for canned asparagus to allow stannous chloride on the list of optional ingredients with the provision that the concentration of stannous chloride in the product be no more than 35 ppm (FDA 2003). A use of 200 ppm of Sn or 337 ppm of stannous chloride in the developed formula treating only the exterior of the fruits would most likely yield a low Sn^{+2} content in final product. In this study, formaldehyde and stannous chloride are used as pigment stabilizers, critical functional ingredients for helping retain the red pigments on the surface of the pears. Hence, further studies are necessary to understand the pigment complexation process, to measure the residual in finished products, to optimize the formula to minimize its use and remove the residual from the outside layers of fruit peels, and to find potential replacement of formaldehyde if necessary

5.5. Conclusion

This study demonstrated that an aqueous formula constituted of stannous, formalin, hydrochloric acid, and tannic acid forms metallic-phenolics complexation and creates a new red co-pigment on pear peels that is highly water insoluble and heat stable. Such pigment is excellent for processed red fruits and vegetables. Although the exact nature of the newly formed adducts are still unknown, stannous was found to be the key component in the complexes. The complexation did not yield a purple color or discoloration as found when treating pears with stannous or with other metal ions alone, suggesting that metallo complexations occurring on the pear peels depend on the presence of other compounds besides metal ions in the environment.

5.6. Acknowledgement

We would like to express our special thanks to Dr. Ronald Wrolstad for his precious insight and constructive discussions on many technical aspects of the project. Our sincere thanks to the Oregon and Washington Pear Commissions and Mr. Dennis Anderson, Green & Green, Inc. for partially funding our research, as well as to Dr. Eric Wilhelmsen for assistance in the initial stage of the procedure development.

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Table 5.1 - Conditions of metallic ion solutions used in the evaluation of metallic ion reactions with de-waxed red D'Anjou pears [a]

Metal ion	Salt source	pH after 15 min immersion
Al^{+3}	$AlCl_3$	4.17
Ca^{+2}	$CaCl_2$	4.32
Cd^{+2}	$CdSO_4$	4.37
Co^{+2}	$CoSO_4$	4.88
Cu^{+2}	$CuCl_2$	4.54
Fe^{+2}	$FeCl_2$	4.69
Fe^{+3}	$FeCl_3$	4.63
Mg^{+2}	$MgCl_2$	4.5
Mn^{+2}	$MnCl_2$	4.56
Ni^{+2}	$NiCl_2$	4.59
Sn^{+2}	$SnCl_2$	4.03
Zn^{+2}	$ZnCl_2$	4.88

[a] Metallic ion solutions were prepared by dissolving various salts into buffer KH_2PO_4 0.1 M adjusted to pH 4.5 by use of hydrogen chloride with a ion concentration of 0.01M.

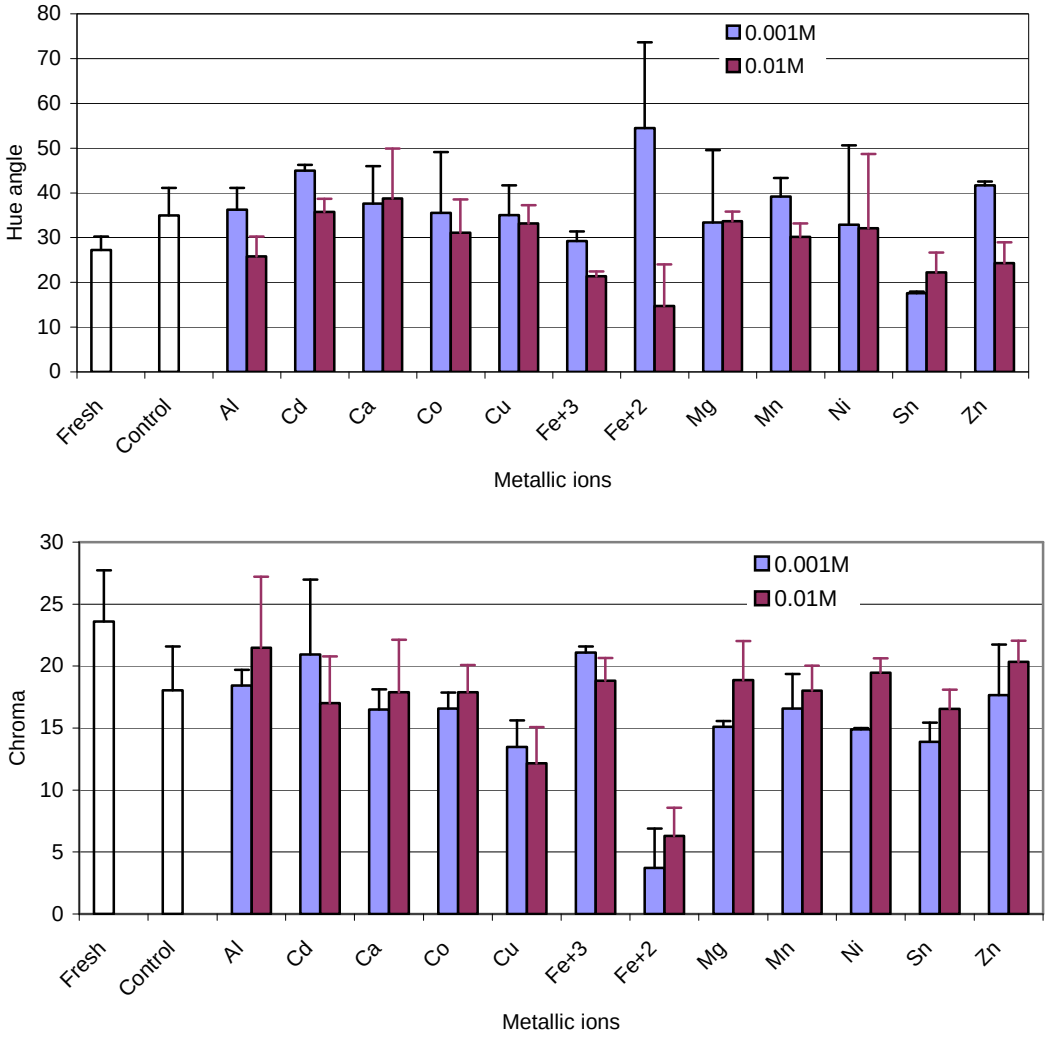
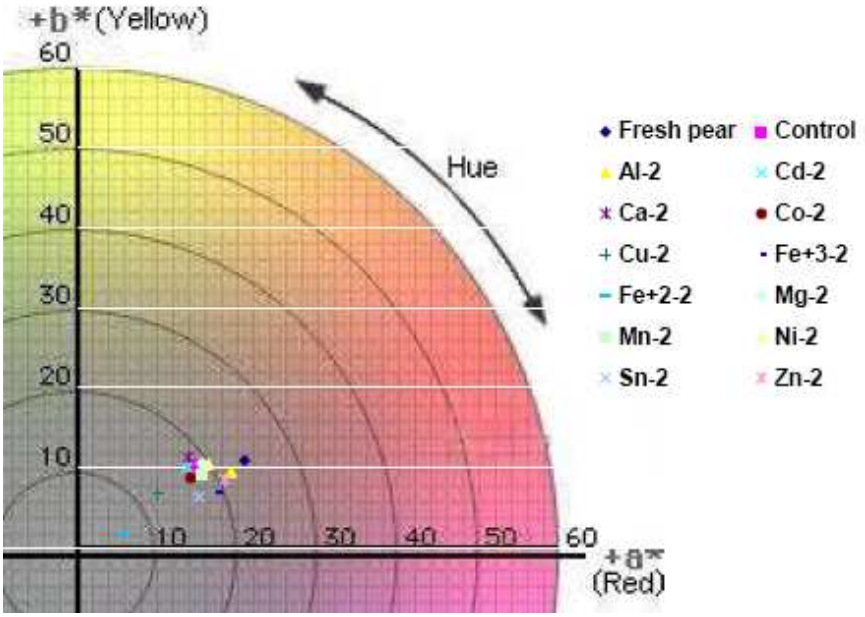


Figure 5.1 - CIEL*C*h color values of red D'Anjou pears immersed in 0.01M or 0.001M metallic ion solutions prepared in phosphate buffer solution with a pH of 4.5 for 15 min. Control: Buffer solution (monopotassium phosphate, 0.1 M, pH = 4.5). Al: aluminum; Cd: cadinium; Ca: calcium; Co: cobalt; Cu: cupric; Fe^{+2}: iron divalent; Fe^{+3}: iron trivalent; Mg: magnesium; Mn: manganese; Ni: nikel; Sn: stannous; Zn: zinc.

a



b

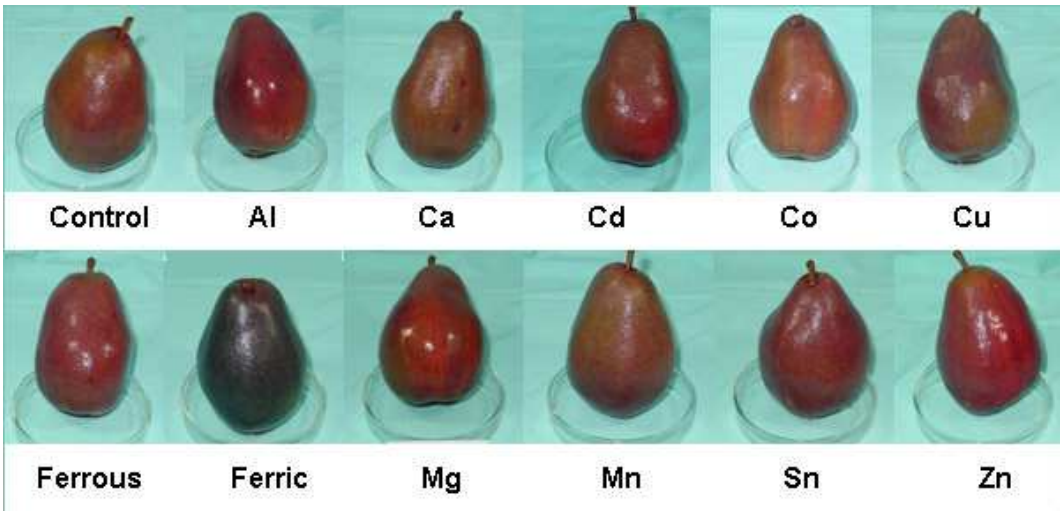


Figure 5.2 - CIEL*C*h color (a) and photo (b) of red D'Anjou pears after immersion in 0.01M metallic ion solutions prepared in phosphate buffer solution with a pH of 4.5 for 15 min. Control: Buffer solution (monopotassium phosphate, 0.1 M, pH = 4.5). Al: aluminum; Cd: cadinium; Ca: calcium; Co: cobalt; Cu: cupric; Fe^{+2}: iron divalent; Fe^{+3}: iron trivalent; Mg: magnesium; Mn: manganese; Ni: nikel; Sn: stannous; Zn: zinc.

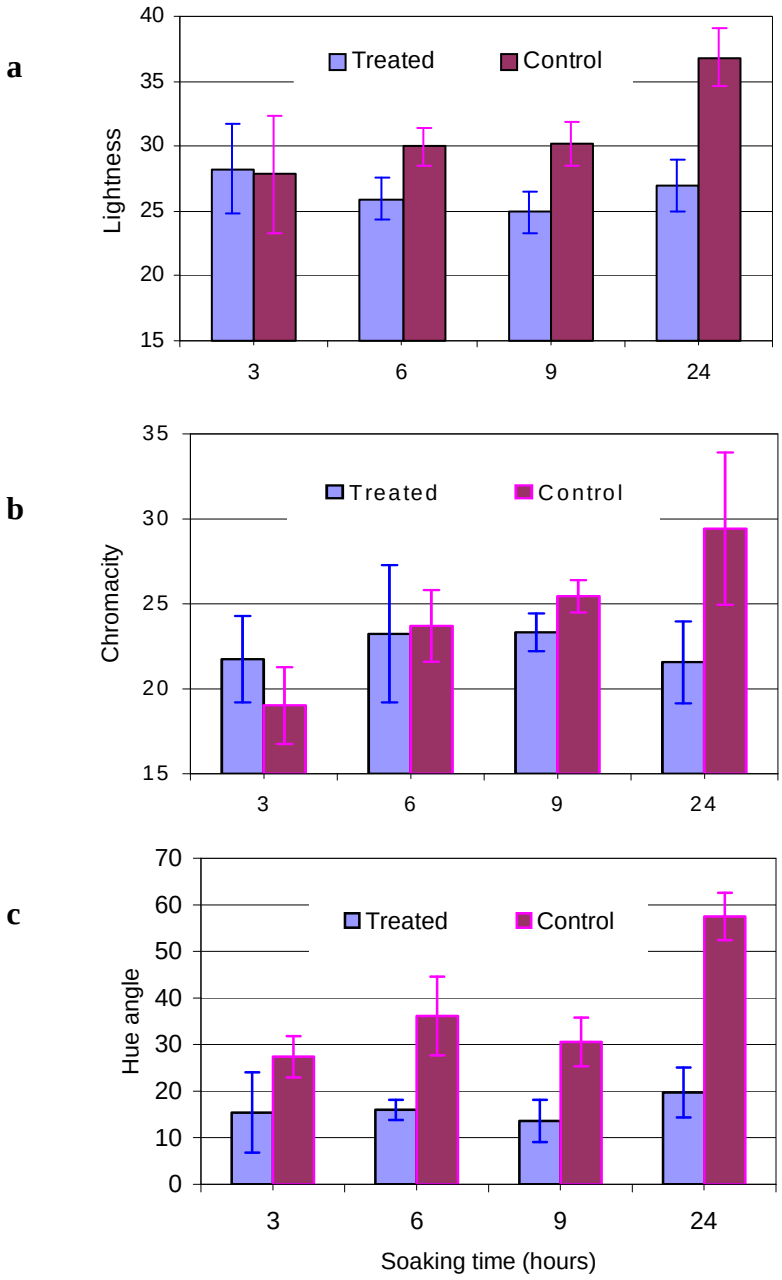


Figure 5.3 - CIEL*C*h values of treated samples and the control during 24-hr of water test. Treated: De-waxed pears were treated in an aqueous formula composed of water, formalin, HCl, tannic acid, and stannous chloride for 4 min before immersing in water solution containing 0.5% of ascorbic acid (pH~3.1) at 3 ^{o}C. Control: De-waxed pear immersed in water solution containing 0.5% of ascorbic acid (pH~3.1) at 3 ^{o}C.

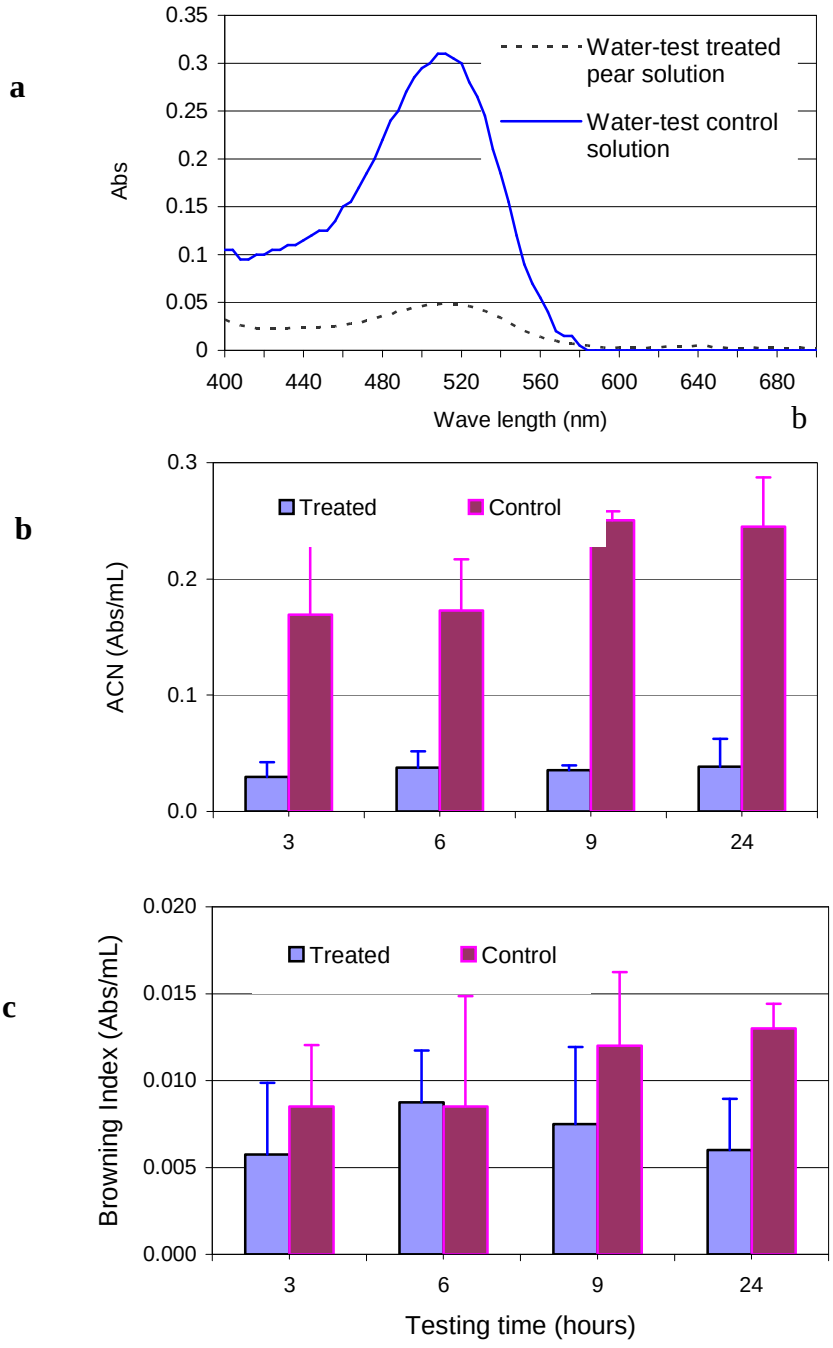


Figure 5.4 - Absorption spectral curves (a), anthocyanin contents (b), and browning index (c) of the water-test solution containing 0.5% of ascorbic acid (pH~3.1) over the time of storage at 3 ^{o}C. Treated: De-waxed red D'Anjon pears were treated in an aqueous formula composed of HCl, tannic acid, stannous chloride, and formalin for 4 min before water-test. Control: De-waxed pears without treatment.

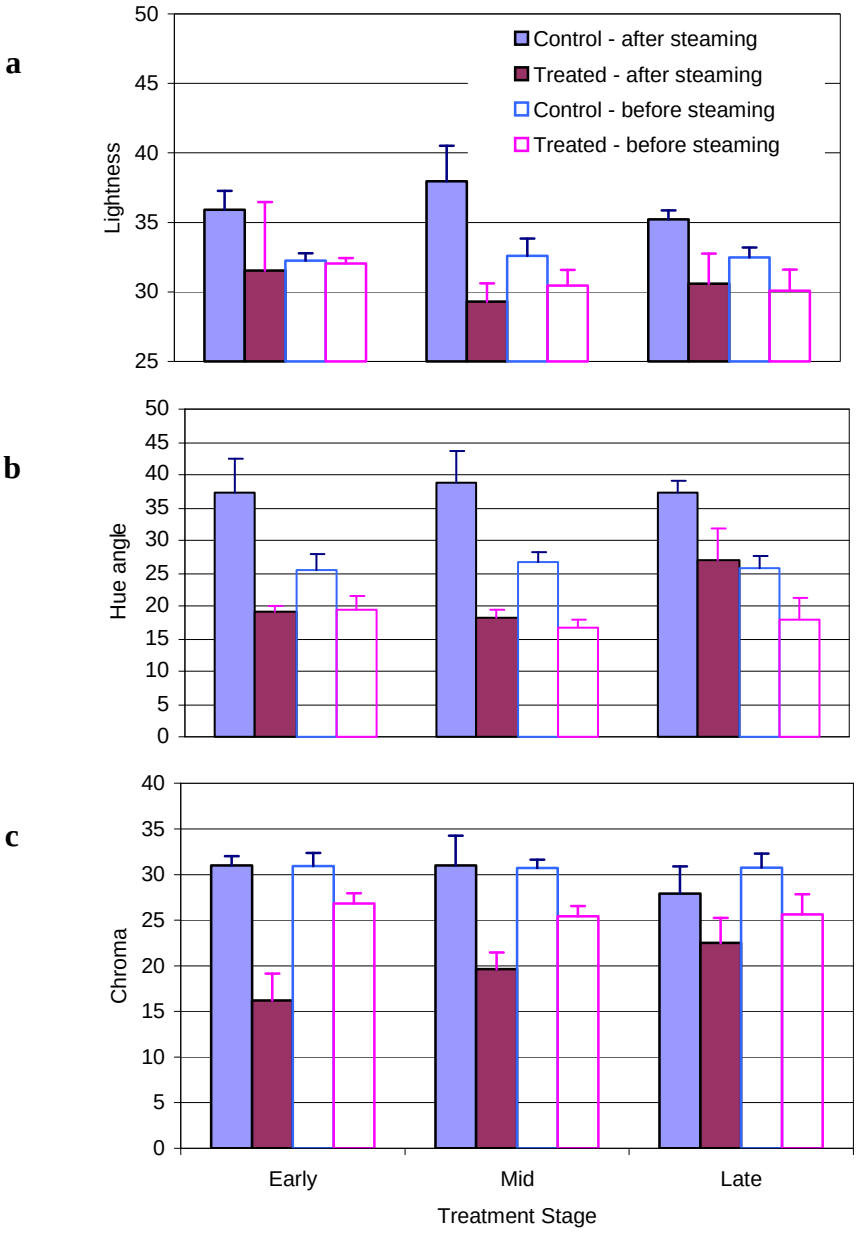


Figure 5.5 - CIEL*C*h values of treated red D'Anjou pears and the control samples before and after steam-heating at 73 °C for 7 min. For the treatment, 400 mL of developed formula (for treated pears) or HCl (for control) was used to treat 12 pears consecutively. The first 4 pears, the next 4, and the last 4 were represented as Early, Mid, and Late.

Figure 5.6 - Thermally processed red Starkrimson pear after storage in glass jar for 6 months at ambient temperature. Pear was pretreated in the developed formula, steam-heating at 74 ^{o}C for 7 min, cooled to room temperature, hot-filled into a glass jar, and thermally processed in 10 ^{o}Brix cane sugar solution at 94 ^{o}C for 7 min.

CHAPTER 6

Stabilization of Anthocyanins in Thermally Processed Red D'Anjou Pear Peels through Stannous-Anthocyanin Complexation and Crosslinking Polymerization

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6.1. Abstract

De-waxed Red D'Anjou pears were treated in formulated treatment solutions containing tannic acid, formaldehyde, stannous (Sn), and hydrochloric acid (HCl) that were prepared either immediately before application or 4 h earlier. Treated pears were drained off the excess solution, steam-heated at 73 ^{o}C for 7 min or not, hot-filled in water and processed at 94 ^{o}C for 7 min. Interactions between Sn and/or HCl and peel phenolics extract were assessed by use of a spectrophotometer. The effects of setting-time of the formulated solutions before applying in a range of 4 h and the time of Sn addition into the solution (0, 1, 2, or 3 h) on the polymerization capacity of the solutions were investigated by monitoring the haze formation of the solutions using a ColorQuest sphere spectrophotometer. Aqueous acetone extract of the peels from fresh and treated pears at major processing steps (immersion, steam-heating, and canning) were subjected to HPLC qualitative analysis on phenolics and anthocyanins. The total phenolics (TP) and anthocyanin content (ACN) of the peels were measured by Folin-Ciocalteu assay and pH differential method, respectively. Polymerization in the solution matrix was influenced by the composition, setting-time, and the time of Sn addition into the formulated solutions. The use of developed solution treatment with steam-heating prior to canning showed its capacity to fix and stabilize anthocyanins on the pear peels, most likely through the development of a crosslinked matrix and Sn-anthocyanin complexation. Cyanidin 3-galactoside together with chlorogenic acid, epicathechin were the major anthocyanin and phenolic, respectively, identified in pear peels. No major newly formed anthocyanins or small phenolics were detected in treated pears. Peels of immersed, steamed, and canned pears had TP of 21.7, 40.5, and 90.7%, respectively, lower than that of fresh pear peels and ACN of 34.3, 62.8, and 98.3%, respectively, lower that that of fresh peels.

6.2. Introduction

Cyanidin 3-galactoside is a major anthocyanin responsible for the red appearance of red pear cultivars (Mazza and Miniati 1993; Dussi and others 1995). This type of glycoside is extremely unstable and is easily destroyed during thermal processing and storage, resulting in loss of red appearance and/or browning. Several mechanisms of anthocyanin stabilization have been proposed, including copigmentation with other phenolics, chemical transformation mediated or not by aldehyde, complexation with metal ions, and incorporation of anthocyanins into aggregates (Lima and others 2002). Among these, the latter has been found to be very effective in preparing highly thermal stable red pigments in non-food application area (Kohno and others 2007). Anthoycanins incorporated or embedded in macromolecule aggregation or matrix is believed to be stabilized by an environmental protection provided by the host, preventing the pigments from water attacks. Recent researches on mature anthocyanin-rich plant cells have a globular inclusion possibly made of proteins to stabilize anthocyanins in synergy with stacking and copigmentation phenomena (Markham and others 2000).

In our previous studies, stannous (Sn) has been found to be a good chelating agent for stabilizing and fixing pear anthocyanins in pear peels. However, the complexation was not highly heat stabilized and most of the created purple pigments were destroyed during typical canning process. Attempts have been made to use tannic acid, formaldehyde, and hydrochloric acid in the formulation to stabilize the pigments. The results of our preliminary study suggested that an appropriate formulated solution containing these four active components combined with a gentle heat process is helpful to improve the performance of stannous.

In wood science, aggregates or resins are artificially made from phenolic source and formaldehyde. Being a phenolic, tannic acids (Figure 6.1) are known to condense initially with formaldehyde in the presence of either acid or alkali to form a phenolic alcohol, and then dimethylolphenol. The second stage of the reaction involves the reaction of the methylol group with other available phenol or methylophenol, leading to the formation of polymers. In the presence of bivalent metal

ions at the second stage, polymerization occurs at a much higher rate than that of the process with conventional acid or alkali catalysis (Pizzi 1983a).

Our hypothesis is that treating de-waxed pears with stannous, anthocyanins are fixed and become water-insoluble but not heat-stabilized. The resin supplied by a formulated solution composed of tannic acid, formaldehyde, and hydrochloric acid will synergy the effects of stannous and form a matrix to enhance the stability of Sn-anthocyanin complexes.

The objectives of this study were to evaluate the polymerization capacity of the formulated solutions composed of Sn, formaldehyde, tannic acid and hydrochloric acid and the interactions between pear peel phenolics and the compounds in the formulated solutions, to investigate the thermal stability of red pigments in pear peels through complexation and catalysis with Sn, formaldehyde, tannic acid, and hydrochloric acid, and to determine the amount and composition of phenolics and anthocyanins on the pear peels during major processing steps of immersion, steam-heating, and canning by use of HPLC and spectral measurement methods.

6.3. Materials and Methods

6.3.1. Materials

D'Anjou red pears (*Pyrus communis* L.) were purchased from a local grocery store in Corvallis, Oreg., U.S.A. Cane sugar was from C&H Sugar Company (Crockett, Calif., U.S.A.). Pears were de-waxed by use of a sugar spraying system as described by Ngo and Zhao (2005) (Ngo and Zhao 2005) to remove the wax layer on the top of the pear peels.

Tannic acid (TA) from Chinese natural nut galls, Folin-Ciocalteu's phenol reagent (FC), gallic acid, and sodium carbonate were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Hydrochloric acid (HCl) (36.5 - 38.0%) and 36 % formaldehyde solution (Formaldehyde, FL) were purchased from Mallinckrodt Baker. Stannous chloride (Sn) was from Em Science (Cherry Hill, NJ, U.S.A.).

6.3.2. Preparation of treatment solutions

The treatment solution was formulated based on our preliminary studies containing tannic acid, formaldehyde, and HCl solution with the addition of Sn ions (Chapter 5). The initial formula was modified from general reaction conditions to create resin from phenolics and formaldehyde presented in the documentation of wood adhesive, such as described by Tahir and others (2002) (Tahir and others 2002).

To make 525 ml of the solution of 4 components, 400 ml of 1% Tannic acid (TA) solution, 100 mL of Formaldehyde (FL), and 25 mL of HCl was mixed together. TA solution was previously prepared by adding 10 g of TA in 1-L of deionized water and stirring until homogeneity. Sn was added at different times to make a final Sn ion concentration of 0.0016 M (~200 ppm). Based on our preliminary study, the formulated solutions need to be allowed to let stand at room temperature for a couple of hours for developing a full capacity of retaining red pigments on processed pears. In this study, the solutions were first allowed to stand for 3.5 h before adding Sn, and then set for another ½ hr before treating the pear samples. This formula was thus named as “Sol-4h” as the total reaction time was 4 h, while “Sol-0h” stands for solutions that were used right after all reagents were mixed together. Table 6.1 lists the other six treatment solutions prepared from different combinations of the reagents. Other than the Sol-4h solution, all others were used immediately after the reagents were well mixed.

For treating the pears, an individual whole pear (~120 g) was immersed in the formula (~ 525 mL) for 30 s, labeled as “immersed pears” throughout this manuscript. Pears were set on a rack to drain and dry with an air flow of about 30 m/min for about 4 min before two more immersion-drying cycles. The same solution was consecutively used for four different pears.

6.3.3. Thermal processes of pears

6.3.3.1. Steam-heating

Individual whole pear was thermally processed at 73 ± 4 ^{o}C for 7 min in a cylindrical chamber (20 cm diameter x 30 cm length) designed in our laboratory with

steam flowing upward from the bottom through the chamber and out through a bell-shaped lid. The temperature inside the chamber was maintained by adjusting the flow rate of the steam input. After steam-heating, pears were transferred to a closed chamber which had the inlet and outlet for nitrogen gas to come in from onside to cool the product and exit at the other side. The gas flow was kept continuously under a pressure of c.a. 100 mmHg. The fruits were cooled to room temperature within 30 min. In processing sliced pears, the cooled pears were sliced into chunks before being canned in the next step.

6.3.3.2. Canning processing

A modified canning procedure for green pears was used (Ngo and Zhao 2005). Previously steam-heated or unheated pears were sliced and hot-filled with boiling water in 300-mL Mason glass jars (Olshen's Bottle Supply Co., Portland, OR, U.S.A.). The jars were sealed and immersed in 94 ^{o}C water in a 60-L steam kettle for 7 min before cooling to room temperature. Another set of the samples were prepared in the same way except that they were steam-heated at 70 ^{o}C before slicing and canning. Similarly, whole pears were processed in 1-L glass jars.

6.3.4. Polymerization of formulated solutions

6.3.4.1. Haze measurement and polymerization

Light going through a solution containing large size soluble molecules can be scattered, which gives a "haze" perception of the solution. In the literature, haze measurement by spectral methods has been found useful to describe the degree of polymerization in a solution since haze formation overtime reflects the relative changes in size and concentration of particles in a solution (Morris 1987; Siebert 2000). Haze concentration was thus used in this research to describe the formation of polymers in the formulated solutions with and without addition of pear peel extracts. the measurement of transmission haze was performed on a ColorQuest HunterLab Spectrophotometer (Hunter Associates Laboratories Inc, Reston, Va., U.S.A.) by using a 2-mm length cell. The results were reported in percentage haze.

6.3.4.2. Formulated solutions

The haze of three formulated solutions (3, 5, and 8 in Table 6.1) composed of various combinations of Sn, HCl, FL, and TA was measured every hour over a time period of 4 hours. A similar approach to make the solutions described for preparing Sol-4h was applied in which water was used to replace any missing reagent to make a final volume of 525 mL of formulation. Reaction time started immediately after the reagents were mixed together. Four other formula had the same composition as that of Sol-4h but the time when Sn was added was different among the formula, i.e., at the same time as HCl, FL, and TA were mixed together, or at 1, 2, and 3 h of setting time. The haze of formulated solutions at zero hour time was measured at the moment of mixing of HCl, FL, and TA.

6.3.5. Compositional changes of phenolics in the peels of treated pears and interactions between pear peel phenolics and selected reagents in formulated solutions

6.3.5.1. Peel sampling

Pear peels were manually removed from fresh, immersed, steam-heated, or canned pears by use of a knife and immediately frozen in liquid nitrogen. It is believed that the very exterior part of the peels, “top-part of peels”, had direct contact with the reagents in the formulated solutions, thus this part of the peels should receive a close monitoring for changes. However, any attempt to separate the peel top from its bottom part was not successful on thermally treated pears. Thus, all heat treated pears (steaming, canning) were sampled as “whole peels”. In a separate effort to look at the composition of the soft tissues rich in red pigments, the top of the peels was gently scaled to remove the red tissue from the fresh and immersed pears.

The collected samples were immediately freeze-dried under a 100 mmHg vacuum pressure for 48 h by use of a Consol 4.5 (The Virtis Company, Inc., Gardiner, NY., U.S.A.). Freeze-dried peels were ground into powders in liquid nitrogen by use

of a blender (Waring Products, Model 34B97, Dynamics Corporation of America, New Hartford, Conn., U.S.A.).

6.3.5.2. Extraction of phenolics. About 2 g or 0.5 g of whole peel powders or top-part peel powders, respectively, was mixed with 30 mL of aqueous acetone (30:70 v/v). The mixture was sonicated, centrifuged, and the supernatant was collected. Three additional extractions of the residue with aqueous acetone were done. Supernatants were combined and partitioned with 50 mL of chloroform and centrifuged for better separation between the organic and aqueous phases. The aqueous phase containing anthocyanins was recovered and transferred to a rotovaporator to remove residual acetone at 40 °C. The extract was diluted with MillQ water to 25 mL and stored at – 75 ^{o}C until analysis.

6.3.5.3. Interactions between peel extracts with Sn and/or HCl and with the formulated solution

To assess the individual interactions between Sn and HCl and peel phenolic extracts, the individual reagent in its concentrated form was added to the extracts. A 10 mL of pear top peel extracts was mixed with either 200 ppm of Sn or 1 or 5% (v/v) HCl. The haze and spectral visual light absorbance of the solutions were measured immediately after mixing. To address the interactions between the formulated solution as a whole and peel phenolic extracts, the extract was added to the formulated solution and, in order to avoid excessive dilution of the formula, the extract:solution ratio was controlled at 1:15. Two mL of top peel extract was added into a 30 mL of formulated solution. Sol-0h and a solution composed of HCl and FL was used. The latter solution was prepared in the same way as preparing Sol–0h solution except replacing tannic acid volume with deionized water. The haze percentage of the latter solution and that of two control solutions prepared without an addition of extracts were monitored during a time range of 20 min. The Shimazu UV160U spectrometer was used to measure the light absorbance in the range from 400 nm to 700 nm with a 1-cm disposable cell. The results were reported in absorbance unit.

6.3.5.4. Analysis of total monomeric anthocyanins (ACN) and total phenolic content (TPC)

Aqueous extracts were diluted to a known volume with deionized water. The total anthocyanin content was determined by using the pH-differential method described by Giusti and Wrolstad (2005) (Giusti and Wrolstad 2005). The ACN was expressed as mg of cyanidin 3-galactoside/g of dried materials. A molar extinction coefficient of 29,900 L/ cm (Giusti and Wrolstad 2005) and molecular weight of 449.2 were used in the calculation. The absorbance was measured at 510 nm and 700 nm. Total phenolic content (TPC) was determined using the Folin-Ciocalteu (FC) assay (Waterhouse 2005). Aqueous extracts were diluted to a known volume with deionized water. A 0.5 mL of diluted extract was mixed with 0.5 mL of FC reagent in 7.5 mL of deionized water. The samples were held for 20 min at room temperature before 3 mL of 20% sodium carbonate (w/v) was added. The well-mixed samples stood for another 20 min at 40 °C before the absorbance was measured at 765 nm by a Shimazu UV160U spectrometer (Shimazu Corp., Kyoto, Japan) with 1-cm disposable cells. Gallic acid monohydrate was used as a standard and expressed as gallic acid equivalents (GAE) in mg/g of dried materials.

6.3.5.5. HPLC analysis of anthocyanin and phenolics

Chromatographic analysis of anthocyanins was done according to the method of Durst and Wrolstad (2005) (Durst and Wrolstad 2005) using a Prodigy ODS-3 column (5 μm), 250 mm x 4.60 mm i.d. (Phenomenex, Torrance, CA), fit with Allsphere 10 mm x 4.6 mm i.d. ODS-2 guard column (Alltech, Deerfield, IL). The analysis was performed on a Hewlett-Packard 1090 Liquid Chromatograph (Agilent Technologies, Palo Alto, CA), equipped with photodiode array detector and Gateway 2000 P5-90 computer with Hewlett-Packard HPLC Chemstation software. Mobile phase A consisted of 100% acetonitrile, and mobile phase B was a mixture of 1% phosphoric acid and 10% acetic acid (glacial) (v/v) in deionized water. Monitoring was performed at 520 nm. The material used as a reference sample is cranberry juice.

The program was as follows: 0 min, 2% A; 0-25 min linear gradient from 2 to 20% A, 25 – 30 min linear gradient from 20 to 40%, 30 – 32 min linear gradient from 40 to 2%.

Phenolic analysis was done using a similar system. The mobile phase A consisted of 100% acetonitrile, and mobile phase B was a mixture of 0.07 M phostphoric acid. The program was as follows: 0 min 10% A, 0 – 5 min linear gradient from 0 to 5% A, 5 – 30 min 35%, 30 – 35 min 55%., 35 – 39 min from 55 to 10%.

6.3.6. Experimental design and statistical analysis

A completely randomized design (CRD) was used for the collection of peel samples for chemical and HPLC analysis and a split-plot CRD was used for the studies of interactions and haze changes in the formulations. All the experiments were duplicated. Statistical analysis was performed using SAS (SAS Inc., Cary, N.C., U.S.A.). Multifactor analysis of variance was applied for the first CRD design with source of variance being processing extent (fresh, immersing, steam heating, and canning) and location within pear peel. Differences between means were tested for significance by using GLM (general linear model) procedure with Duncan test, using a level of significant of $P < 0.05$.

6.4. Results and Discussion

6.4.1. Polymerization capacity of formulated solutions

Figure 6.1 describes the evolution in transmission haze of the formulated solutions containing formaldehyde with various combinations of other three reagents. Formulated solution type and reaction time both had significant effects on the haze percentage of the solutions ($P< 0.05$). There was a linear increase in haze percentage of the solutions that had both formaldehyde and tannic acid together in the solution. Without tannic acid, there were no significant changes in the haze of the solution over a 4 h of time period as seen in the curve of the HCl/FL/Sn solution (Figure 6.1). The critical role of the tannic acid for haze formation in formulated solution supports the known function of tannic acid as raw material in building phenolic based polymers or

macromolecules (Pizzi 1983b). Among the other three formulated solutions, the one that consisted of all four reagents (HCl/FL/TA/Sn or Sol-0h) had a high increase in haze and that without Sn and HCl had the lowest increase, while the formula composed of HCl/FL/TA had its haze percentage between the two of the latter formulas. In summary, the increases in the haze percentage during preparation of the formulated solutions represent the building up of large size molecules from tannic acid. Both HCl and Sn accelerate the process. This finding echoes the catalysis roles of acid and bivalent metal ions in polymerization of phenolics mediated by formaldehyde reported by Pizzi (1983) (Pizzi 1983b). Based on the chemical structure of tannic acid, there are two activated *o*-hydroxyl positions on each unit of gallic acids (Figure 6.2) that can participate in electrophilic aromatic substitutions similarly to phenol case catalyzed either by hydrogen H^+ through activation of formaldehyde or by metal ions (Figure 6.3a and b, respectively) (Pizzi 1983b). Figure 6.3c describes the two steps of the initial process of polymerization between galloyl units (Özacar and others 2006): the first step is electrophilic substitution on an *o*-hydroxyl group of a galloyl unit to form a hydroxylmethyl compound; the second step results in a crosslinking through a methyl bridge between the latter newly formed compound and another galloyl unit (Figure 6.3c). Further crosslinking among galloyl units is expected and results in polymerization and haze increase in the formulated solutions.

Figure 6.4 shows the dependence of transmission haze of Sol-4h solution on the addition of Sn. The addition of Sn significant increased haze percentage in formulated solution and the reaction time of the Sn with other 3 reagents was also a significant factor ($P< 0.05$). This result indicated that preparation procedure is expected to change the nature of formulated solution for better or worse. For effectively establishing a matrix with pear peel materials to trap the pigments, the size of activated tannic acid, base macromolecules, could be a determining factor. An expectation is that if being too large the polymer molecules formed from activated tannic acids can’t easily rearrange themselves to form crosslinking with the cell wall, while being too small a molecule, such as initial tannic acid, don’t have long branches to promote a good matrix.

6.4.2. Interactions between pear phenolics with Sn and HCl in formulated solutions

Figure 6.5 shows that the absorbance of pear extracts was significantly increased when HCl was added, representing the hyperchromic effects (increased absorption) reported in the literature for acid and metal ions (Brouillard and others 1997). When Sn was added alone, very large bathochromic and hyperchromic shifts (increased wavelength of peak absorption of around 20 nm), named bluing effects were observed (Figure 6.5) as a result of the increase in the colored quinonoidal forms accompanying metal complexation (Wrolstad 2000). The purple color described in our earlier study of Sn treated de-waxed red pears echoed this finding (Chapter 5). Sn might also complex with other phenolics in the pear extracts and created large size complexes as seen in a significantly increased haze percentage of the treated pear extracts (44%) compared to that of the untreated sample (7.8%) (Table 6.3).

Metal-anthocyanin complex formation is known to depend on pH (Brouillard and others 1997). In this research, the bathochromic effect of Sn was found to be limited or annulled if HCl was present together with Sn. As shown in Figure 6.5, the absorbance curve of the peel extract mixed with Sn and 1% HCl shifted to a lower range of wavelength, very close to the absorbance curve of the extract containing HCl only. This finding suggested that the typical bathochromic effect of metallo-complexation was drastically reduced. In fact, the absorbance curve of the pear extract mixed with Sn and 5% HCl (data not shown) coincided completely with that of the pear extract mixed with HCl only. Similarly, the haze percentage of the latter solution was not significantly different from that of the Sn and HCl-mixed solution (Table 6.3). The complexation of metal ions with phenolics in general is thus believed to be insignificant at highly acidic condition used in the formula (e.g. 5% HCl, v/v). Nevertheless, the hydrochloric acid added to the pear peels was expected to be removed through evaporation and dilution during steam heating and canning pears after the immersion in the formulated solutions; stannous might subsequently exercise its major impact on fixing and stabilizing anthocyanins and phenolics.

Figure 6.6 shows that there was a rapid increase in haze percentage in the mixture of the extract added to developed formula (Extract+HCl/FL/TA/Sn (Sol-0h)) and in the solution composed of HCl and formaldehyde shortly after the addition of pear phenolics extracts, while the control solutions without the extract showed no significant changes in the studied time period. This indicates that formaldehyde has the capability to polymerize pear peels in a short period of time. This may be explained by the presence of various phenolics in pear peels, such as epicatechin and proanthocyanidins that can participate in electrophilic substitution, reactions mediated by formaldehyde in the presence of acid (Pizzi 1983b). Figure 6.6 shows that, compared to the HCl/FL solutions, the presence of tannic acid and Sn in the Sol–0h solution enhanced haze formation or the polymerization in the latter solution. However, at which level did Sn and/or tannic acid involved in the polymerization of pear phenolics is unclear at this point.

6.4.3. Treatment effect on the color of canned pears

Figure 6.7 shows finished sliced D'Anjou pears previously treated in 7 different formulated solutions with 2 processing schemes. The two tested schemes were immersion of dewaxed pears in prepared formula prior to thermal processing by hot-filling and canning (Figure 6.7, row A) or by steam-heating prior to hot-filling and canning (Figure 6.7, row B). The pears treated in no-formaldehyde solutions (formula 1 and 4) yielded the lightest color with little or no redness retained on the peels while the pears treated in formaldehyde containing solutions had a dark color. Some samples showed purple red, particularly those steam-heated prior to caning (Figure 6.7, row B). Although Sol-0h and Sol-4h treatments (formula 6 and 7, respectively) were identical in reagents used in the treatment solutions, the approaches used to prepare the solutions were different in terms of setting time, where Sol-4h had a 4 h of solution setting time while Sol-0h had none. The pictures 6 and 7 in Figure 6.7 show that the Sol-4h in combination with steam-heating result in better color retention in canned pear chunks, the evenly red purple color on their peels.

As it has been elaborated early, Sol-4h and Sol-0h solutions might have a very different nature in terms of molecule size and activated reagents. During solution preparation and/or setting, the tannins had been activated and crosslinked together to become larger activated molecules. When pears peels come in contact with these activated macromolecules, specifically during immersion and drying, the latter would interact with phenolics in pear peels as explained earlier as well as cell wall materials such as lignin, a phenolic-based material embedded in plant cell walls (Nada and others 2003). The reactions would have been accelerated during steam-heating due to high temperature condition leading to an intensive matrix formed between tannic-based macromolecules and phenolics and plant materials. The formed matrix would trap and stabilize the anthocyanins. There was a possibility that the cation caused precipitation of anthocyanins and phenolics, making these small size molecules become large size clusters so that they are retained inside the structure. Another possibility is that Sn can act as a link to tie anthocyanin onto the matrix, since the ion is a bivalent chelating agents that can bind to several phenolic molecules at the same time (Özacar and others 2006). Thus, the matrix would not just enclose the anthocyanins, but also link to the pigments through Sn ions.

Figure 6.8 illustrates the whole red D'Anjou (1) and Starkrimson (2) pears treated by Sol- 4h solution and processed by steam-heating prior to canning. The pictures were taken when the products had been stored for 1 month and 10 months, respectively.

6.4.4. Anthocyanin and phenolics content and profile in treated pear peels

Table 6.2 shows the TP and ACN in fresh and treated pear peels. For each g of dried material, a fresh red D'Anjou pear had the highest TP and ACN in the top part of the peels, 47.06 mg EPA and 2.04 mg cyanidin galactoside, respectively, which were significantly higher than 17.76 mg EPA and 0.47 mg cyanidin galactoside in whole peels, respectively. This finding confirmed the general belief that the top layer of the pear peels is concentrated with phenolics and anthocyanins. This location, in fact, makes it possible to manipulate the pigments through immersion pears in a formulated

solution where the phenolic-rich part directly interacts with the reagents in formula. Compared to green D'Anjou pear peels that was reported to contain c.a. 12 mg EPA/ g dried material, red D'Anjou having a 58% higher TP highlights the general belief that fruits rich in red anthocyanins have higher phenolic contents.

Data in Table 6.2 also shows that simply immersing the pears in the acidic formulated solution caused c.a. 21.7% and 34.3% reduction in TP and ANC, respectively in the top peels. The TP and ACN of steam-heated whole pears were 10.56 mg EPA and 0.17 mg cyanidin galactoside, respectively, while they were 1.65 mg EPA and 0.01 mg cyanidin 3-galactoside, respectively for steam-heated and then canned pears, a substantial loss in a range of 40.5% to 90.7% in phenolics and anthocyanin of whole peels of steam-heated and canned pears. However, Figure 6.9 shows that the extraction with aqueous acetone could not dissolute and exhaust the pigmented materials as their fibers still retain purple chromophores after the four extractions. The immersed pears peels, on the contrary, become yellow white after three extractions. The phenolics and anthocyanins hence were not completely dissolved and the reported analysis results underestimated their amounts in the thermally processed pears. The low solubility of the phenolics in processed pear peels compared with that of non-thermally treated samples (fresh or treated fresh pears) suggested a formation of some physical or chemical protective barriers due to thermal treatment, specifically steam-heating.

Figure 6.10 presents chromatography analysis results on the anthocyanins of peel extract of pears at different steps of processing. Their chromatographic analysis results are presented in Figure 6.11. Cyanidin 3-galactoside was the major anthocyanin in pear peels while peonidin 3-galactoside was the largest among the minor ones (Figure 6.10a), which agrees well with previously published data (Mazza and Miniati 1993; Dussi and others 1995). These two anthocyanins were found in significant amount in the peels of pears after steaming (figure 6.10c). For phenolic analysis, the overall chromatography spectrum of fresh and treated pear peels stayed unchanged suggesting no major changes the profile of the simple phenolics (figure 6.11). Some of the phenolic acid and flavonols reported in the literature for pear skin, such as

chlorogenic, epicatechin, and quercetin were identified on the chromatography spectrum (Figure 6.11). As no new major peaks in the chromatographic spectra of anthocyanins neither of phenolics were observed for pear peels during the major steps of immersion and steam heating, the results of this study do not support the hypothesis of a major chemical transformation of simple phenolics and anthocyanins due to addition of formaldehyde and acidic condition. However, there is a possibility that the formed compounds were in polymerized form and thus not extractable, or detected in chromatogram. The low values of TP and ACN would simply reflect the loss of phenolics due to processing and ineffective extraction. As described earlier, the extraction for peel phenolics and anthocyanins of finished pears was extremely poor and hence no HPLC analyses were performed for them.

6.5. Conclusion

Treating pears in a developed formula containing 100 mL of formaldehyde, 200 mL of 1% tannic acid, 25 mL of hydrochloric acid, and 200 ppm stannous chloride and steam-heating treated pears at a temperature about 73 ^{o}C have been proved to be capable in stabilizing red pear anthocyanins on the pear peels after canning process. Exposing the treated pears to a gentle heat treatment was believed to enhance the formation of tannic acid-based matrix which in turn retained and protect anthocyanins during canning through inclusion phenomenon reported by various authors (Markham and others 2000; Mulinacci and others 2001; Kohno and others 2007). A key compound catalyzing the polymerization reactions was Sn which is also expected to enter in complexation with phenols and anthocyanins and to contribute in detaining anthocyanins in the matrix. Although it was still unclear about the nature of the pigments retained on the finished pears since its extraction from the treated pears was not effective, the current research results suggest that they are likely unchanged in nature but significantly reduced in amounts. Some major disadvantages of the formulated treatment include the color shifting of pear anthocyanins from red to red purple or purple and the pear peels were loosen on the fruit surfaces due to the reaction of HCl. As a phenolic resin (prepolymer) can be prepared under a neutral

condition and the catalysts are divalent metal ions (Tigani and others 2000), future research can look at replacing HCl with organic acids.

6.6. Acknowledgement

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

6.7. References

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Table 6.1 – Formulated treatment solutions with various combinations of hydrochloric acid (HCl), formaldehyde (FL), tannic acid (TA) and stannous (Sn)

	Formulated treatment solutions	Symbols
1	HCl	HCl
2	HCl + Formaldehyde	HCl/FL
3	HCl + Formaldehyde + Tannic acid	HCl/FL/TA
4	HCl + Stannous	HCl/Sn
5	HCl + Formaldehyde + Stannous	HCl/FL/Sn
6	HCl + Formaldehyde + Tannic acid + Stannous (Solution-0 h)	HCl/FL/TA/Sn (Sol-0h)
7	HCl + Formaldehyde + Tannic acid + Stannous (Solution-4 h)	HCl/FL/TA/Sn (Sol-4h)
8	Formaldehyde + Tannin acid	FL/TA

Notes: Formula 1 – 6 were used for treating pears immediately after listed reagents were mixed together, thus Formula 6 was presented as “Sol-0h”. For the Formula 7, HCl, FL, and TA were first mixed and set for 3.5 h, and Sn was then added and set for another 0.5 h before used for treating pears, represented as “Sol-4h”.

Table 6.2 – Anthocyanin and phenolic contents in the peels of fresh and treated pears at different processing steps

Samples	Pears*	Phenolics mg EGA/g[g]	Mono-anthocyanin mg cyanidin 3-galactoside/g[h]
Top part of the peels[a]	Fresh[c]	47.06 ± 0.84	2.04 ± 0.08
Peels[b]	Fresh	17.76 ± 1.16	0.47 ± 0.05
Top part of the peels	Immersed in formulated solution[d]	36.85 ± 5.07	1.34 ± 0.19
Peels	Steam-heated after immersion[e]	10.56 ± 0.74	0.17 ± 0.04
Peels	Steam-heated, then canned after immersion[f]	1.65 ± 0.04	0.01 ± 0.00

* Pear peels were de-waxed before analysis. Means ± standard deviations derived from 2 replications with a combination of 4 pears per replication. Means within the same column followed by the same letter were not significantly different (LSD test, $P<0.05$).

[a]Top part the peels: only the first layers consisting soft tissue on top of the peels of dewaxed pears are sampled.

[b] Peels: the peels consist both the pigment rich top layer and the hard epidermal cells. Some of the adjacent flesh tissue was also sampled together (hypodermal layer).

[c] Pears were de-waxed with peels-on.

[d] Pears were de-waxed, immersed in Sol-4h and drained.

[e] Pears were de-waxed, immersed in Sol-4h, drained, and steam heated at 73 ^{o}C for 7 min.

[f] Pears were de-waxed, immersed in Sol-4h, drained, steam heated at 73 ^{o}C for 7 min, and canned at 94 ^{o}C for 7 min.

[g] Anthocyanin content was expressed as mg of cyanidin 3-glycoside/g of dried sample.

[h] Phenolic content was expressed as mg equivalent gallic acid (EGA) /g of dried sample.

Table 6.3 – The haze percentage of pear extracts when mixed with stannous and hydrochloric acid

Formulated treatment solution	Stannous (ppm)	Hydrochloric acid (%, v/v)	Haze concentration (%)
1	0	0	7.76
2	200	0	44.42
3	200	1	17.92
4	200	5	10.56
5	0	1	9.52

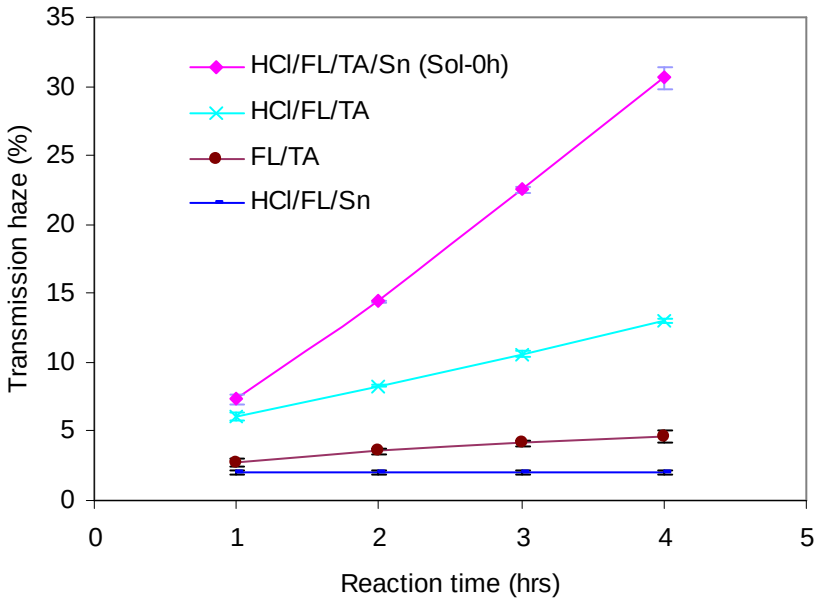

Figure 6.1 - Evolution in haze of various formulated solutions composed of stannous (Sn), hydrochloric acid (HCl), formaldehyde (FL), and/or tannic acid (TA). A solution of 525 ml of HCl/FL/TA/Sn (Sol-0h) was made by mixing 400 ml 1% TA solution, 100 mL FL, 25 mL HCl, and 0.0016 M Sn together. Other solutions were made in the similar way by replacing the missing reagents with water to make a final volume of 525 mL. Reaction time started as soon as the reagents were mixed together.

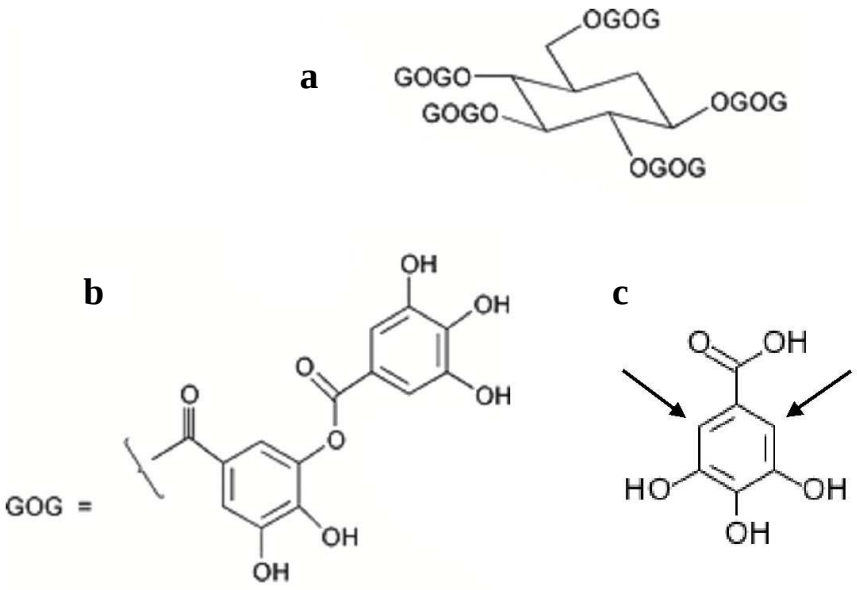

Figure 6.2 – Structure of tannic acid (gallotannin). It is a polygalloyl glucose (a): a penta-*o*-galloyl-β-D-glucose attached other galloyl groups (b). Each galloyl unit has two free activated sites on the ring (c).
Modified from (Whiting 2001)

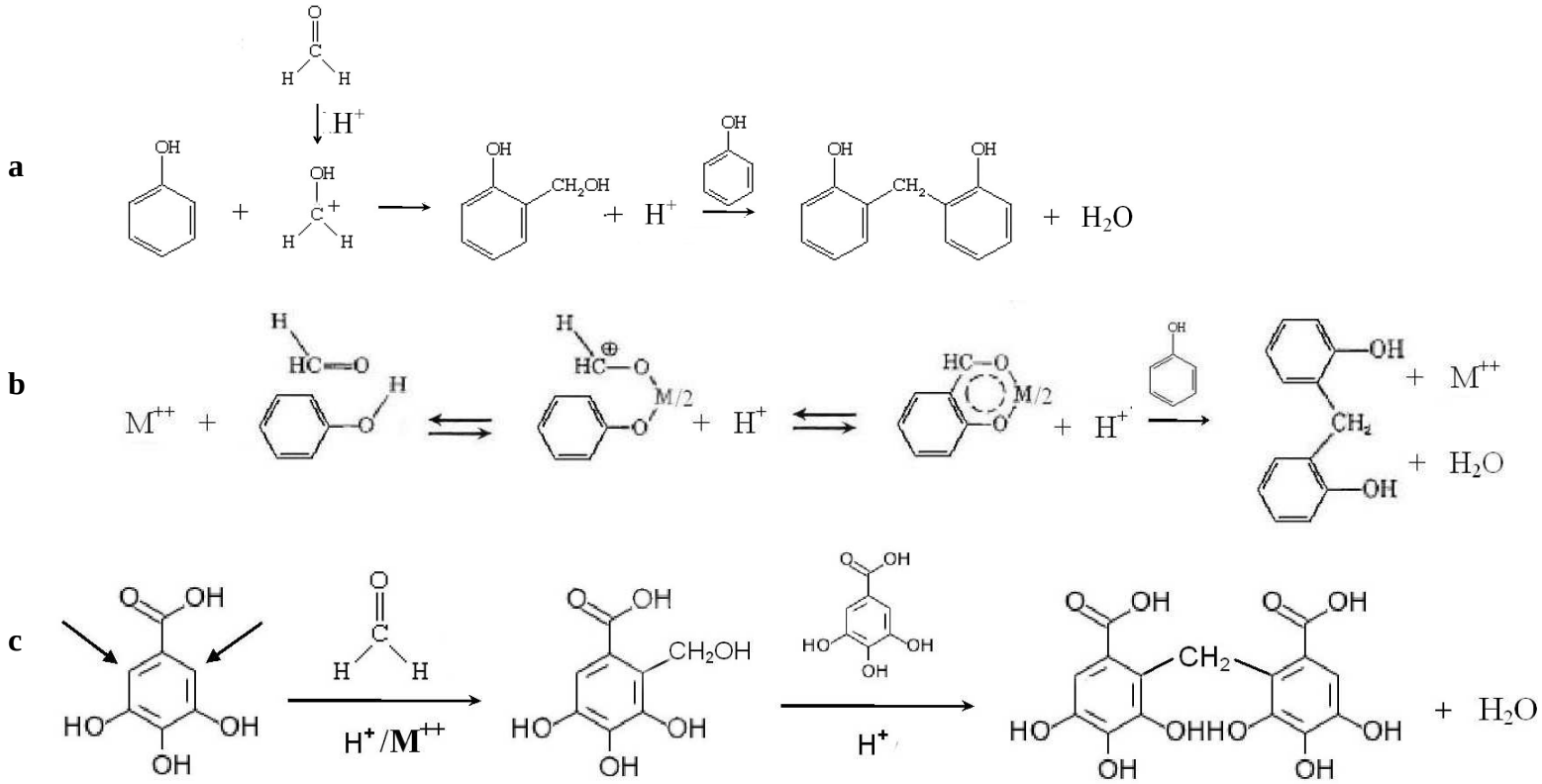

Figure 6.3 - Crosslinking formation between aromatic rings through two steps: electrophilic aromatic substitution and condensation. Phenolic crosslinking formation between phenols catalyzed by proton H^+ (a) and divalent metal ion (b) with formaldedhyde as a crosslinking agent and crosslinking formation between galloyl units through two steps: electrophilic aromatic substitution and condensation (c).
Modified from Pizzi (1983a) and Özacar et al. (2006).

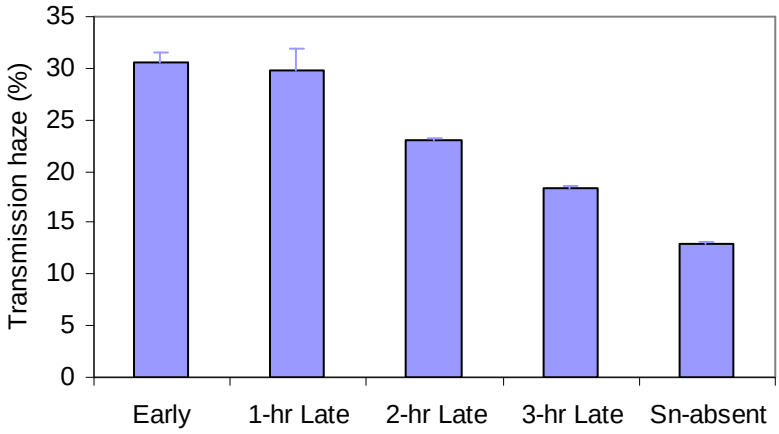

Figure 6.4 - Dependence of haze formation on the moment of the addition of stannous (Sn) in a 4-h process of formulation preparation/reactions. To prepare 525 ml of the formulated solution, 400 ml of 1% tannic acid solution, 100 mL of formaldehyde, 25 mL of HCl were mixed together. Early: 0.0016 M of Sn was mixed together with other 3 reagents and set for 4 h before measurement (Sol-4h). 1-hr Late, 2-hr Late, and 3-hr Late: 0.0016 M of Sn was added into the 3-reagents mixture after 1, 2, and 3 h, and then set for another 3, 2, or 1 h, respectively to have a total 4 h setting and reaction time before the measurement. Sn-absent: No addition of Sn ions. The solutions were gently stirred during mixing the reagents and let set still. The reaction time was 4 h counted since the initial mixture of reagents.

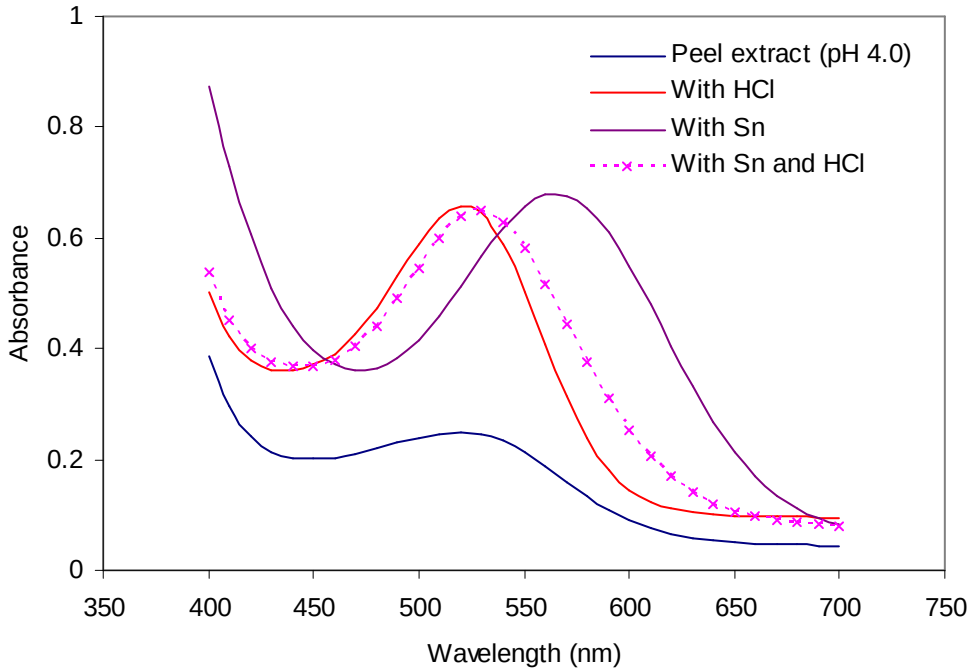

Figure 6.5 - UV-spectral curves of aqueous pear peel extracts mixed with hydrochloric acid (1%, v/v) and/or stannous (Sn, 200 ppm).

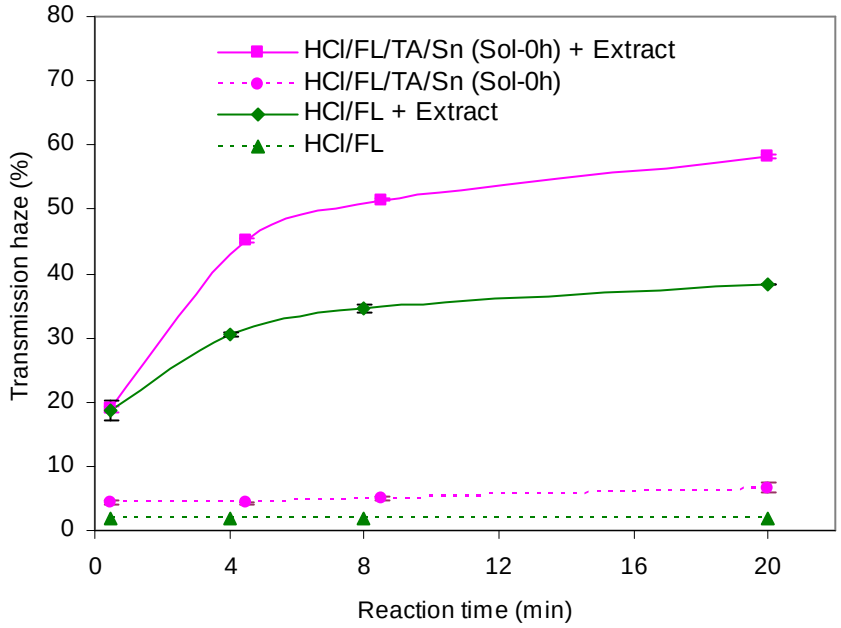

Figure 6.6 - Evolution in haze over time in a mixture of formulated solutions consisting of hydrochloric acid, formaldehyde, tannic acid, and stannous (Sol-0h) or only hydrochloric acid and formaldehyde with and without addition of peel pear phenolic extracts. Each mixture was made from 30-mL formulated solution and 2-mL pear peel phenolic extract. To prepare 525 mL of the Sol-0h, 400 ml of 1% tannic acid (TA) solution, 100 mL of formaldehyde (FL), and 25 mL of 1% hydrochloride acid (HCl) were mixed together. Stannous chloride (Sn) was shortly added to make a 200 ppm. For the formula composed of HCl and Formaldehyde (HCl/FL), TA was replaced by deionized water and no Sn was added. Reaction time was counted immediately after the solutions were made. For samples with added extracts, extracts were added immediately after the solutions were made and reaction time was counted. Notes: the standard deviations are in the range of less than 1 % and are thus not obvious on the graphs at the presented used scale)

Figure 6.7 - Thermally-processed sliced D'Anjou pears stored in glass jars. The whole pears were previously immersed in formulated treatment solutions, drained by convection air flow prior to slicing into chunks, and canned (row A) at 94 ^{o}C for 7 min or steam-heated first at 73 ^{o}C for 7 min and then canned (row B). Canning solution was plain water.
Pears were treated in the solutions composed of: 1. Hydrochloric (HCl) solution; 2. HCl and Formaldehyde (FL); 3. HCl, FL, Tannic acid (TA); 4. HCl and Stannous (Sn); 5. HCl, FL, Sn; 6. HCl, FL, Sn, TA; 7. Same as 6 except that the formula was set for 4 h before use, in which HCl, FL, and TA were first mixed and set for 3.5 h, and then Sn was added and set for another ½ h. To make 525 ml formulated solution containing all 4 reagents, 400 ml of 1% TA solution, 100 mL of FL, 25 mL of HCl, and 0.0016 M Sn were mixed together. To make a solution of less reagents, deionized water was added to make up total 525 mL.

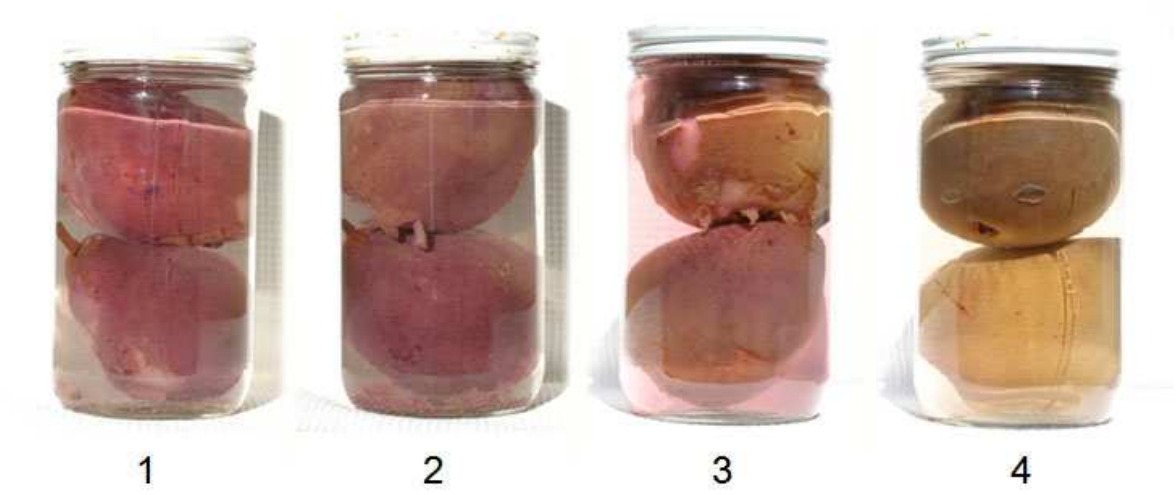

Figure 6.8 –Thermally processed whole pears stored in glass jars. The pears were previously treated in formulated solutions, drained by convection air flow prior to steam-heating at 73 ^{o}C for 7 min, and then hot-filled and canned at 94 ^{o}C for 7 min. All pears were de-waxed briefly prior to processing.
1. Starkrimson pears treated in a formula containing hydrochloric acid (HCl), Formaldehyde (FL), Tannic acid (TA), and Stannous (Sn); 2. D'Anjou pears treated in a solution similar to case 1; 3. D'Anjou pears treated in HCl, FL, and TA; 4. Untreated D'Anjou pears. To make 525 ml of the formulated solution, for 1 and 2, 400 ml of 1% TA solution, 100 mL of FL, 25 mL of HCl were mixed and set for 3.5 h, and then 0.0016 M Sn were mixed before the solution was set for another ½ hr. For 3, no Sn was added and the solution was set for 4 h before used for the treatment.

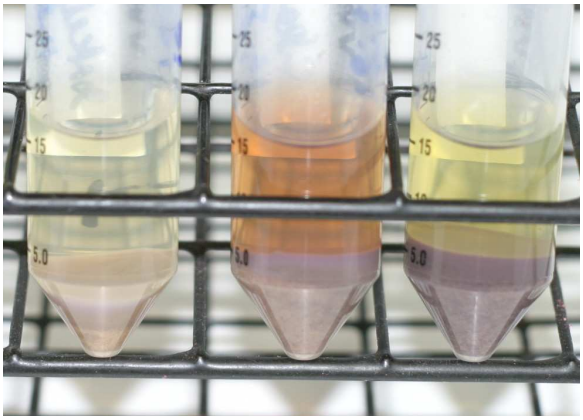

Figure 6.9 – Appearance of the fibers and aqueous extracts of the peel powders from treated and non-treated (fresh) pears at their third phenolic extraction stage. Aqueous acetone was composed of 70% of acetone and 30% of milli-Q water. From left to immediately: Whole peel extraction of fresh pears, steam-heated treated pears, and steam-heated and canned treated pears. Treated pears were briefly immersed in formulated solution composed of tannic acid, formaldehyde, hydrochloric acid, and stannous.

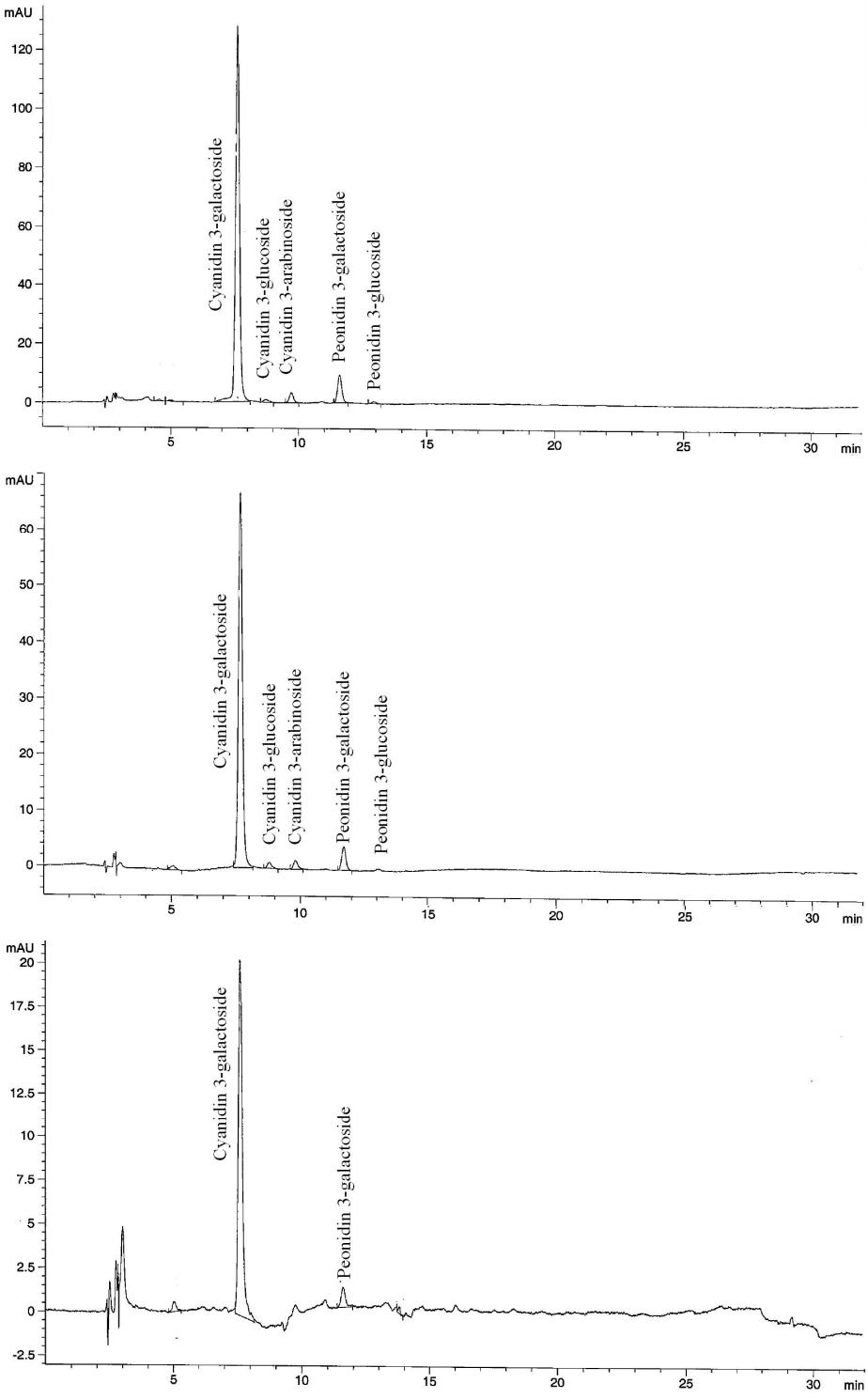

Figure 6.10 – Chromatography spectrum of HPLC anthocyanin analysis of phenolic peel extracts of fresh and treated pears monitored at 520 nm. a: Fresh pear peels; b: Peels of pears after immersion; c: peels of pears after immersion and steam-heating.

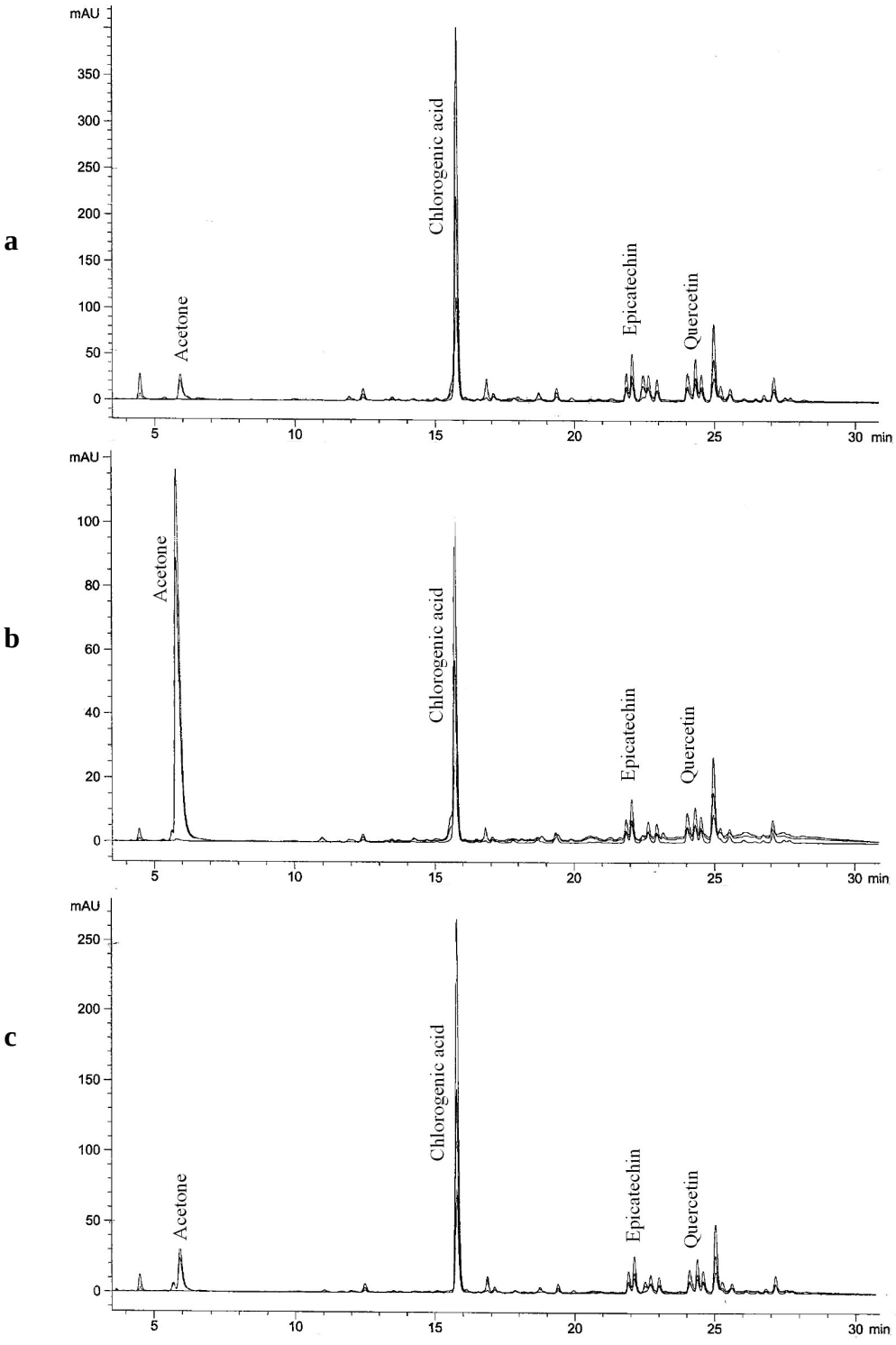

Figure 6.11 – Chromatography spectrum of HPLC phenolic analysis of fresh and treated pear peel extract monitored at 520 nm, 320 nm and 280 nm. a: Fresh pear peels; b: Peels of pears after immersion; c: peels of pears after immersion and steam-heating.

CHAPTER 7
General Conclusions

Preserving the green and red pigments, known as chlorophyll and anthocyanin, respectively, on the peels of processed pears has been a challenging task due to the extreme sensitivity nature of these pigments to environmental changes during and after fruit processing. Additionally, the anthocyanins are water soluble, making it even more difficult to retain on the peels of canned pears.

The use of zinc ion was studied to stabilize chlorophyll derivatives formed on pear peels during blanching. Zinc ion concentration of 1,300 ppm or above and blanching time up to 12 min are necessary for the formation of zinc chlorophyll complex, mostly Zn pheophytin a and a'. Surface pretreatment before zinc application to remove the wax and part of the cuticle layers is essential for the reactions of zinc ions with chlorophyll in the peel tissues. In developed procedures, zinc ion was used as a processing aid, thus significantly minimizing zinc content in the final products which can be packed in conventional canning syrup. The green color of the peels-on thermally processed (canned) green pears stored in a transparent package was highly stable during storage for 19 weeks at 38 ^{o}C under intensive illumination or for at least 35 weeks at 10 ^{o}C.

The developed technology using an aqueous formula constituted of stannous, formalin, hydrochloric acid, and tannic acid together with steam-heating has shown to be successful in retaining the red color on canned pear peels. The red purple color of finished peels-on canned pears was found to be stable during a 2-month storage in transparent packaging at room condition. The developed formula was found to be capable of building up polymers at high rate with phenolics from red pear peels. Stannous, a key component in the formula together with tannic acid, was shown to cause a purple precipitate itself when added into pear extracts. However, it is uncertain at this point whether a chemical transformation of anthocyanins occurs and to what extend the formed pigment products contribute to the color of finished products. Cyanidin 3-galactoside, the major anthocyanin in fresh red pear peels, was detected in

the peels of steam-heated pears and was expected to contribute to the color of canned red pears. The anthocyanin would be found in the form of anthocyanin-metallo complex embedded in a matrix formed from the added tannic acid and/or its polymerization products and phenolics and cell materials such as lignin. The results of this study suggest that the overall stabilization of anthocyanins on pear peels is a result of the synergistic effects of stannous and the created polymer matrix. However, only monomers and oligomers can be analyzed by HPLC, while the large molecules that may carry covalently bonded anthocyanins may have a poor resolution with HPLC methods as seen with wine tannin. To better understand the phenomena explaining the stability of the pigments, phenolic and anthocyanin changes associated with formulating and processing red pears should be more appropriately approached. The extraction method also needs to be improved so that the extract can be subjected to depolymerization to give access to the nature of its consecutive units. Such assays will help to clarify whether a typical part of anthocyanins has been chemically transformed and stabilized.

In the context of this research, both the peels of peels-on red and green processed pears did not retain the high extractable-phenolics content of the fresh peels. In the case of green pears, this would be due to the dissolution of the phenolics into canning solution and loss during processing. For processed red pears, anthocyanins and phenolics would have been attached onto the peels as the result of formulation treatment and heating process. More research is necessary to evaluate the effects of treatment procedures on the enzymatic digestion of the fruit peels.

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APPENDIX

Color Quality of Oregon Strawberries - Impact of Genotype, Composition and Processing

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Publication in Journal of Food Science
525 W. Van Buren, Suite 1000, Chicago, IL 60607
2007, 72(1): C025-C032

Abstract

This investigation was to evaluate fruit color and study the effect of processing on color quality of strawberry products. Three color instruments with different viewing angles, viewing areas, and sample presentation geometries were compared for their effectiveness in measuring CIEL*C*h^o color values for fresh fruits of six strawberry genotypes. There were significant differences between genotypes as well as between instruments. Fruits from the Totem genotype were frozen, canned, and made into jam. Color changes were measured along with the following compositional determinations: total monomeric anthocyanins (ACN), total phenolic content (TPC), and % polymeric color. ACN in fresh strawberries ranged from 21.2 mg to 69.9 mg per 100 g of fresh fruit. Freezing resulted in an apparent increase in ACN, and transfer of 70.2% of the anthocyanins from the berries into juice. Physical transfer of pigments to syrup also occurred with canning: there was approximately 70% loss in ACN, about 20% increase in polymeric color, and 23.5% decrease in TPC. Pronounced color change and substantial losses in ACN and TPC of strawberry jams occurred during processing and 9 weeks of storage. Storage of jams at 38 °C compared to 21 °C over a period of 9 wks resulted in marked losses of ACN and TPC.

Key words: Strawberries, color, genotype, freezing, canning, jam

Introduction

Strawberries are a rich source of polyphenolics (Sun and others 2002; Aaby and others 2005), with anthocyanin pigments being responsible for their appealing, bright red color (pelargonidin-3-glucoside is the major pigment, Garzón and Wrolstad 2002). Researches on strawberry extracts have shown that strawberry phenolic extracts have high antimicrobial activities (Nohynek and others 2006) and high levels of antioxidants (Aaby and others 2005; Rababah and others 2005). The phytochemicals present in strawberry extracts had also been found to have a potent inhibitory effect *in vitro* on $HepG_2$ cell proliferation (Meyers and others 2003).

Unfortunately, the attractive color of fresh strawberries does not normally prevail during processing and storage (Garzón and Wrolstad 2002). Compared to other berries, strawberries are relatively low in pigment content (Skrede and others 1992; Clifford 2000), ranging in total anthocyanin (ACN) typically being from 10 to 80 mg/100 g of fresh weight (Rein 2005). Thus degradation during processing and storage can have a major impact on the color of finished products.

The freezing process triggers the formation of ice in cellular fruits, which increases the volume of the fruit (Fennema 1996) and damages the integrity of the cell leading to fruit structure breakdown. Large drip loss found in the thawed product (Han and others 2004) will have a major effect on the appearance of the product. Another adverse consequence of freezing is that nonaqueous constituents become concentrated in the unfrozen phase (Fennema 1996). Thus, besides lowering reaction rate by lowering temperature, freeze-concentration can increase reaction rates, resulting in decreased anthocyanin and ascorbic acid contents in frozen stored strawberries (Larsen and Poll 1995; Sahari and others 2004).

Processing strawberries by canning or manufacturing into jam necessitate high temperature treatments that can alter and damage color quality of the finished product. Normal commercial exhaust and sterilization procedures during canning have been shown to have little effect on anthocyanin degradation; the process, however, caused leaching of anthocyanins out of the berries into the syrup (Adams and Ongley 1973). During jam manufacture at atmospheric pressure, anthocyanin losses in the final product varied from 10 to 80% when boiling time ranging from 10 min to over 15 min (García-Viguera and Zafrilla 2001). Under vacuum pressure condition, loss was approximately 40% during a 15 min process (García-Viguera and others 1999). Maillard reaction products, ascorbic acid, glucose and fructose with their degradation products may accelerate the color loss catalyzed by high temperature and oxygen (von Elbe and Schwartz 1996; Stintzing and Reinhold 2004; Wrolstad and others 2005).

Since color is a critical quality parameter in food purchases, color measurement has gained much attention from food scientists and industry. To investigate color quality in a systematic way, it is necessary to objectively measure color as well as pigment concentration. Method for measuring total anthocyanins and indices for polymeric color and browning (Bakker and others 1994; Giusti and Wrolstad 2005) has been well established and used in research and in industrial control applications (Wrolstad and others 2005). The instrumental specification of color using CIEL*a*b* system is most commonly performed by use of tristimulus colorimeters and spectrophotometers. The color perception often alters when viewing angels and illumination change and when the sample is rotated, where changes in color, gloss, or surface texture pattern might occur (Hutching 1999). Francis (1987) stated that one of the major problems in comparison of tristimulus data obtained from food is the use of instruments with different designs. In addition, food samples are not flat, nor perfectly opaque/transparent, and nor of a single uniform color, the physical environment can sometimes be more influential than the colorant itself (Joshi 2001). Previous studies have evaluated color measurement of food in liquid (Kent 1987) and powder forms (Brimelow 1987; McDougall 1987). For fruits and fruit products with high anthocyanins, researches have emphasized on developing methods to monitor

pigment indices, but not on color instrumental specification, particularly on fresh strawberry and its processed products.

The major objective of this study was to compare the effectiveness of three color measurement instruments that have different optical geometric arrangements (observation and illumination angles) and different features for sample presentation (viewing port size and location), for measuring strawberry color. Another objective was to measure the color and total pigment changes that occur when strawberries are processed into frozen, canned and jam products. Changes in polymeric color, browning, and total phenolics were also monitored.

Materials and Methods

Chemicals and reagents

Folin-Ciocalteu's phenol reagent (FC), gallic acid, and sodium carbonate were obtained from Sigma Chemical Co. (St. Louis, MO., U.S.A.). Ascorbic acid was from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, U.S.A.). Sodium bisulfite was from Mallinckrodt Inc. (Paris, KT, U.S.A.). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was from TCI America (Portland, OR, U.S.A.). Anhydrous citric acid was from Integra Chemical Co. (Renton, WA., U.S.A.). Sugar was from C&H Sugar Co. (Crockett, Calif., U.S.A.). Pectin was Pacific 150 grade fast set pectin (Pacific Pectin, Inc. Oakhurst, CA, U.S.A.). All solvents were HPLC grade (EM Science, Gibbstown, NJ, U.S.A.).

Strawberry samples

Three commercial strawberry (*Fragaria x ananassa*) genotypes — Ovation, Totem, Puget Reliance, and three experimental selections, 2273-1, 1843-1, and 1723-1 (2.5 kg/each genotype)

were obtained at their commercial ripeness in June 2005 from the Oregon State University (OSU) North Willamette Research and Extension Center, Aurora, OR. These samples were selected because they represented a wide range in visual lightness/darkness and hue from panel assessments of thawed frozen experimental

selections the previous season. Another lot of Totem was purchased from a commercial berry farm in Dayton, OR for the processing trials. Fruit was picked at commercial harvest maturity, and immediately transported to the Department of Food Science and Technology pilot plant, OSU, Corvallis, OR, and stored at 2 ^{o}C before subsequent sampling and processing.

Freezing process and storage

Strawberries were washed, carefully sorted to remove any damaged ones, individually quick frozen (IQF) in an air-blast freezer at -23 ^{o}C, subsequently transferred into glass jars, and stored at -12 ^{o}C. Frozen fruits from the Totem genotype was stored for one month before color measurement and extraction, and for six months before making into jam. Samples were stored for one month before extraction and compositional determinations.

Canning process

The canning process was adapted from processing procedures described by Chaovanalikit and Wrolstad (2004). Fresh fruits of the Totem genotype (2 kg) were washed and placed into cans (Nr 303 x 406 with dark fruit enamel), hot filled with sucrose syrup (C&H sugar diluted to 20 ^{o}Brix with water) at 95 ^{o}C. Cans with lids on were placed on a running belt and exhausted in a steam tunnel before sealed on a manual can seamer (Automatic Canning Devices Inc., Manitowoc, Wis., U.S.A.). The cans were immersed in boiling water for 15 min, then immediately put in running water (20 ^{o}C) to be cooled to room temperature before being stored at room temperature for 60 d. The process was replicated twice.

Manufacture of strawberry jams

Jams were made from strawberries of the Totem genotype following a procedure similar to the one described by Kim and Padilla-Zakour (2004). Frozen berries (14 kg) were thawed overnight at 2 ^{o}C and manually crushed into puree by use of a potato smasher. The jam formulation was 52% fruit, 47.5% sugar, and 0.5%

pectin. Crushed berries were continuously stirred and brought to 50 ^{o}C in a steam kettle before sugar addition. The pH was measured and adjusted to 3.2 by addition of citric acid. The mixture was boiled within 20 min to a concentration of 66 to 67 ^{o}Brix (approximately 104 ^{o}C) before adding pectin, previously mixed with a small amount of sugar. The jam was hot-packed in 250 ml mason jars, immediately sealed with plastisol lined metal lids, and inverted for 3 min to sterilize the lids, then returned to normal position for air-cooling. Manufacture of jam was replicated twice.

Color measurement of fresh strawberries

The three color instruments evaluated in this study were: Minolta CR-300 colorimeter (Minolta Corp., Ramsey, N.J., U.S.A.), LabScan II and ColorQUEST spectrophotometers (Hunter Associates Laboratories Inc, Reston, VA., U.S.A.). The features of these colorimeters are presented in Table 1. The instruments were calibrated every two hours by use of standard white/black calibration plates included with the instruments. The L*C*h^{o} coordinate system was used for LabScan II and ColorQUEST. Undamaged whole fresh fruits from each genotype were randomly selected, grouped into two sets containing from 40 to 50 fruits per set, and used for all

color measurements with the three instruments. For the Minolta CR-300 and LabScan II, single measurements were made on the reddest area of individual berries. For the ColorQUEST, a circular optical glass cell 130 mm in diameter x 50 mm in height was used. The cell was filled with berries from each set (from 2 to 3 layers of fruit), with their reddest area facing the port view before taking the color reading. A box with its interior covered with black absorbing paper was used to cover the sample. After the first reading, the cell was emptied and re-filled with another sample of fruit to make a total of three readings.

Color analysis of processed Totem strawberries

All color measurements of frozen and canned fruits, and jams utilized the glass cell and the ColorQUEST instrument (Table 1). For frozen berries, the same 40 – 50 fruits that were measured for color prior to processing (in fresh form) were re-

analyzed with the same method of sample presentation as done on fresh fruit. The frozen berries taken from the freezer were kept in sealed jars to prevent moisture condensation onto the fruit surface, and were exposed to the air only for less than 45 s when filling the glass cell for color measurement. About 400 g of frozen fruit was thawed overnight at 2 ^{o}C in plastic bags. Thawed berries were then drained and weighed. Canned berries were also drained, and both the drained berries and the liquid weighed. For color measurement of drained frozen and canned fruits, the cell was filled with 2 layers of fruit. Color of drained liquids (from thawed frozen berries) and canned syrup were determined using a 2.5 mm pathlength optical glass cell (Hellma, Germany) utilizing the ColorQUEST sphere instrument with a view port of 25.4 mm. For jam measurements, 210 g of jam was placed in the large cell, approximately 12 mm depth and read using the ColorQUEST (Table 1).

pH measurement

Six strawberries were blended with deionized water (1:9 fruit:water, w/w) for 1 min. The pH of filtered juice was determined using a Corning pH meter 125 with an Orion Epoxy body, non-refillable pH electrode (Thermo Electron Corp., Waltham, MA, U.S.A.).

Extraction of anthocyanins and polyphenolics

A modified method described by Rodriguez-Saona and Wrolstad (2005) was used. One hundred grams of frozen and canned berries were thoroughly homogenized by powdering in liquid nitrogen. About 5 g of powder was mixed with 20 mL of acetone. The mixture was centrifuged and the supernatant was collected. An additional extraction of the residue was done with aqueous acetone (30:70 v/v). Supernatants were combined and partitioned with 50 mL of chloroform and centrifuged for better separation between the organic and aqueous phases. The aqueous phase containing ACN was recovered and transferred to a rotovaporator to remove residual acetone at 40 ^{o}C. The extract was diluted with MilliQ water to 25 mL. For jams, about 200 g of sample was homogenized in a 0.25 l glass jar using a 12 speed Osterizer blender

(Sunbeam-Oster Company, Fort Lauderdale, FL, U.S.A.). After blending, 10 g of MilliQ water was added to approximately 5 g of sample and vortexed before addition of acetone. Subsequent additional extractions were done as described for fresh berries above.

Analysis of total monomeric anthocyanins and polymeric pigments

Aqueous extracts were diluted to a known volume with de-ionized water. The total anthocyanin content was determined by using the pH-differential method described by Giusti and Wrolstad (2005) and expressed as mg pelargonidin-3-glucoside per 100 g of initial fresh weight with molecular weight of 433.2 g/mol and a molar absorptivity of 15,600.

Percent polymeric color (PPC, sum of the absorbance at 420 nm and 496 nm of bisulfite-treated extract/sum of the absorbance at 420 nm and 496 nm of berry extract) and browning index (absorbance at 420 nm of bisulfited-treated extract, reported as absorbance per 100 g of initial fresh materials) were determined by using the method described by Giusti and Wrolstad (2005).

Analysis of total phenolic content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu (FC) method (Waterhouse 2005). Aqueous extracts were diluted to a known volume with de-ionized water. A 750 μL of diluted extract was mixed with 0.5 mL of FC reagent in 7.5 mL of de-ionized water. The samples were held for 20 min at room temperature before 0.5 mL of 20% sodium carbonate (w/v) was added. The well mixed samples stood for another 30 min at 40 ^{o}C before the absorbance was measured at 765 nm by a Shimazu UV160U spectrometer (Shimazu Corp., Kyoto, Japan) with 1-cm disposable cells. Gallic acid monohydrate was used as a standard and expressed as gallic acid equivalents (GAE) in mg per 100 g of initial fresh materials.

Shelf-life evaluation of Totem jam

Twelve randomly selected jars of jams were assigned to one of the 3 different storage temperatures (10 ± 3 ^{o}C, 21 ± 2 ^{o}C, 38 ± 2 ^{o}C) for evaluation over a 9 wk period in the dark. Jams were analyzed for anthocyanin content, percent polymeric color, and browning index as well as CIEC*L*h^{o} color values. The measurements were replicated twice.

Experimental design and statistical analysis

A completely randomized design with two replications was used. Statistical analysis was performed using the SAS (Statistical Analysis System Institute Inc., Cary, NC, U.S.A.). Multifactor analysis of variance was applied with source of variance being genotypes and color measurement instruments. Differences between means were tested for significance by using GLM procedure with Duncan test, using a level of significant of $P < 0.05$.

Results and Discussion

Color of fresh strawberries

Preliminary experiments on berry color measurements were conducted with the main objective to determine the most appropriate means for berry presentation. It was noticed that some berries have both yellow-colored and red-colored areas with very different local L*, C*, h^{o} color values. In the common practices, the overall color presentation of a fruit is more important than the single area of color on the fruit. While using an instrument with small view port to obtain average color of fruits with both red and yellow areas, it is advised to rotate single fruit and make multiple readings (Francis 1987). In this study, most samples have uniform color distribution dominated by red with very limited yellow areas (Figure 2). In addition, the high fragility of some selected genotypes did not permit excessive manipulation. To compare the performance of the color measurement instruments in monitoring the color of the six strawberry genotypes, it was thus decided to make readings only on the reddest area of a fruit. Using instruments with small port view diameter, less red areas on a fruit were ignored and the collected data represented, in fact, the average of

the reddest area of a fruit pool. Tests on the ColorQUEST using the large cell showed that three readings would be sufficient to acquire data with small standard deviations. The surface of individual berries was measured on the LabScan II with its small viewing port. Color measurements of 20 vs. 50 berries yielded data with almost the same means and standard deviations (data not shown). Readings from 40-50 berries were done in this study, since that number of fruits was needed to take three readings using the large cell, and the author desire was to use the same sample for all instruments.

Figure 1 shows L*, C* and h^o values generated by the Minolta CR-300 and LabScan II for single fruit of the six different genotypes, and by the ColorQUEST for whole fruit pools of each genotype. All three instruments show a general trend for reduction in L*, C*, and h^o in the order of Ovation, 2273-1, 2384-1, Puget Reliance, Totem, and 1723-2. There are obvious differences, however, in color values among the instruments (Figure 1). L* values generated by Minolta CR-300 were higher than that by LabScan II. As shown in the Table 1, the two instruments both have small viewing port area (8 mm in Minoltad CR-300 and 6.35 mm in LabScan II), but different degrees of viewing angel (2^o in Minoltad CR-300 and 10^o in LabScan II). According to Hutchings (1999), the 2^o and 10^o observers should give close tristimulus values, thus can not be the significant cause of the difference in L* values. In this case, it was believed that the differences are the result of different optical features in these two instruments. With a d/0 geometry characterized by a diffuse illumination and observation angle of 0^o in Minolta CR-300, the reflected light from the sample would include some specular light which is reflected without becoming selectively absorbed by the sample surface pigments. Unlike the d/0 geometry, a 0/45 geometry, standing for illumination angle of 0^o and observation angle of 45^o, in the LabScan II excluded specular light. The higher L* values reported by the Minolta CR-300 were thus mainly due to the integrated specular light which increased the brightness of the light reflected on the sample surface. While the LabScan II and ColorQUEST have similar optical features, 45/0 vs. 0/45 (Table 1), the L* values from LabScan II were higher than those obtained by ColorQUEST (Figure 1). Even though both instruments had

specular light excluded, there might still be a considerable variation between the two instruments when specular component was excluded (Kent and Smith 1987). However, the fact that the ColorQUEST having a view port area significantly larger than that of the LabScan II (89.9 mm vs. 6.9 mm) could be the main explanation for the differences in their reported color values. On the other hand, C* and h^o values reported by the three instruments were very similar (Figure 1). The similarity suggests that the average color of a fruit pool of the studied genotypes were close to the average color of reddest area on fruits or there were limited color variance on the red side of studied fruits as it was assumed earlier.

For smaller view port size, i.e. LabScan II and Minolta CR-300, measurements can only be conducted on a single fruit. The surface area of strawberries is not flat, and includes yellow achenes and reddish hair-like structures. There is considerable berry to berry variation for the same genotype, as illustrated by 2273-1 or 2284-1 genotype (Figure 2). Fruit surface characteristics varied considerably from berry to berry. Instrumental color values generated by LabScan II and Minolta CR-300 fluctuated accordingly and resulted in large standard deviations (Figure 1). For high precision, multiple readings on different individual objects are thus needed when taking measurement on single berries, whereas with the large viewing port of ColorQUEST, readings were done on a group of objects. This variation was thus averaged, and data showed small standard deviations (Figure 1) with as few as three readings. Still, the curved shape, pillowing form, and size variation of berries (Figure 2) can modify and complicate the direction of reflected light, and thus affect the amount of light projected from fruit onto the sensors to some extent.

In the composite picture of all six genotypes (Figure 2) taken with the same camera under the same condition of D2 light, camera view angle and distance, it can be seen that the visual appearance of berries varies from berry to berry and from genotype to genotype. Actually, these genotypes were selected because they represented a wide range in visual lightness/darkness and hue from panel assessments of thawed frozen experimental selections the previous season. As it can be seen in

Figure 2, 1723-2 berries had the darkest and reddest color and Ovation the brightest and the most orange-colored.

It was anticipated that L*, C*, and h^o values of the six strawberry genotypes would be in a reasonably wide range. Figure 1 shows that the color values measured by ColorQUEST were significantly different ($p<0.05$). L* values increased from 21.3 to 29.1, C* from 24.3 to 41.9, and h^o from 24.4 to 32.6 in the order of Ovation, 2273-1, 2384-1, Puget Reliance, Totem, and 1723-2 with a few exceptions (Figure 1). Within this area on the solid color sphere, the lower L* and h^o values represent darker sample color with a bluish-red hue while higher values are lighter and more orange. It can be concluded from Figure 1 that 1723-2 was the bluish genotype and Ovation the most orange-colored, which is well reflected with the results described above.

The pH and total ACN values of 6 selected strawberry genotypes varied significantly (Table 2), which was not surprising because of the wide range in visual appearance. The observed pH values in the range of 3.25 – 3.80 reflected well with the results described by Wrolstad and others (1970). The total ACN content of strawberries in this study, 37-122 mg/100g fresh weight, were higher than the range of 30-100 mg/100 g reported in a study of over 18 genotypes (Wrolstad and others 1970). The total ACN content among 6 tested genotypes is in the decreasing order of Ovation→Puget Reliance→2384-1→2273-1→Totem→ 1723-2. Interestingly, the 1723-2 selection with the highest ACN had the lowest L* and h^o value and the Ovation with the lowest ACN and the highest L* and h^o value. However, as expected, no significant linear correlations were observed between anthocyanin content or pH and color values (Pearson's r^2 was less than 0.7, data not shown). As anthocyanin color is from different parts of the fruits, including the pericarp of whole uncomminuted material as shown on Figure 2, the achenes, and the inner part of the fruit (Aaby 2005), there was a difference between the anthocyanin color of whole fruit samples and the anthocyanin nitrogen powders. Visual assessment showed that all samples, except for Ovation, had pigmentation on their flesh.

Effects of processing on color, pigment content, and antioxidant properties of strawberries

Table 3 compares the color values of fresh and processed fruits from the Totem genotype, all measurements being taken on the ColorQUEST with the large cell except for the liquid (drip from thawed fruits and syrup from canned berries) which was measured with the ColorQUEST sphere. The compositional data of processed products are presented in Table 4. As in the earlier analysis of ACN, all chemical analyses in this study were based on nitrogen powdered material and the results are expressed as per 100 g of berries in the various products. The content expressed in 100 g of finished products can be obtained from these values through a simple conversion associated to each product given in the footnote of Table 4.

Frozen berries. There were marked differences in the visual appearance of frozen berries compared to fresh fruit with respect to "gloss" that is not evident from L*, C* or h° data presented in Table 3. Gloss is the property by which a material appears shiny or lustrous as a high proportion of light impinging on the food surface reflected. Thus, gloss is dependent on the refractive indices and on the size of discontinuities present on the involved food surfaces (Hutchings 1999). Wax layers and deposits are the components of the surface cuticle of fruits having natural gloss (Nussinovitch and others 1996). During freezing process and frozen storage, the berry surface characteristics must have changed, such as more discontinuities in the cuticle layers because of the crystallization and fruit volume increase. All made the frozen fruits much less shiny compared to the fresh fruits, which was not reflected on the L*, C*, and h^o measurement. The glossiness of fruits and vegetables can be measured by a glossimeter (Nussinovitch and others 1996). The ACN of frozen fruits from the Totem genotype (69.7 mg/100g, Table 4) is higher than the 50.3 mg/100 g reported by Pilando and others (1985) for fresh Totem genotype. TPC fell in the range of 300 to 341 mg GAE/100 g fresh weight reported by Aaby and others (2005). An assumption was made at the beginning of the study that no major compositional changes and pH would occur in frozen strawberries during short-term storage at low storage

temperature. While the temperature of -12 ^{o}C may not be sufficiently low, no analysis of fresh berries was conducted to permit comparisons for any compositional changes with short-term storage. Thawed berries had significantly higher L* and lower h^{o} values than that of fresh or frozen berries ($P<0.05$) (Table 3), indicating a light red color compared to frozen or fresh ones. This higher hue angel value might suggest that ACN in thawed fruits (61.7 mg/ 100g, data not shown) may be concentrated on the fruit surface when juice leached out during thawing. An increase in lightness may be attributed to the physical changes resulting from collapse of thawed berry structure. The drip solution had an ACN value of 11 mg/ 100g (data not shown).

Canned berries. Canned fruits analyzed after 60 d of storage at room temperature had similar L* ($P>0.05$) but higher h^{o} values ($P<0.05$) compared to those of fresh berries (Table 3), representing a shift from red toward more orange color. Total ACN in canned strawberries (8.8 mg ACN /100 g initial weight) was lower than in the syrup (13 mg ACN /100 g initial weight) (Table 4). Compared to ACN value in the fresh samples, the total level of combined ACN in canned strawberries and in the syrup decreased markedly, the reduction being as high as 68.8%. The great loss and leaching of anthocyanin explain well the change to yellow-colored appearance of the canned berries.

Table 4 shows that along with the canned berries, the syrup also contained considerable high levels of total phenolics. Compared to a 100% of TPC (313.6 mg EGA/ 100g) in frozen berries, the calculated TPC of combined canned fruit and syrup (239.7 mg EGA/ 100g) represents a loss of 23.5 %, which might reflect the loss in anthocyanins.

Heat processing and storage also increased PPC content in the final products, leading to a total PPC of 33.20% in canned fruits, while the value was 7.16% on frozen berries. An increase in PPC is indicative of condensation reactions of anthocyanins with other phenolic compounds, such as procyanidins to form colored polymer pigments (Monagas and others 2005), reducing ACN in canned fruits. A

significant increase in browning index was also found in canned liquid and fruits compared to that of frozen fruits (Table 4).

As anthocyanins are thermally labile, an extended heating at 100 ^{o}C for 15 min would be the main reason causing significant degradation of red pigments. Adams and Ongley (1973) found that anthocyanin degradation can take place to an important extent if fruits are heated at 100 ^{o}C for over 12 min at commercial canning conditions. Ascorbate degradation could also significantly involve in the color and chemical changes of canned product. Studies have shown that most ascorbic acid in strawberries is destroyed during thermal process (Skrede and others 1992) and furfural is a major product (Tatum and others 1969). Catechin and anthocyanins, which are flavonoids available in large quantity in strawberries (Aaby and others 2005), have been shown to form condensated dimeric products mediated by furfural (Es-Safi and others 2000). In nature, dimeric complexes between anthocyanidin and flavanols in strawberries have been found recently (Fossen and others 2004). These reactions can eventually contribute to the darkening of the fruits (Es-Safi and others 2000). During storage of canned products, pelargonidin-3-glucoside, the main anthocyanin in strawberries, can still be hydrolyze by acid to perlargonidin and further broken into hydroxybenzoic acid (Stintzing and Carle 2004).

Jam. L*, C*, and h^{o} color values of jam were distinctly lower than those of fresh and other processed products ($P<0.05$) (Table 3), indicating an increase in darkness, decrease in chroma and hue angle caused by jam manufacture. Besides compositional and chemical changes, one of the main factors to take account for when comparing the colors of jam and fruits is that the physical differences between these two forms of products. When made into jam, the product's light reflectance properties are no longer similar to that of berries.

Total ACN and TPC values of jams were significantly lower than those of frozen berries ($P<0.05$) (Table 4). Based on ACN differences between jam and frozen fruits, approximately 70% loss of ACN occurred during manufacture of frozen berries into jam. This compares to a loss of 36% for strawberries processed using lower

temperatures and a vacuum process (Garcia-Viguera and others 1999). For jam making, the stability of anthocyanins during processing are mainly affected by cooking temperature and heating time (Garcia-Viguera and Zafrilla 2001; Schmidt and others 2005).

As shown in Table 4, PPC of jams was 27.7%, significantly higher than that of frozen berries (7.2%). Its BI was about 3 fold higher than those of other processed products (Table 4). Compared to frozen berries (100 g), TPC of jams was reduced by 16.4% after processing, close to the data reported by Amakura and others (2000), where a reduction of about 10% after jam processing was observed based on DPPH radical scavenging assay. These data suggested that processing strawberry jams in open air generally increase BI and PPC, but reduce TPC of the products.

The increase in BI is mostly like due to the formation of melanoidin pigments from reactions of reducing sugars, amino acids, and ascorbic acid during open air processing (von Elbe and Schwartz 1996; Stintzing and Carle 2004). The products of ascorbic acid oxidation in the presence of oxygen have also known to trigger the formation of dark-color compounds from catechin (Bradshaw and others 2001). In addition, the browning could be contributed by strawberry peroxidase and polyphenol oxidase activity (Lopex-Serrano and others 2002) on phenolic compounds and ascorbic acid during thawing and maceration (Wroldstad and others 1990; Bakker and Bridle 1992; Garzon and Wrolstad 2002; Stintzing and Carle 2004). Simple phenolics such as hydroxycinnamic acid (Kosar and others 2004) could be oxidized to quinones, then entering Maillard reactions (Cilliers and Singleton) and finally contributing to browning of the product. The phenolic autoxidation together with enzymatic oxidation of phenolics could be one of the main reasons for the loss in phenolic compounds represented by reduced TPC. The TPC reduction could also be due to the changes in the nature of some phenolics such as anthocyanin and other flavonoids which were believed to be converted to catechol at high temperature (Mitani 1983, von Elbe and Schwartz 1996).

Color changes in jams during storage at different temperatures

Figure 3 shows pronounced color change of jams stored at 38 ^{o}C during 9 wk of dark storage. The major increase in h^{o} value occurred within the first 3 wk of storage ($P<0.05$) at 38 ^{o}C while it took 9 wk for changes in h^{o} to become obvious at 21 ^{o}C ($P<0.05$).

Figure 4a shows a reduction in anthocyanins in jams during storage and the loss was more pronounced at higher storage temperatures. While the ACN in the fresh jams was about 20.1 mg/ 100 g of initial fruits, the jams stored after 9 wk at 10 ^{o}C, 21 ^{o}C, and 38 ^{o}C contained significantly lower ACNs ($P<0.05$), about 12.9, 11, and 5.4 mg/100 g of initial fruits, respectively. Thus, the stability of anthocyanins in jams was markedly influenced by temperature (von Elbe and Schwartz 1996; Guarcia-Viguera and others1999). Figure 3c and 4a show that a major loss of anthocyanins had occurred before any significant changes in hue angle of the jams was observed. These results would indicate that ACN is not a good measure of color quality of strawberry jam, as concluded by Abers and Wrolstad (1979). In stead, the role of browning pigment formation was emphasized over the amount of ACN loss as the major cause of the color deterioration of jams (Abers and Wrolstad 1979).

Storage time and temperature had significant effects on PPC and BI of jams, which increased with storage time and temperature (Figure 4b, c). Abers and Wrolstad (1979) proposed that catechin and proanthocyanins played an important role in degradation of anthocyanins and in polymeric browning of strawberry jam. It is possible that the products of ascorbic acid oxidation during jam making could induce browning of catechin during product storage under anaerobic condition (Bradshaw 2001). The condensations between flavanols and anthocyanins as described earlier (Abers and Wrolstad 1979; Monagas and others 2005) might explain for the increase in PPC of jams after processing and during storage. Some of these products could form black compounds (Es-Safi 2000). Sugars at high concentrations as found in jams are known to stabilize anthocyanin presumably by lowering water activity (von Elbe and Schward 1996). This protective effect might operate here but is counterbalanced or offset by sugar degradation as the reducing sugars produced during processing can

react with free amino acids (Abers and Wrolstad 1979) accelerating Maillard browning.

Conclusions

Total monomeric anthocyanins, total phenolic content, percent polymeric color, browning index, and CIEL*C*h^o color values were usefully combined to represent the color of fresh strawberry and strawberry products and to track the anthocyanin changes during processing and storage. It was found that the pigment content was not the dominant indicator of the color of strawberry and processed strawberry products. The red pigment in strawberry is very labile and easily degraded during processing and storage. Fortification with anthocyanins as shown by Skrede (1992) may improve the product color. Besides, the matrix compounds should be brought into consideration to enhance the stability of the pigments and to prevent browning reactions.

When measuring color of similar samples using color measuring instruments, the measurement system and sample presentation must be carefully matched and standardized since there are interactions between instruments and samples as defined by sample presentation and instrument geometry. The sample glossiness is not represented by the CIEL*C*h^o color system, thus a glossimeter is necessary when monitoring similar samples that are different in gloss as found on fresh and frozen fruits. When measuring color of samples with unevenly colored surfaces, it is advisable to use large-area color measuring instrument to minimize manipulative acts. For products with un-flat surfaces, there is most likely a complication of light reflecting and scattering for both colorimeter or spectrophotometer, which raises the concern about the accuracy of the defined fruit color. The best practice on an instrument is to develop a standard sample presentation and be consistent in the operation.

Acknowledgments

We are grateful to the Oregon State University North Willamette Research and Extension Center and the Oregon Strawberry Commission for supplying strawberry samples. Special thanks to Mr. Brian Yorgey for the help in processing strawberries into frozen, canned and jam products. The authors are grateful to Drs. Andrew S. Ross and Henrik U. Stotz for allowing us to use their color measurement instruments and to Ian McLaughlin for the photograph.

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Table 1 - Features of the three color instruments (Minolta CR-300, LabScan II, and ColorQUEST)

Colorimeter /Spectrophotometer	Sample presentation	Optical feature (degree/degree)	Illuminant	Viewing port diameter (mm)	Viewing angle (degree)
ColorQUEST	Glass cell[a]	45/0[c]	D_{65}	88.90	10
LabScan II	Berry surface[b]	0/45[d]	D_{65}	6.35	10
Minolta CR-300	Berry surface[b]	d/0[e]	D_{65}	8.00	2

[a] An optical cylinder glass cell (120 mm x 70 mm, diameter x height) was used to present samples.
[b] The berries were in direct contact with the instrument view port and were individually read.
[c] Illuminating from an angle of 45 ± 2 degree and detecting light at 0 ± 10 degree (specular light excluded).
[d] Illuminating from the direction of 0 ± 10 degree and detecting light at 45 ± 2 degree (specular light excluded).
[e] Illuminating the sample diffusely and detect the light at 0 ± 10 degree (specular light included).

Table 2- Total anthocyanin content and pH of different berry genotypes[a]

Berry genotypes	ACN[b]	pH
Ovation	37.1 ± 4.4	3.28 ± 0.07
2273-1	71.8 ± 2.3	3.25 ± 0.01
2384-1	62.1 ± 0.4	3.80 ± 0.09
Puget Reliance	50.9 ± 2.8	3.40 ± 0.02
Totem	76.0 ± 4.0	3.58 ± 0.01
1723-2	122.3 ± 2.3	3.46 ± 0.02

[a] Frozen whole fruits were used in the analysis to represent the fresh fruits.
[b] Total anthocyanin content was expressed as mg of pelargonidin-3-glucoside per 100 g of initial materials, determined by a pH differential method.

Table 3- CIEL*C*h^{o} color values of fresh fruit from the Totem genotype and processed products (Frozen, Thawed, Canned, and Jam)

Samples	L*	C*	h^{o}
Fresh fruit[ab]	23.35 ± 2.18	31.1 ± 0.740	27.38 ± 0.23
Frozen fruit[ab]	25.03 ± 0.05	31.13 ± 0.44	26.90 ± 0.45
Thawed fruit[ad]	30.64 ± 0.59	30.60 ± 0.14	24.79± 0.11
Drip[e]	66.54 ± 0.22	88.96 ± 0.09	49.44 ± 0.03
Canned product			
Fruit[ac]	23.99 ± 0.25	28.58 ± 0.46	31.73 ± 0.01
Syrup[e]	80.91 ± 2.81	38.40 ± 4.69	35.84 ± 0.64
Jam[ad]	3.47 ± 0.31	12.46 ± 0.59	12.46 ± 0.87

[a] Measurement with the ColorQUEST 45/0, view port diameter of 89.9 mm using a cell to present samples.
[b] Measurement cell was filled up with fruits (about 2 to 3 layers of fruits).
[c] Measurement cell was filled with 2 layers of fruit.
[d] 210 g of jam was used (approximately 1.2 cm in depth).
[e] Measurement with the ColorQUEST sphere, view port size of 25.4 mm using a cuvette (2.5 mm in length).

Table 4- Total phenolics, antioxidants, anthocyanins, and browning and polymeric pigment in canned, frozen, and jams of strawberries from the Totem genotype[a]

Samples	Total Phenolics[b]	ACN[c]	Browning Index[d]	Percent Polymeric Color
Frozen fruit[e]	313.6 ± 17.7	69.7 ± 4.2	1.14 ± 0.13	7.16 ± 0.68
Canned product (total)	239.7 ± 18.1	21.7 ± 3.4	--	--
Fruit	118.9 ± 13.4	8.8 ± 1.7	2.10 ± 0.7	33.20 ± 2.5
Syrup	120.9 ± 4.6	13.0 ± 1.7	1.91 ± 0.05	27.4 ± 1.9
Jam	262.1 ± 4.9	20.6 ± 1.4	5.00 ± 0.74	27.7 ± 3.3

[a] Values are presented in Mean ± SD of two replicates.
[b] Total phenolics was determined by use of DPPH method and expressed as equivalent mg of gallic acid per 100 g of initial fresh materials.
[c] Total anthocyanin content was expressed as mg of pelargonidin 3-glucoside per 100 g of initial materials, determined by a pH differential method.
[d] Browning index was expressed as calculated absorbance unit based on 100 g of initial fresh materials.
[e] Data reported for frozen fruit are based on fresh fruit. Contents per 100 g of finished product can be obtained from values in the table by a division of the latter by a conversion factor of 0.49, 1.03, and 1.41 for canned fruit, syrup and jam, respectively.

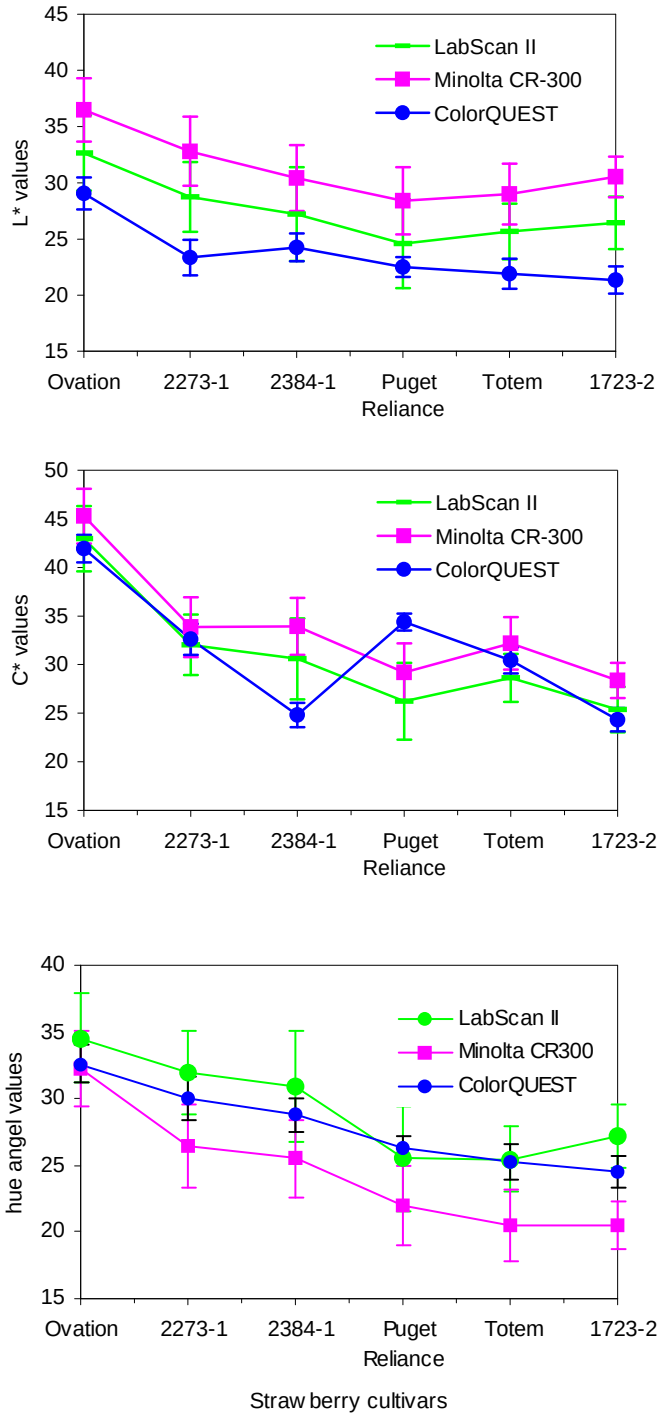

Figure 1 - CIEL*C*h^{o} color values of 6 strawberry genotypes measured by 3 different color instruments.

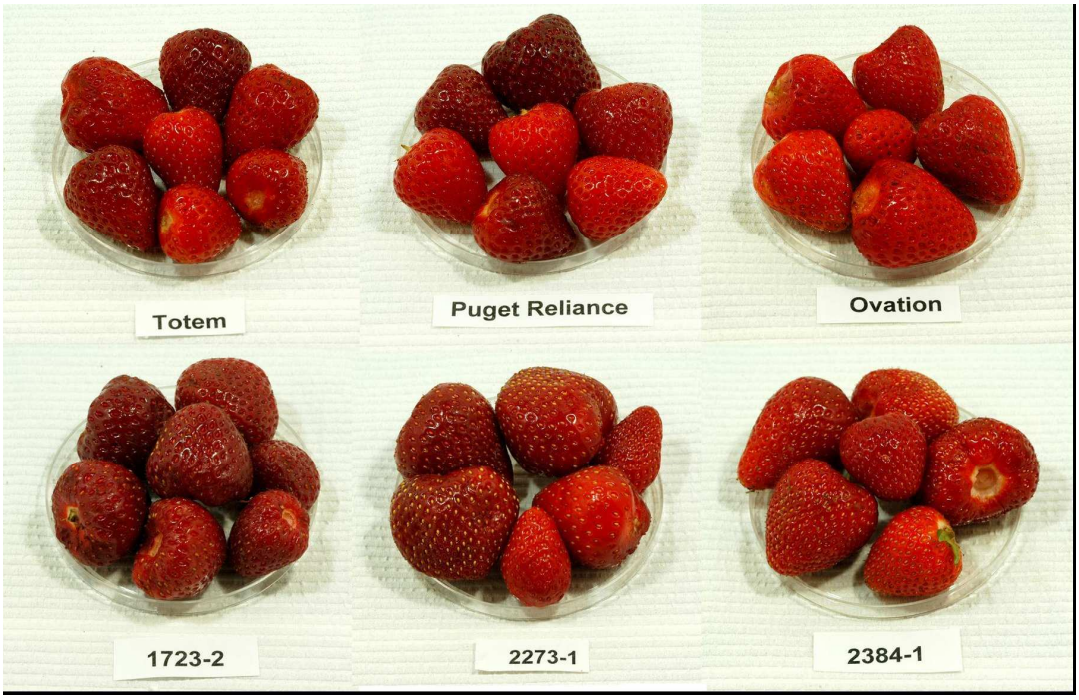

Figure 2 - Photos of 6 fresh strawberry genotypes harvested in 2005 season at the Oregon State University North Willamette Research and Extension Center (Aurora, OR, U.S.A.).

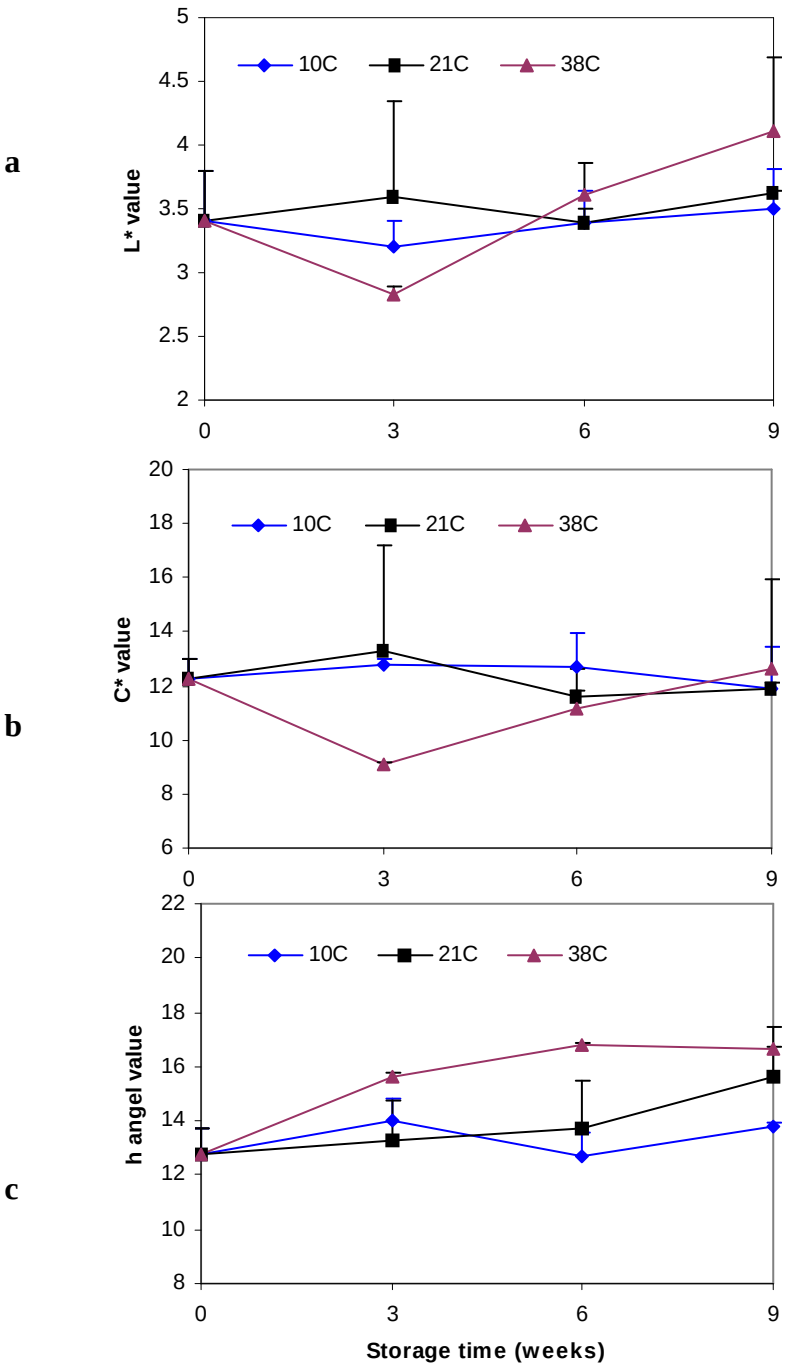

Figure 3 - Changes in CIEL*C*h*color values of jams made from strawberry of the Totem genotype during storage at 10 °C, 21 °C, and 38 °C for 9 wk.

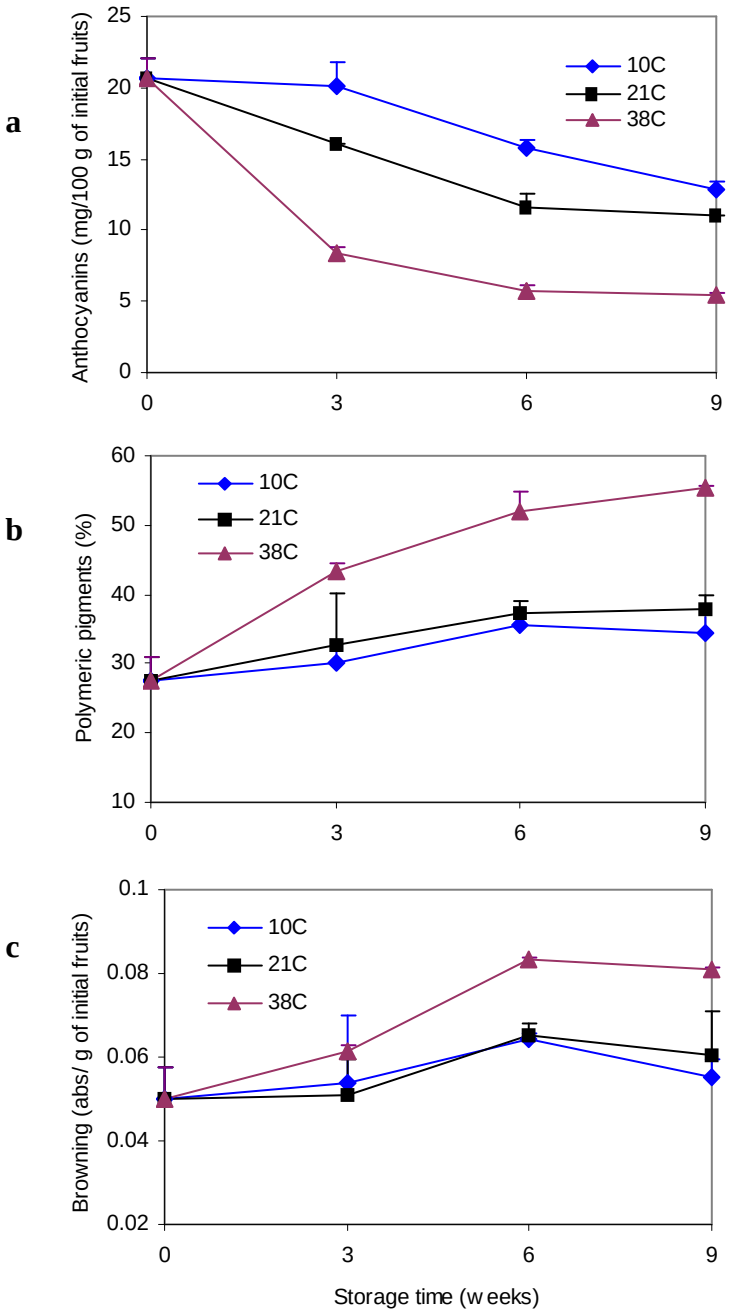

Figure 4 - Changes in anthocyanins, percent polymeric pigments, and browning of jams made from strawberry of the Totem genotype during storage under dark at 10 °C, 21 °C, and 38 °C for 9 wk.