EFFECTS OF FLAVONOIDS AND ASCORBIC ACID DERIVATIVES ON NON-ENZYMATIC BROWNIG IN PEACHES

Silvia E. Peralta Arribasplata

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Sean O'Keefe, Chairman

Joseph Marcy

William Eigel

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ABSTRACT

Non-enzymatic browning (NEB) due to ascorbic acid degradation is one of the most common reasons the shelf life of many processed foods is reduced. Different methods to minimize or retard the formation of browning pigments have been studied; however, to date, refrigeration is still the most preferable. Unfortunately, the use of low temperatures to preserve food is not always available in many parts of the world. Indeed, an area of concern due to NEB has been identified in meal-ready-to-eat (MRE) individual military rations, specifically diced peaches with syrup. This product was once part of soldiers' menus; however, it was removed due to browning and textural deterioration that occurred when stored under field conditions. We examined two general approaches to reduce NEB: the replacement of ascorbic acid by a more stable form and the use of flavonoids as antibrowning antioxidants in peach systems. These approaches were studied in three objectives. In our first objective, ascorbyl-2-phosphate showed better stability than ascorbic acid at 40°C in peach puree model systems, but not at 50 or 60°C. In the second objective, after the evaluation of the effect of two forms of vitamin C (ascorbic acid and ascorbyl-2-phosphate) and Pycnogenol (0%, 0.01% and 1%) on the quality of diced peaches in retortable pouches, we concluded that neither ascorbyl-2-phosphate nor pycnogenol resulted in improved color or ascorbic acid stability. Finally, in our third objective, after the evaluation of the effect of peach source (fresh, individually quick frozen and canned), addition of calcium chloride, and the addition of a water soluble flavonoid (α-glucosylrutin, α-GR) in diced peaches packaged in retortable pouches stored at 24, 40 and 51°C, there were no significant effects of α-GR on any of the peach sources at 51°C. However, at 40°C, α-GR improved the quality of diced peaches in pouches made of individually quick frozen and canned peaches, but not for fresh peaches. Quality was assessed by color (CIELAB system), which was measured using a handheld colorimeter, and ascorbic acid levels of peaches, which was determined using high performance liquid chromatography.

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LIST OF ABBREVIATIONS AND TERMS

A2P	Ascorbyl-2-phosphate
AA	Ascorbic acid
BI	Browning index
C	Variable content studied at time t
C_0	Variable content at time zero
DHAA	dehydroascorbic acid
E_a	Energy of activation
k	Rate constant
k_0	Zero order kinetic constant
k_1	First order kinetic constant
k_2	Second order kinetic constant
R	Universal gas constant (8.314J/molK)
RMSE	Root mean square error
t	time
T	Temperature (°Kelvin)
αGR	α-Glucosylrutin
β	Shape parameter

CHAPTER 1: INTRODUCTION

Ascorbic acid (AA) is frequently added to food products because of the importance of this vitamin in human health and its utility in food processing. AA is added during the manufacture of many food products due to its properties as a reducing agent. For example, this vitamin is used in the prevention of browning in fruit and vegetables, for stabilization of meat color, and inhibition of oxidation in beer, wine, milk and other products (Wong 1989). Unfortunately, due to the low stability of this compound, its lifetime is short. This degradation causes not only loss of nutrients, but also the appearance of brown pigments. The rise of these undesirable characteristics reduces shelf life of many food products.

Many studies have been conducted to identify the reactants, pathways and conditions by which AA is degraded (Sawamura and others 1991; Shinoda and others 2004; Roig and others 2000; Yuan and Chen 1998). However, scientists still do not fully understand all of the mechanisms involved since they are highly sensitive to environmental conditions, such as concentration of oxygen, metals, amino acids and pH. (Hui 2006; Fennema 1996). Currently, most of the literature classifies AA loss into aerobic and anaerobic degradation pathways (Wong 1989; Fennema 1996; Hui 2006). It is known that aerobic loss occurs at rates much higher than anaerobic degradations; however, the study of the latter is of great importance since many shelf-stable food products follow this deterioration route after all dissolved oxygen is consumed (Fennema 1996) .

Research on non-enzymatic browning has been conducted on many food systems such as citrus juices, pears, fruit puree, fruit pulps, among others (Roig and others 2000; Chutintrasri and Noomhorm 2007; Ibarz and others 1999; Garza and others 1999; Lozano and Ibarz 1996; Buglione and Lozano 2002). In addition to these products, diced peaches in syrup and packaged in retortable pouches has been identified as an area of concern. The retort pouch package was invented and introduced by the US Army Natick Laboratory as a replacement for the metal can due to the advantages that pouches offer (lighter weight, lower cost, lower total heat processing required, etc). Retort pouches are soft containers which do not injure soldiers when the food is carried in pockets; also, the sensory quality is higher than in metal cans due to fact that the length of heat treatment is reduced (Clark 2009). Unfortunately, the stability of some of the products

packaged in this type of container due to AA loss is still a challenge for food scientists. In fact, diced peaches in syrup in retortable pouches used to be part of the soldier menus; however, due to the AA browning that developed during storage, especially when refrigeration is not available, it was removed. Just a few studies examining non-enzymatic browning of food products manufactured in retort pouches have been published to date (Clark and others 2002; Kluter and others 1996; Kluter and others 1994; Olivas and others 2002; Rodriguez and others 2003).

Different methods to minimize or retard the formation of browning pigments due to non-enzymatic reactions have been studied. Some of these techniques involve the use of chemicals such as thiol compounds, sulfites and maltitol (Roig and others 1996; Naim and others 1997; Naim and others 1993; Davies and Wedzicha 1992; Koseki and others 2001). Physical methods such as modified-atmosphere packaging, microwave heating and ultrasound-assisting thermal processing have been also examined for the stabilization of ascorbic acids in foods (Howard and Hernandez-Brenes 1998; Villamiel and others 1998; Zenker and others 2003). Based on literature reports, two methods appear promising for the delay of AA loss in shelf-stable food products: the use of more stable AA derivatives and the addition of flavonoids.

The use of other forms of AA which have substituted groups at carbons 2 and 3 has been proposed as a replacement for AA, due to improved chemical stability (Iacobucci and Sweeny 1980). Indeed, one of the ascorbic acid derivatives that has been shown to have promising results is ascorbyl-2-phosphate (A2P) (O'Keefe 2001). For example, ascorbyl-2-monophosphate and ascorbyl-2-polyphosphate had better stability than AA in carbonated beverages at 15°C, 25°C and 35°C (Wang and others 1992). In mashed potatoes after processing and storage, the losses of AA and A2P were 50% and 20%, respectively (Wang and others 1995). In addition, higher stability of A2P has been shown in cosmetics and aquatic feeds (O'Keefe 2001; Austria and others 1997). Even though A2P still does not have approval for use in foods, additional study is warranted because of its reported higher stability in different food systems.

The addition of flavonoids in food systems in order to delay the AA browning has not been studied yet. These compounds are secondary plant metabolites that have been shown to have good antioxidant properties, due to their free radical scavenging and metal chelation

activities. In fact, their protective role against ascorbic acid degradation has been known since 1936, when Szent-Gyorgyi and coworkers found that flavonoids, at that time called Vitamin P, had an ascorbate protective role (Rice-Evans and Packer 1998). Subsequently, research *in-vitro* has confirmed this protective role (Bors and others 1995; Rice-Evans and Packer 1998; Sivonova and others 2006; Cossins and others 1998). There is also evidence that shows that juices with high content of phenolic compounds exhibit good AA stability (Clegg and Morton 1968; Rice-Evans and Packer 1998).

Among flavonoids, a blend of procyanidns and phenolic acid glycosylation products, which is sold under the name of Pycnogenol, has been demonstrated to have a better ascorbate protective activity than other compounds (Cossins and others 1998). Pycnogenol is a tradename for an extract of pine bark developed originally by French scientist, Jack Masquelier (Masquelier 1987). Most research on Pycnogenol has focused on its antimicrobial and antioxidant properties (Ahn and others 2006; Torras and others 2005). One study in yogurt demonstrated the stability of Pycnogenol during storage in this acid product (Ruggeri and others 2008). Even though the ascorbate protective role of Pycnogenol was noted more than one decade ago (Cossins and others 1998), Pycnogenol has not been evaluated as a potential additive to attenuate ascorbic acid degradation in food products.

In addition to Pycnogenol, flavonoids that have been modified by the addition of glucose groups are of interest. These new types of phenolic compounds allow the study of flavonoids in food systems due to their greatly improved solubility (Anonymous 2006; Schonrock and Kruse 2004). One of the modified flavonoids that is currently commercially available is α-glucosylrutin (Anonymous 2006). The antioxidant activity of α-glucosylrutin (α-GR) has been demonstrated in skin care products. Indeed, some properties of this glucosylated flavonoid are the inhibition of the initial formation of free radicals and the scavenging of intermediates that occur in the skin aging process (Anonymous 2006). Also, the use of α-GR in cosmetics has been proposed due to the protective role this compound has against AA oxidation, which can occur during storage of cosmetics and dermatological preparations (Schonrock and Kruse 2004). Even though there is no information on the use of α-GR in food products, there is evidence related on rutin that suggests that this flavonoid can exert an ascorbate protective role. In fact, in a model

system composed of AA and ascorbate oxidase, rutin was the compound which best extended the ascorbate radical lifetime after Pycnogenol and an extract of green tea (Cossins and others 1998)

Research objectives

The main goal of this study was to investigate the effectiveness of two possible methods of delaying AA loss in food systems. In addition, since diced peaches packaged in retortable pouches stored at high temperature is an area of concerned for the US army, this study will also permit a better understand the kinetics involved in the development of browning compounds and loss of ascorbic acid in this food product.

Objective 1: Compare the stability of ascorbyl-2-phosphate and ascorbic acid during high storage temperatures (40°C, 50°C and 60°C) in a peach puree model system.

Objective 2: Evaluate the effect of two forms of vitamin C (ascorbic acid and ascorbyl-2-phosphate), the concentration of Pycnogenol (0%, 0.01% and 1%) and their interactions on the quality of diced peaches in retortable pouches.

Objective 3: Evaluate the effect of peach source (fresh, individually quick frozen and canned), addition of calcium chloride, and the addition of a water soluble flavonoid (α-glucosylrutin) in diced peaches packaged in retortable pouches.

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CHAPTER 2: REVIEW OF LITERATURE

L-ASCORBIC ACID

L-Ascorbic acid (AA) is a carbohydrate-like vitamin with two enol groups built into a 5 membered heterocyclic lactone (Fig. 1) (Kall 2003). The enediol group is responsible for the strong reducing activity of this compound. AA is characterized by its high solubility in water due to its high polarity; in addition, as a consequence of the ionization of the carbon 3 hydroxyl group (Pk_{a1}= 4.04 at 25°C), AA is acidic. A second dissociation of the carbon 2 hydroxyl group is not favorable because of the high pK (Pk_{a2}=11.4) (Fennema 1996).

Since AA has two chiral carbon atoms, carbons 4 and 5, it can also exist in any of its four stereoisomer forms: L-AA, D-ascorbic acid, D-isoascorbic acid and L-isoascorbic acid (Kall 2003). However, L-AA is the only form that occurs almost exclusively in nature and has nutritional value (Fennema 1996). Because of the reducing and antioxidant activity of L-isoascorbic acid, D-ascorbic acid (Fennema 1996) and D-isoascorbic acid (Liao and Seib 1988), these compounds are used as food ingredients, and are good substitutes for AA from a functional, non-nutritive standpoint. However, AA has some properties that are not present in its stereoisomers, including bread-improving action and vitamin C activity. In fact, the use of D-isoascorbic acid in foods can interfere with the bioavailability of AA (Liao and Seib 1988).

AA is found in many fruits and vegetables. It has also been reported in animal tissues and animal-derived products, but to a lesser extent (Fennema 1996). The importance of AA consumption is based on the fact that humans are not able to synthesize it. AA participates in several important reactions in our bodies, such as collagen synthesis, conversion of 3,4-dihydroxyphenylethylamine to noradrenaline, and protection against free-radical damage (Wong 1989). A deficiency of AA in the human diet can cause scurvy (Belitz and others 2004). Ranges of human requirements are not well defined, nevertheless a range of 45-75 mg/day has been reported (deMan 1999). The recommended daily intake (RDI) for adults is 60 mg.

In food processing, AA is used because it has reducing and antioxidant activities. As an antioxidant, AA is able to donate electrons, eliminating free radicals such as those formed during

the damage of lipids. The oxidation products are semidehydroascorbic acid and dehydroascorbic acid (DHAA) (Kall 2003). The principal uses of vitamin C in the food industry include: (1) prevention of browning in fruit and vegetables, (2) inhibition of oxidation in beer, wine, vegetable oil, milk and dairy products, (3) stabilization of meat color and (4) improvement of bread dough. AA may be added to foods either as its undissociated acid or as the neutralized sodium salt (sodium ascorbate) (Wong 1989). When AA is conjugated with hydrophobic compounds, it acquires lipid solubility. For example, fatty acid esters such as ascorbyl palmitate and ascorbic acid acetals are lipid soluble; consequently, they can be used as antioxidant agents in lipid environments (Fennema 1996).

LOSS OF L-ASCORBIC ACID

AA browning result from the decomposition of vitamin C under both aerobic and anaerobic conditions and the presence or absence of amino compounds (Hui 2006). Even though the route of AA browning depends on factors such as temperature, pH, and oxygen level, the types of reactions are usually divided into aerobic and anaerobic pathways (Wong 1989).

Types of degradation

Aerobic degradation

Fig. 2 shows a proposed pathway for the degradation of AA under aerobic, acidic conditions (Wong 1989). First, AA is oxidized to DHAA. This reaction can happen either by two one-electron transfer processes (2-1-e^-) or a single two-electron reaction (1-2-e^-). In the 2-1-e^- transfer, the first product formed is semi-dehydroascrobic acid ($AH^\bullet$). Subsequent loss of a second electron yields DHAA. The 1-2-e^- reaction results in the formation of an ascorbate-oxygen-metal catalyst complex. The formation of DHAA is direct with a two-electron process (Fennema 1996). This last reaction increases when acidity is decreased (i.e., pH increased) from pH 1.5 to 3.5 (Liao and Seib 1988).

DHAA is not stable and its lactone ring is easily cleaved to 2,3-diketogulonic acid (Wong 1989). This hydrolysis to 2,3-diketogulonic acid, which is irreversible, is responsible for loss of vitamin C activity. In fact, DHAA presents approximately the same vitamin activity as AA due to its reduction to AA by enzymes in some tissues. It has been shown that this

hydrolysis is favored by alkaline conditions. Indeed, at pH 2.5-5.5, DHAA has its maximum stability. At pH>5.5, the stability of DHAA is very poor. In addition, there are results that demonstrate that this reaction is not affected by the presence of oxygen, but it is affected by an increase in temperature. Because of the facile formation of DHAA, this reaction represents an important and, frequently, rate-limiting aspect of the aerobic degradation of AA (Fennema 1996).

L-xylosone is formed after the decarboxylation of 2,3-diketogulonic acid. After a 2,3-enolization and dehydration, a tricarbonyl compound, 3-keto-4-deoxypentosulose is produced. This same compound can be obtained from a 3,4-enolization of the 2,3- diketogulonic acid. This last route seems to be the major pathway (Fig. 2) (Wong 1989).

Anaerobic degradation

Under anaerobic conditions, the reactions involved are slow compared with oxidative degradation; however, they can be accelerated by lowering the pH. In fact, the maximum rate of anaerobic degradation occurs in the pH range of 3-4. This mildly acidic environment indicates the importance of the pH on the opening of the lactone ring (Fennema 1996). In the initial step, the lactone ring is cleaved to form a 2,3-enediol. After dehydration and rearrangement, this compound produces a 2,3-diketo acid. Decarboxylation of the diketo acid produces the 3-deoxy-L-pentosulose, which corresponds to the 3-deoxyglycosulose in the maillard reaction. As we can see in Figure 3, furfural is formed after dehydration of the 3-deoxyglycosulose (Wong 1989).

Factors that enhance the degradation of the L-ascorbic acid

Several factors can affect the rate, mechanism and qualitative nature of AA browning; however, pH, oxygen concentration and the presence of metal catalysts have the most influence (Fennema 1996).

Metal ions

As mentioned earlier, DHAA is formed either by a two sequential one-electron transfer mechanism or by a single two-electron reaction (Fennema 1996). Metal ions can participate in

either of these two mechanisms (Wong 1989). In the first type of reaction, the metal ion acts as the oxidizing agent while in the second type, it acts as a catalyst of the formation of the ascorbate-metal-dioxygen complex. In this last reaction, hydrogen peroxide and DHAA are formed (Wong 1989).

Evidence indicates that oxidation uncatalysed by metals is not significant, and that the oxidative degradation is mostly via pathways involving trace metals (Fennema 1996). In fact, it has been reported that degradation of AA is observed to be first order with respect to the concentration of metals, and the rate constant is $6x10^{-7}$ sec^{-1} in an air-saturated solution at pH 7.0 (Buettner 1988). Among metal ions, Cu^{2+} and Fe^{3+} are the most important participants (Belitz and others 2004).

The potency of the reactions depends on the metal involved, its oxidation state and the presence of chelators. In reference to the chelators, their role is not predictable, since Fe^{3+} shows an enhanced catalytic activity when it is chelated by EDTA while Cu^{2+} is inhibited under the same conditions (Fennema 1996). Also, it has been noted that browning is enhanced and retarded by the chelators nitriltriacetic acid (NTA) and diethylenetriamine pentaacetic acid (DTPA) respectively. Finally, another metal chelator that has been shown to enhance AA degradation is citric acid (Shinoda and others 2004; Clegg 1964).

pH

AA has different ionic forms that exhibit different susceptibilities to oxidation. The ascorbate dianion (A^{2-}) has the greatest susceptibility to oxidation, followed by the ascorbate monoanion (AA^{-}), and subsequently AA. The concentrations of the different ionic forms depend on environmental pH. Since most foods containing ascorbic acid are acidic (AA is unstable under neutral or alkaline conditions), AA oxidation is mostly governed by the concentrations of AA and AA^{-} (pK_{a1}= 4.04). At pH 8, the rate of oxidation increases, since there are significant concentrations of DHAA (pK_{a2}= 11.4); in fact, at pH>5.5 the stability of DHAA is very poor (Fennema 1996). Under anaerobic conditions, the rate of oxidation is maximal at pH 4 and minimal at pH 2 (Belitz and others 2004).

Oxygen

Over the range of 1.0-0.4 atm O_2, the rate of metal-catalyzed oxidation is proportional to the partial pressure of dissolved oxygen. Nevertheless, at partial pressures < 0.2 atm, the rate of oxidation is independent of the oxygen concentration. Reactions involving metal chelators are also independent of oxygen concentration (Fennema 1996).

Contradictory results on the acceleration of the AA degradation due to oxygen concentration have been reported. Reportedly, when bottles of model orange juice were degassed using nitrogen or had no headspace left, the degree of browning was greater compared to bottles that had 25 ml of oxygen; the reasons for these results are not clear (Shinoda and others 2004).

Other environmental conditions

In general, enzymes that have copper or iron in their prosthetic groups catalyze AA degradation. Among the most important are ascorbic acid oxidase, phenolase, cytochrome oxidase and peroxidase (deMan 1999). However, the importance of these enzymes in food processing is minimal (Wong 1989).

Water activity also influences the rate of AA degradation. For example, it has been reported that over the range of 0.10-0.65 water activity, the rate of oxidation increases as water activity increases. This behavior is apparently due to water acting as a solvent for reactants and catalysts (Fennema 1996).

Temperature is another factor that affects the rate of AA degradation. In fact, there is a study which reports that lower temperatures inhibited AA degradation and limited the accumulation of various degradation products (Yuan and Chen 1998). Light irradiation is also reported to accelerate the process (Fennema 1996).

The type of sugar that is present also influences the rate of oxidation. For example, ketoses can increase the rate of anaerobic degradation while other sugars and sugar alcohols show a protective effect in oxidative degradation (Fennema 1996).

Citric acid has also been suggested as a contributor to browning in acidic products containing AA. However, the role of citric acid is still under investigation (Roig and others 2000; Clegg 1964). In a model solution of orange juice, the degree of browning was reduced between 40 to 60% when citric acid was removed (Shinoda and others 2004). Other compounds such as sugars, amino acids and increasing concentration of AA have been reported to enhance browning in orange juice (Kacem and others 1987). How these compounds interact with AA degradation products is still unclear.

Products of L-ascorbic acid Degradation

Polymerized intermediates, unsaturated carboxylic acids of five- and six-carbon chain length, and fragmentation products having five or fewer carbons have been identified as AA degradation products. These new compounds are responsible for the changes in flavor and odor in citrus juices during storage or processing (Fennema 1996). Also, there is evidence that suggests pigmented materials are formed when the degradation products and/or sugars or other carbonyl compounds, polymerize or react with amino acids to produce browning materials (Sawamura and others 1991). The types and concentrations of the compounds and the reactions involved are influenced by several factors such as temperature, pH, water activity, concentrations of oxygen and metal catalysts, and presence of active oxygen species (Fennema 1996).

As illustrated in Fig. 2, the intermediates and products of AA degradation under aerobic conditions include DHAA, carbonyls and dicarbonyl compounds (Wong 1989). DHAA can form browning products in both the presence or absence of amino acids (Liao and Seib 1988). In the presence of amino acids, DHAA can produce L-scorbamic acid, which is an important intermediate in the browning reaction. In fact, scorbamic acid can react with other molecule of DHAA to produce a red pigment, which undergoes further reactions with more molecules of scorbamic acid, resulting in brown reaction products (Liao and Seib 1988). In addition, it has been reported that the three browning fractions of low molecular weight (lower than DHAA) are probably products of the reaction of amino acids with DHAA. Furthermore, in the absence of amino acids, in a model system composed just of DHAA, at least four different brown pigments and three colorless products were identified. The four brown pigments identified had molecular weights lower than DHAA (Sawamura and others 1991). In more recent work, it was reported

that one of the brown compounds was 3,4-dihydroxy-5-methyl-2(5H)-furanone (molecular weight of 130) and one of the colorless compounds was identified as 2-furoic acid. This research noted an earlier publication that showed that browning developed in a DHAA aqueous solution faster under nonoxidative than oxidative conditions. Consequently, the authors of this paper suggest that if the DHAA products follow oxidative reaction, the development of brown pigments is suppressed (Sawamura and others 1994). 3-Hydroxy-2-pyrone was also identified as an aerobic degradation product of AA (Yuan and Chen 1998) and furfural was identified as a degradation compound of AA via DHAA (Shinoda and others 2004).

Furfural is an AA degradation product under anerobic conditions (Wong 1989). As shown in Fig 3, 3-deoxy-L-pentosulose is also produced. This compound corresponds to the 3-deoxyglycosulose in the maillard reaction; consequently, it can further react producing brown pigments. Finally, there is evidence that under anaerobic conditions, the products are carbon dioxide, furfural, and 2,5-dihydro-2-furoic acid (Wedzicha 1984).

In a model solution of orange juice stored at 50°C, 3-hydroxy-2-pyrone, 5-furfural, 2-furoic acid, hydroxymethylfurfural and 5-hydroxymaltol were identified. The first three compounds were produced from DHAA, while the last two were derived from fructose (Shinoda and others 2004). In the same study, but in retail orange juice samples stored at 50°C, the production of the same five compounds was also observed. In order to study the relationship of these five compounds with brown pigments, these compounds were stored under the presence and absence of amino acids. 3-Hydroxy-2-pyrone produced brown pigments with and without amino acids. Furfural required amino acids for browning. 5-Hydroxymaltol, hydroxymethylfurfural, and 2-furoic acid did not brown in any of the model systems. Other studies also note that hydroxymethylfurfural cannot be used as an index of browning since it is not a precursor of browning pigments (Roig and others 2000).

Studies of L-ascorbic acid browning in food products

In food products, anaerobic degradation of AA is not significant compared to the aerobic degradation. In fact, evidence shows that anaerobic loss occurs at 2 or 3 orders of magnitude less than oxidative degradation. However, anaerobic degradation is a concern in canned food

products after depletion of the residual oxygen (Fennema 1996). For example, in citrus juice packed in tetrabrick cartons, ascorbic acid loss mainly occurred during the first few days due to the presence of residual oxygen. After the oxygen was depleted, the rate of degradation decreased (Roig and others 2000).

Many studies examining non-enzymatic browning due to AA have been published (Burdurlu and others 2006; Buedo and others 2000; Roig and others 2000; Chutintrasri and Noomhorm 2007; Rattanathanalerk and others 2005). Most of these studies have applied kinetic models to make predictions and to understand how different storage conditions impact browning. For example, in citrus juices stored at 28, 37 and 45°C, AA loss followed first-order kinetics (Burdurlu and others 2006). In citrus juice stored at a wide range of temperatures (4-105°C), at lower temperatures (4-37°C) AA loss followed zero order models, while at higher temperatures of 76°C and 105°C, the loss followed first and second order models, respectively (Roig and others 2000). During storage of peach juice concentrate at 3, 15, 30 and 37°C, the absorbance values (indicative of browning) throughout storage fit a parabolic model (Buedo and others 2000). In addition, non-enzymatic browning developing during thermal treatments has also been reported. For instance, during heating of pineapple puree at 70-110°C, it was reported that L and b values followed first order models while ΔE^*, and browning index fitted zero order models (Chutintrasri and Noomhorm 2007). In a similar study but heating pineapple juice at 55-95°C, it was observed that a and b values fit first order models, and browning indexes and hydroxymethylfurfural followed zero order models (Rattanathanalerk and others 2005).

In addition to the simple kinetic models (zero, first and second orders), there are studies that recommend the application of other equations, since these new models more accurately describe AA degradation. For example, the Weibull model fit AA degradation data better than zero, first or second order models in fresh cut watermelon and citrus juice heated under aerobic conditions (Oms-Oliu and others 2009; Manso and others 2001). The Weibull model considers a scale parameter (α) as a time constant (inverse of the rate constant), and a shape parameter (β) as a behavior index (Hui 2005). Due to the use of α and β parameters, this model is extremely flexible (Manso and others 2001). Furthermore, in reference to color characteristics, the combined model has been applied successfully in several studies (Lozano and Ibarz 1996; Ibarz

and others 1999; Garza and others 1999; Rattanathanalerk and others 2005). This model is based on the theory that there are two stages associated with color changes. The first stage is a color formation which follows a zero order kinetic model and the second stage is a color degradation which follows a first order kinetic model (Ibarz and others 1999). Even though reaction 1 is intended to describe color changes due to the maillard reaction, it is possible that that color change due to AA-related browning can also be explained using the same equation. In fact, it is known that some intermediates of AA degradation enter the maillard reactions (Wong 1989).

CONTROL OF L-ASCORBIC ACID DEGRADATION

L-Ascorbic acid derivatives

AA derivatives have been produced commercially since this vitamin has several reactive positions (Packer and Fuchs 1997). The esterification of AA at one of its hydroxyl groups has overcome solubility problems in pharmaceutical and food industries (Austria and others 1997; O'Keefe 2001). For example, when AA is conjugated with fatty acids such as palmitate or acetate, vitamin C acquires lipid solubility and can be used as an antioxidant in lipid environments (Fennema 1996). Also, when derivatives have substituted groups at carbons 2 and 3, the vitamin become more stable against oxidative reactions (Iacobucci and Sweeny 1980).

The use of AA derivatives with substituted groups at carbons 2 and 3 is appealing because it can attenuate the loss of vitamin C during the production or storage of food products (Wong 1989; Liao and Seib 1988). To date, the most effective derivatives developed are ascorbyl-2-sulfate and ascorbyl-2-phosphate esters (Fig. 4) (O'Keefe 2001). The esterification of vitamin C at other carbons also results in improved stability; however, the stability is not as high as with the esterification of the enediol group (Austria and others 1997; Schonrock and Kruse 2004).

Currently, most applications of ascorbyl-2-phoshphate and ascorbyl-2-sulphate are in cosmetics, pharmaceuticals and aquaculture feeds (Austria and others 1997; O'Keefe 2001; Fermann and Gripp 2004). For cosmetics, a more stable form of AA is advantageous, since this vitamin is used as an antioxidant in skin formulations; AA is easily depleted during skin exposure to the sun (Fermann and Gripp 2004). Currently, sodium ascorbyl phosphate is

distributed under the name of Stay-C 50 for use in skin formulations. There is evidence showing that this compound is highly stable at 43°C at pH 7 or higher. In a study conducted at pH 4 with solutions and topical formulations, it has been demonstrated that ascorbyl palmitate and magnesium ascorbyl phosphate were more stable than AA. In fact, between ascorbyl esters, the ascorbyl phosphate was more stable. Here it was reported that ascorbyl phosphate had 95% retention after 60 days at 42°C in darkness; while after 2 months at room temperature and at darkness, ascorbyl palmitate had 27% retention (Austria and others 1997). In addition to the application of these AA derivatives in cosmetics, there are several published papers which emphasize the advantages of these compounds over AA in aquaculture. In fact, this industry requires methods to preserve AA in feeds, since many fish species can develop scurvy and, consequently, produce serious economic losses. For example, it has been reported that after diet processing and storage of shrimps, magnesium ascorbyl-2-phosphate was more stable than AA (Shigueno and Itoh 1988). Similar studies are reported elsewhere (O'Keefe 2001)

Even though neither ascorbyl-2-phosphate nor ascorbyl-2-sulphate are currently Generally Recognized as Safe (GRAS) by the FDA, some research in food systems has been conducted. For example, in a study on white bread, after 3 days the samples fortified with L-ascorbate-2-polyphosphate and L-ascorbate-2-monophosphte retained 10-15% more vitamin C than samples which were fortified with AA (Park and others 1994). In another study in bread, samples fortified with ascorbyl-2-monophosphate and AA were retained at 40% and 5%, respectively, after 6 days at 25°C (Wang and others 1995). In addition, solutions of ascorbyl-2-monophosphate and ascorbyl-2-polyphosphate (0.3%) injected into ground turkey and ground beef were more effective than AA or sodium ascorbate for the preservation of flavor and aroma of cooked ground turkey and beef after 3 days storage at 4°C (Craig and others 1991). In the production of mashed potatoes, in samples fortified with AA, 96% was lost during preparation and storage of potato flakes and during holding of the reconstituted product on the steam table. On the other hand, samples with ascorbyl-2-monophosphate and ascorbyl-2-polyphosphate were retained around 68% at the point of consumption (Wang and others 1992). In carbonated beverages, samples fortified with ascorbyl-2-monophosphate lost about 8% of vitamin C after 28 days of storage at 15°C-35°C while samples fortified with AA lost 18-36%. Under these conditions, ascorbyl-2-polyphosphate also had greater stability than AA at 15°C and 25°C

storage. At 35°C, there was no difference between ascorbyl-2-phosphate and AA. After 25°C storage at 40% moisture for 7 months, bran flakes fortified with AA, ascorbyl-2-monophosphate and ascorbyl-2-polyphosphate lost 5%, 0% and 11% of vitamin C, respectively (Wang and others 1995). Finally, in acidic model systems with an anthocyanin and different AA derivatives, after 50 hours of storage, the systems that had AA had a color loss of over 23% while the systems that contained ascorbyl-2-phosphate and ascorbyl-2-sulfate showed less than 7% color loss (Iacobucci and Sweeny 1980).

Research comparing bioavailability of ascorbyl esters with AA has been reported (Iacobucci and Sweeny 1980). Ascorbyl-2-sulfate has been shown to have the same antiscorbutic activity as AA in rainbow trout; however, in studies done in catfish, vitamin C activities in blood and liver tissues were higher in those fed with AA than with acorbyl-2-sulfate. Phosphate esters of AA appear to have better bioavailability than ascorbyl-2-sulfate according to investigations in guinea pigs, rhesus monkey, shrimp, among other species (O'Keefe 2001).

Flavonoids

Flavonoids are secondary plant metabolites that have potential benefits for human health. All flavonoids contain the same backbone structure, differing in the hydroxylation, methoxylation, or glycosylation patterns. To date, there are approximately 3000 known flavonoids; however, only few are widely present in plants. The major classes of flavonoids are the flavonols, catechins, anthocyanidins and dihydroflavonols (Rice-Evans and Packer 1998).

To date, scientists have concluded that the antioxidant properties of flavonoids are due to their capacity to reduce radicals and to chelate metals (Pietta 2000; Rice-Evans and Packer 1998; Perez 2003). In addition, it has been reported that flavonoids inhibit oxidase enzymes (Perez 2003). In order to predict antioxidant activities of flavonoids, studies have focused on the determination of pK values, scavenging and decay rates of radicals, and redox potentials (Bors and others 1995; Cossins and others 1998; Jovanovic and others 1996). A study that identified the decay constants of different radicals ($^{\cdot}OH$, $N_3^{\cdot}$, $O_2^{\cdot -}$, $LOO^{\cdot}$, $tBuO^{\cdot}$, and sulphite) and the stability of the antioxidant radicals reported that three criteria were important for effective radical scavenging in flavonoids (Bors and others 1990):

1. The o-dihydroxy structure in the B ring, which confers higher stability to the radical form and participates in electron delocalization.
2. The 2, 3 double bond in conjunction with a 4-oxo function in the C ring, responsible for electron delocalization from the B ring (phenoxyl radicals produced are stabilized by the resonance effect of the aromatic nucleus).
3. The 3- and 5-OH groups with the 4-oxo function in A and C rings result in maximum scavenging potential.

According to these criteria, quercetin is the flavonoid that should have the best antioxidant properties. In addition to the research noted above, these criteria have been confirmed by another study where antioxidant properties of several flavonoids using the Trolox equivalent antioxidant activity (TEAC) assay were compared (Rice-Evans and Packer 1998). Studies conducted to determine optimal inhibition of lipid peroxidation using flavonoids also confirmed these characteristics (Rice-Evans and Packer 1998). Furthermore, in a pulse radiolysis study, quercetin was identified as the best electron donor (measured as $E_{10,8}$= 0.09V and E_7= 0.33V) of all of the investigated flavonoids. In this study, it was reported that this favorable electron-donating property is originated in the 3-hydroxyl group in the C ring, which conjugates to the catechol (B ring) radical through the 2,3 double bond (Jovanovic and others 1996). On the other hand, in an oxygen radical absorbance capacity (ORAC) study, quercetin had a lower antioxidant capacity than other flavonoids such as rutin (quercetin rutinoside), catechin, epicatechin, among others. This study reported differences in the antioxidant activity values obtained from different methods. Also, it is seen that the ORAC values show lower correlations with electrochemical responses (Aaby and others 2004). Solubility of quercetin is low, and may explain the better antioxidant capacity of rutin, which is a quercetin-sugar compound.

Interaction of flavonoids with ascorbate radicals

The protective role of flavonoids against the degradation of AA has been noted since 1936, when Szent-Györgyi and coworkers found a compound, called vitamin P at that time, with an ascorbate protective role. Research conducted in the 1930s showed that only flavanones had this protective role (Rice-Evans and Packer 1998). Years later, studies conducted with different

flavonoids in model systems at pH of 2.9 demonstrated that flavones and flavonols also protect AA from oxidation; in fact, these studies demonstrated that quercetin was a better antioxidant than kaempferol and rutin under the conditions used (Harper and others 1969; Clegg and Morton 1968). Anthocyanins, such as cyanidin-3-rhamnoglucoside and delphinidin-3-glucoside, have been reported to accelerate AA degradation (Clegg and Morton 1968). Similar results were obtained in a study at pH of 2.8, where cranberry anthocyanins accelerated the oxidation of AA, and quercetin and quercitrin had protective effects on both anthocyanin and AA degradation (Shrikhande and Francis 1974). At pH of 3.8, catechin slowed AA oxidation but not anthocyanin degradation (Poei-Langston and Wrolstad 1981).

The protective role of phenolic compounds against AA degradation has also been suggested from fruit juices and extract studies. For example, in a study where AA degradation in juices was compared, it was observed that the juice containing blackcurrant had higher AA stability than the one made with lemon. It is known that blackcurrant juice is rich in phenolic compounds with antioxidant properties (Clegg and Morton 1968). In a later research, in order to identify which phenolic compounds exert these antioxidant properties, model systems with ascorbic acid at pH of 2.9 were prepared. The treatments were composed of different phenolic compounds that occur in blackcurrant juice. In this study it was observed that quercetin, dihydroquercetin, kaempferol and rutin presented antioxidant activity while cyanidin-3-rhamno-glucoside and delphinidin-3-glucoside accelerated oxidation (Harper and others 1969). Other reports note that, in juices fortified with AA, the ascorbate protective role was in the order of black currant>orange>apple (Rice-Evans and Packer 1998). It has been reported that acai anthocyanins fortified with AA had higher AA stability than a similar system composed of acerola extract (De Rosso and Mercadante 2007). In this study, the researchers attributed this result to the 10 times higher flavonoid content in the acai extract.

In more recent and specific studies using pulse radiolysis, it was concluded that flavonoids containing the B-ring catechol group and the 2,3 double bond had a higher redox potential than ascorbate, while flavanones such as dihydro-quercetin were able to reduce the ascorbyl radical at pH 8.5 (Bors and others 1995). According to these results, flavonoids such as quercetin, rutin and kaempferol would not be able to protect the ascorbate radical, contradicting

previous studies. Subsequent research confirmed that the results of Bors and others (1995) were erroneous, since the kinetics were obtained at a very high doses, and there were nonequilibrium experimental conditions (Jovanovic and others 1996). Flavonoids such as rutin, catechin and epigallocatechin have higher reduction potentials than ascorbate in the pH range of 0-14. This study also noted that the quercetin radical had the lowest reduction potential of the flavonoids studied (rutin, catechin, epigallocatechin, hesperidin and galangin); however, reduction potential was still higher than ascorbate between pH 7-14 (Jovanovic and others 1996). Finally, pure flavonoids (rutin, quercetin, kaempferol, among others) and blends of flavonoids (Pycnogenol and green tea extract) were able to increase the ascorbate radical life time when they were incubated with an AA-ascorbate oxidase model system at pH of 7.4 (Cossins and others 1998).

From the studies noted above, we can observe that even though flavonoids have higher redox potential than ascorbate, they are still able to protect vitamin C in both food systems and buffer solutions. In an attempt to explain this, it has been proposed that the recovery process of radicals due to flavonoid activity is not driven by reduction potential of the compounds involved (Packer and others 1999). Consequently, it is clear that more research is needed in order to clarify the mechanisms of the protective role of flavonoids against AA degradation.

A study to determine if flavonoids are able to recycle AA from DHAA consisted of adding three different flavonoids (polyphenon, theafavin and Pycnogenol) to a system where all of the AA had been consumed by ascorbate oxidase at pH of 7.4. The results obtained showed the generation of ascorbate radical signal. Consequently, this study proposed that the mechanism by which flavonoids exert their protective role is as follows:

$$FlOH + DHA \leftrightarrow FlO^{\cdot -} + Asc^{\cdot -} + 2H^{+}$$

Also, in the same research paper it was reported that myricetin exerts an ascorbate protective role by competing with AA for ascorbate oxidase. In fact, the myricetin radical was detected, confirming a reaction between the enzyme and the flavonoid. Finally, the metal chelating properties of flavonoids has also been proposed as the reason for the ascorbate protective role (Cossins and others 1998; Harper and others 1969; Clegg and Morton 1968).

α-Glucosylrutin

The use of flavonoids is often hampered by their low solubility in water (Shrikhande and Francis 1974; Clegg and Morton 1968). This characteristic restricts the commercial use of these compounds. In order to counteract this disadvantage, some flavonoids, such as rutin, have been modified by the addition of glucose groups (Schonrock and Kruse 2004; Anonymous 2006). Indeed, a new molecule called α-glucosylrutin (Fig. 5) is obtained by using a microbial enzyme that transfers a molecule of glucose to rutin. As a result, the solubility is increased from 0.01 g/100 g water to 120g/100 g water (Anonymous 2006).

The antioxidant activity of α-glucosylrutin has been demonstrated in skin care products. In fact, some properties of this glucosylated flavonoid are the inhibition of the initial formation of free radicals and the scavenging of intermediates that occur in the skin aging process (Anonymous 2006). Also, the use of α-glucosylrutin in cosmetics has been proposed due to the protective role this compound has against AA oxidation, which can occur during storage of cosmetics and dermatological preparations (Schonrock and Kruse 2004).

Even though there is no information related to the use of α-glucosylrutin in food products, there is evidence related to rutin, which suggests that this flavonoid can exert an ascorbate protective role. In fact, in a model system composed of AA and ascorbate oxidase, rutin was the compound which best extended the ascorbate radical lifetime, after Pycnogenol and an extract of green tea (Cossins and others 1998)

Pycnogenol

Pycnogenol is a tradename for an extract of pine bark developed originally by the French scientist, Jack Masquelier (Masquelier 1987). This substance is a blend of procyanidins extracted from the bark of the French maritime pine, *Pinus maritime*. The components of this blend have not been completely characterized; however, it is known that phenolic compounds divided into monomers (catechin, epicatechin and taxifolin) and condensed flavonoids (classified as procyanidins/ proanthocyanidins) are the main components. Also, in lower amounts, pine bark extract contains phenolic acids (for example, caffeic, ferulic and p-hydroxybenzoic acids) and glycosylation products (Packer and others 1999).

Studies have shown that Pycnogenol has strong free radical-scavenging activity against reactive oxygen and nitrogen species. In fact, in comparison with flavonoids, the antioxidant activities are superior, due to the higher number of target sites for free radicals in the oligomeric compounds (Rohdewald 2002). Pycnogenol has been shown to have better AA protecting properties than other flavonoids such as quercetin, rutin and kaempferol. Also, it was demonstrated that Pycnogenol is able to regenerate ascorbate radical from DHAA in model systems (Cossins and others 1998).

In food systems, studies have shown the antioxidant and antimicrobial properties of Pycnogenol (Ahn and others 2006; Ruggeri and others 2008; Torras and others 2005). For example, in a study of lipid oxidation in cooked meat, it was demonstrated that Pycnogenol reduced the amount of *E. coli* 0157:H7 and *Salmonella typhimuriun*, and slowed the growth of *Listeria monocytogenes* and *Aeromonas hydrophila*. Pycnogenol helped retain the red color in cooked beef during storage. Also, this mixture of phenolic compounds reduced the production of hexanal and thiobarbituric acid-reactive substances in the cooked meat sample (Ahn and others 2006). One study in yogurt demonstrated the stability of Pycnogenol during storage in this acidic product (Ruggeri and others 2008). Even though the ascorbate protective role of Pycnogenol was noted more than one decade ago (Cossins and others 1998), Pycnogenol has not been evaluated as a potential additive to avoid ascorbic acid degradation in food products.

Sulfites

Sulfites are the compounds with the longest history of use for the prevention of enzymatic and non-enzymatic browning (Roig and others 1996). It is known that browning due to anaerobic and aerobic degradation of AA is inhibited by sulfite species (Wedzicha 1984). Under anaerobic environments, the reactions involved in the inhibition of maillard and AA browning by sulfite agents are similar. In both systems, 3-deoxyosuloses seems to participate in the inhibition. In reference to AA browning, studies have shown that sulfites do not react sufficiently with reactants, and the loss of sulfites seems to be consequence of the reaction with an AA degradation intermediate called 3,4-dideoxypentosulos-3-ene. The product formed after this reaction has been identified as 3,4-dideoxy-4-sulphopentosulose (Wedzicha 1984). In more recent studies, results suggest that sulfite is able to react directly with AA; in fact, there is

evidence that infers that sulfites are able to catalyse the hydrolysis of the AA lactone ring (Davies and Wedzicha 1992).

Under aerobic conditions, it is believed that sulfites can inhibit the degradation of AA by two mechanisms: (1) inhibition of autoxidation of AA and (2) prevention of browning due to DHAA acid and other carbonyl degradation products (Wedzicha 1984). In reference to the first mechanism, there are results that show that, in mixtures of AA and sulfites, AA increased the rate of sulfite loss. The results in this study infer that semidehydroascorbic acid is reduced by the sulphite ion (Davies and Wedzicha 1992). In the second type of mechanism, DHAA reacts with sulfite species forming hydroxysulphonate. The reactivity of this new product is much lower; as a consequence, the tendency of reaction with amines and the development of red colors are decreased. There is no evidence that shows that the formation of hydroxysulphonate is irreversible (Wedzicha 1984).

Research conducted in food systems has proved the effectiveness of sulfites for the inhibition of AA degradation. For example, in single-strength reconstituted citrus juice in tetrabrik cartons, sodium metabisulfite decreased the development of browning, especially at 76 and 105°C, where there was a great accumulation of 5-hydroxymethylfurfuraldehyde (Roig and others 1996). Also, in aqueous model systems, the effect of sodium bisulphate was to inhibit the anaerobic degradation of AA, but only at low temperatures (24, 33 and 45°C) (Rojas and Gerschenson 1997). In addition, in a DHAA aqueous solution stored at 37°C for 28 days, it was observed that sulfites (40mM) inhibited the development of browning pigments (Sawamura and others 2000).

The application of sulfites in food products is not desired due to the fact that some people (especially sulfite-sensitive asthmatics) have negative reactions when exposed to these compounds. For this reason, there is a tendency to replace sulfites with other compounds with similar antioxidant properties. Studies conducted with sulfhydryl-containing amino acids have shown the effectiveness of these compounds against AA degradation. In fact, *in vivo,* thiol compounds work as nucleophiles and scavengers of free radicals. Among the sulfur containing amino acids, L-cysteine, N-acetyl-L-cysteine and gluthanione have been the most studied. In

orange juice stored at 45°C for 7 and 14 days, it was reported that levels of L-cysteine and N-acetyl-L-cysteine at 2.5 mM or lower reduced browning and 5-hydroxymethylfurfural formation (Naim and others 1993). Similar results were obtained in another study of fortified orange juice, where glutathione, L-cysteine and N-acetyl L-cysteine at concentrations below 4.0 mM inhibited AA degradation during pasteurization. Also, in this same study, the same thiol compounds reduced the production of p-vinylguaiacol and browning during 25°C storage for 12 weeks (Naim and others 1997).

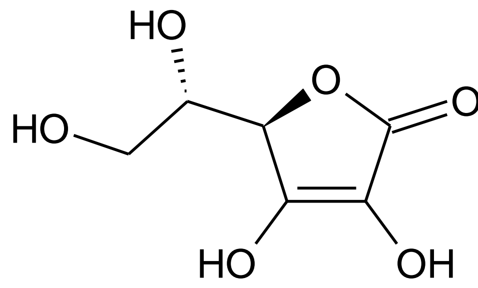

Fig. 1: L-ascorbic acid

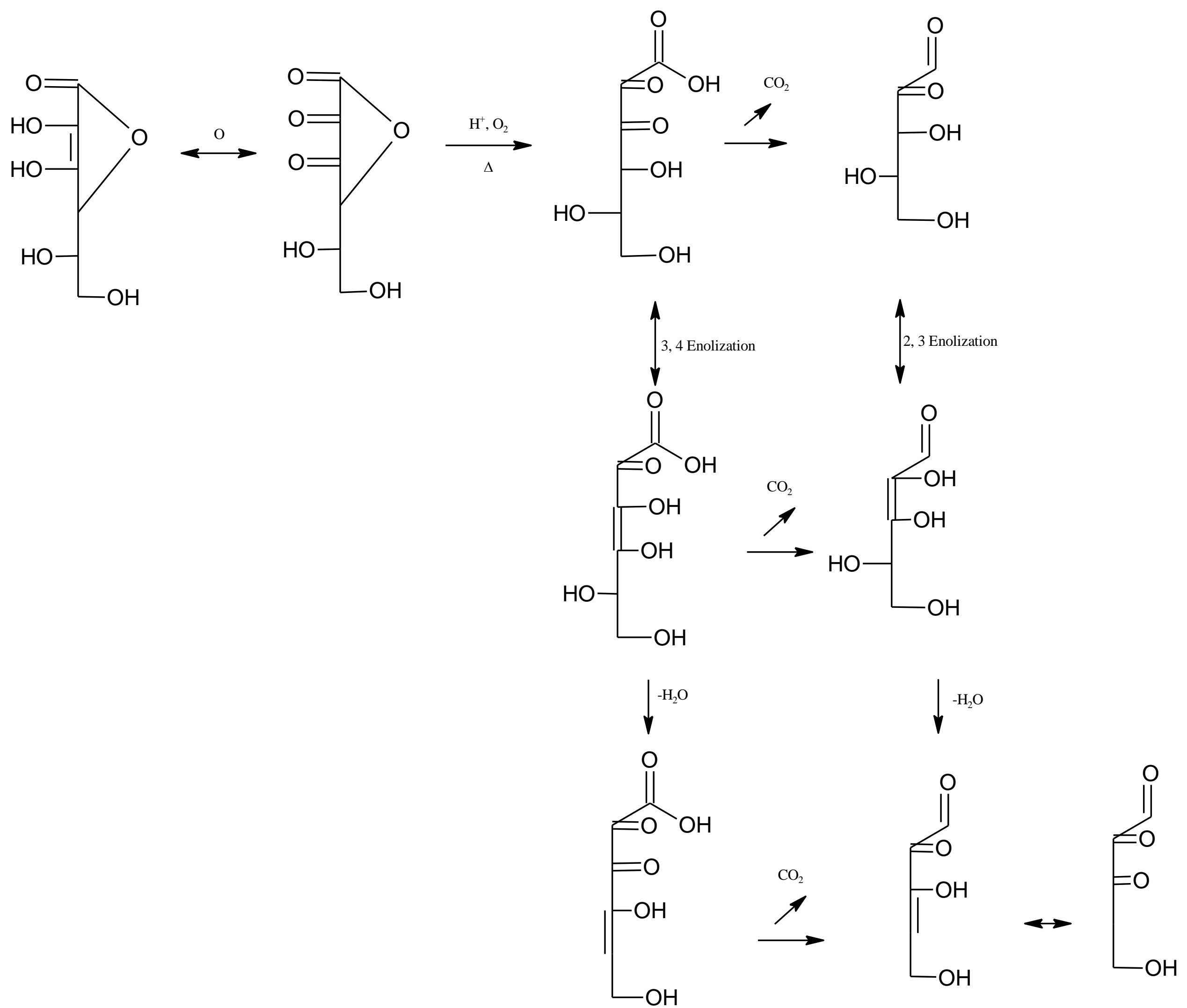

Fig. 2: Aerobic degradation of L-ascorbic acid

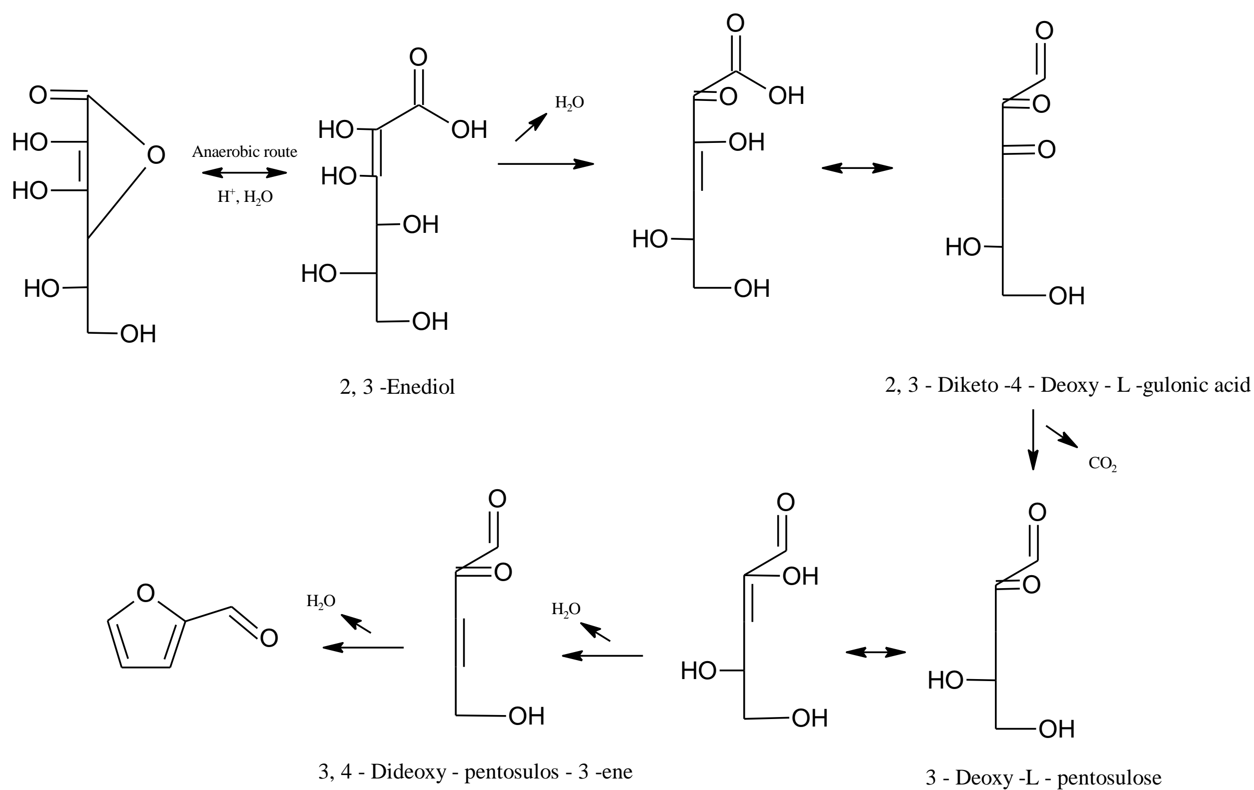

Fig. 3: Anaerobic degradation of L-ascorbic acid

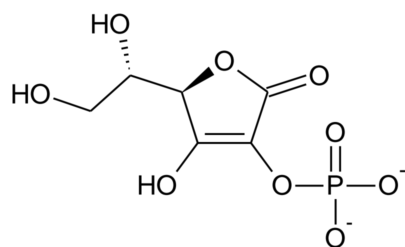

Fig. 4: L-ascorbyl-2-phosphate

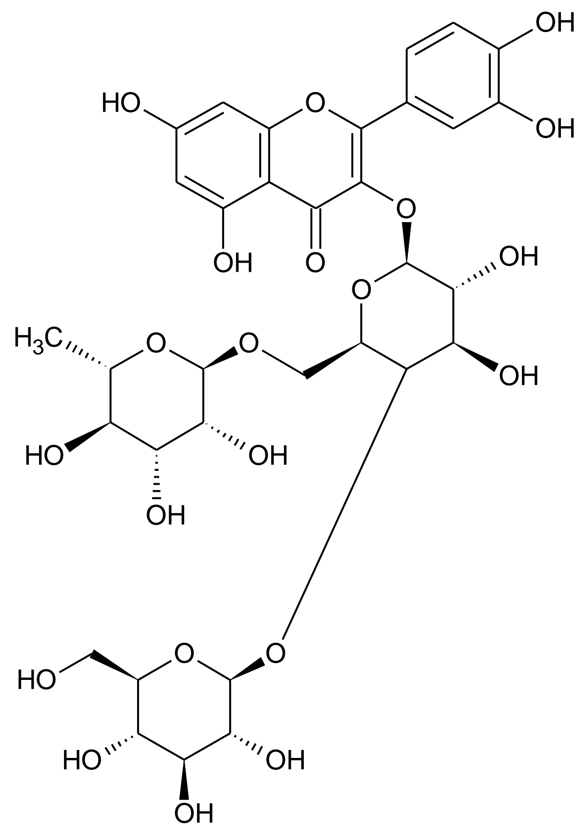

Fig. 5: Chemical structure of α-Glucosylrutin

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CHAPTER 3: KINETICS OF NONENZYMATIC BROWNING IN PEACH PUREE DURING STORAGE

ABSTRACT:

Ascorbic acid (AA) is often added to foods for its vitamin activity as well as antioxidant and reducing effects. The effects of ascorbic acid and ascorbyl-2-phosphate (A2P) on non-enzymatic browning were studied in a peach puree model system. Samples were stored at 40°C, 50°C and 60°C for 29 days under darkness. Dissolved oxygen, vitamin C concentrations and color (CIE Lab) were measured at different times during storage. Kinetic models were applied to the vitamin C content and color parameter (L*, a*, b* and ΔE*) data. Dissolved oxygen in both AA and A2P samples was consumed immediately. Kinetic models could not be applied successfully for all color parameters at all temperatures due to data variability. The Weibull model fit the changes in vitamin C better than zero, first or second order models. At all temperatures, the rate constants of vitamin C degradation were higher in the AA containing samples than in A2P. A2P was hydrolyzed during storage to AA.

KEYWORDS:

Peaches, non-enzymatic browning, ascorbic acid, ascorbyl-2-phosphate, color, kinetics

INTRODUCTION

Ascorbic acid (AA) is often used in food processing due to its vitamin activity and chemical properties (reducing agent, antioxidant). However, due to its low stability, ascorbic acid degradation is common in many foods. AA degradation involves not only loss of nutrients, but also the appearance of undesirable characteristics, such as non-enzymatic browning. Research on non-enzymatic browning has been conducted on many different food systems (Garza and others 1999; Burdurlu and others 2006; Chutintrasri and Noomhorm 2007; Roig and others 2000; Lozano and Ibarz 1996). Different methods to minimize or retard the formation of browning pigments due to non-enzymatic reactions have been studied. Some of these techniques involve the use of chemicals such as thiol compounds, sulfites and maltitol. Physical methods such as modified-atmosphere packaging have been examined as well as rapid thermal processing such as microwave heating, ultrasound-assisting thermal processing and pulsed electric field processing for the stabilization of ascorbic acids in foods (Hui 2006).

The use of other forms of AA that have substituted groups at carbons 2 and 3 has been proposed as replacement for AA, due to the improved stability of substituted AA forms (Iacobucci and Sweeny 1980). One of the ascorbic acid derivatives that has been shown to have promising results is ascorbyl-2-phosphate (A2P) (O'Keefe 2001). For example, ascorbyl-2-monophosphate and ascorbyl-2-polyphosphate had better stability than AA in carbonated beverages at 15°C, 25°C and 35°C (Wang and others 1992). In mashed potatoes after processing and storage, the losses of AA and A2P were 50% and 20%, respectively (Wang and others 1995). In addition, higher stability of A2P has been shown in cosmetics and aquatic feeds (O'Keefe 2001; Austria and others 1997). Furthermore, animal studies have shown that A2P and AA have the same bioavailability (O'Keefe 2001). Even though A2P still does not have approval for use in foods, additional study is warranted because of its reported higher stability in different food systems.

A good approach to study quality aspects of food, such as those involving non-enzymatic browning, is through kinetic models (van Boekel 2008). In fact, kinetic models in food systems can allow predictions of chemical changes during both processing and storage (Van Boekel 1996). Many studies have examined kinetics of non-enzymatic browning during heating and

storage. Simple kinetic models such as zero and first order have been applied in the changes of color parameters (L*, a* and b*) as well as in the AA contents (Chutintrasri and Noomhorm 2007; Burdurlu and others 2006; Garza and others 1999; Buedo and others 2000; Roig and others 2000). On the other hand, other research points out that these simple kinetic models do not successfully explain reactions involving non-enzymatic browning (Garza and others 1999; Manso and others 2001).

Equations such as the combined and Weibull models have been recommended for application to analysis of non-enzymatic browning kinetics in foods. The combined model is based on the theory that during non-enzymatic browning, there are two stages associated with color changes. The first stage is a color formation which follows a zero order kinetic model (reaction 1) and the second stage is a color degradation which follows a first order kinetic model (reaction 2) (Ibarz and others 1999). Even though reaction 1 is intended to describe color changes due to the maillard reaction, it is possible that that color variations due to AA degradation and browning can also be explained by using the same equation. In fact, it is known that some intermediates of AA degradation enter the maillard reaction (Wong 1989).

$$Sugars + Amino\ acids \xrightarrow{k_0} Colored\ polymers \qquad \text{(Reaction 1)}$$

$$Pigments \xrightarrow{k_1} Non\ colored\ products \qquad \text{(Reaction 2)}$$

The Weibull model reportedly fits AA degradation better than zero, first or second order models (Oms-Oliu and others 2009; Manso and others 2001). The Weibull model considers a scale parameter (α) as a time constant (inverse of the rate constant), and a shape parameter (β) as a behavior index (Hui 2005). Due to the use of α and β, this model is extremely flexible (Manso and others 2001). No studies have examined these various models in modeling the color and ascorbic acid degradation in a peach puree model system.

The objective of this research was to study the stability of A2P during storage at 40°C, 50°C and 60°C in a peach puree model system. Ascorbic acid, A2P contents and color parameters (CIE Lab) were measured at different periods of time during 29 days of storage.

Different models were applied to evaluate accuracy of kinetic parameters. At the same time, we prepared model systems to compare stability of A2P and AA.

MATERIALS AND METHODS:

Peaches of the flavorich variety were obtained from the Pearson farm (Georgia, USA) in the summer of 2010. Flavorich is a popular, early, clingstone variety grown widely in the southeastern United States. The peaches were washed with tap water, peeled and blended for 40 seconds. Three separate batches of peach puree were prepared. Each of the batches corresponded to a different treatment. The first treatment did not have any form of additional vitamin C, while the second and third treatment had 3.98 μmol/g of AA or A2P. L-ascorbic acid (99%) and ascorbyl-2-phosphate, trisodium salt, were obtained from Fisher Scientific (Pittsburgh, PA) and Sigma-Aldrich (St. Louis, MO), respectively. Both vitamin C forms were first dissolved in 3 milliliters of distilled water, and then they were added to one 200g batch of puree. The batches were heated at 92°C for 1 min, and the puree of each treatment was placed into vials of 12 ml capacity and filled to the very top leaving no headspace. Each vial had an O_2xyDot® (©OxySense, Inc., Dallas, Texas) located on the wall of the top ¼ part of the vial in order to measure the dissolved oxygen changes during storage. The vials were sealed with Teflon-lined silicon septa to prevent evaporation or oxygen ingress. The samples were stored in darkness at three different temperatures: 40°C, 50°C and 60°C for 29 days. The analyses were conducted at days 0, 10, 20 and 29. Three repetitions were prepared for each temperature and treatment.

Dissolved oxygen determination

An OxySense ® 4000B (©OxySense, Inc., Dallas, Texas) non-invasive oxygen analyzer system was used. All oxygen readings were obtained after the calibration of the equipment with a vial containing 1% of sodium sulfite solution (0% oxygen), and a vial containing some drops of water (21% oxygen). The readings were obtained as percentage oxygen.

Ascorbic acid determination

A high performance liquid chromatography (HPLC) procedure based on an earlier report (Gokmen and others 2000) was used to quantify ascorbic acid and ascorbic acid-2-phosphate in

peach samples. Peach puree was combined with a solution of methaphosphoric acid (5%) in a ratio of 3:1 (product:methaposphoric acid solution). This mixture was homogenized for 1.5 minutes using a Virtis homogenizer-Model TP 18/10 (Wilmington, NC) at medium speed. The homogenized samples were filtered through Whatman No. 4 (Maidstone, UK) and 0.45 μm filters (Millipore, Massachusetts, USA). The samples were protected from light during filtration. The clarified samples were analyzed by high performance liquid chromatography (HPLC). A model 1200 HPLC consisting of degasser, pump, refrigerated autosampler, column oven and diode array ultraviolet-visible (UV-VIS) detector was obtained from Agilent (Santa Clara CA). The mobile phase was pH 2.4 20mM aqueous phosphate buffer. The column (Luna 5μ C18(2) 100Å, 250mm x 4.60mm, i.d.) was obtained from Phenomenex (Torrance CA) and was used at a flow rate was 0.5 ml/min. The detector was set to 245nm and UV spectra from 190-300 NM were recorded.

Colorimetric Analysis

A Chroma Meter CR-200 tristimulus color analyzer (Minolta, Osaka, Japan) was calibrated with a standard plate (L*: 52.06, a*: 42.13, b*: 19.38) before use. The color of the vials containing peach puree was obtained after the colorimeter measuring head containing the vial was covered with a cardboard box to avoid stray light effects. All readings were obtained in the CIELAB scale (L*, a*, b*). The ΔE* was used to quantify the variation of color throughout the storage; ΔE* is defined by the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*) + (\Delta a^*) + (\Delta b^*)}$$

pH and °Brix

The pH and °Brix were measured directly from the puree after opening the vials. The pH and °Brix were measured using a potentiometer XL-20 (Fisher Scientific) and an Abbe Mark II refractometer (Reichertai Inc,. Buffalo, NY), respectively.

Statistical analysis

The kinetic parameters were obtained by a two-step regression method (Arabshahi and Lund 1985). In the first and second step regressions, rate constants (*k*) and the energy of

activations (E_a) were calculated, respectively. The color parameters were evaluated through equations (1), (2), (3) and (4). In the case of ΔE*, instead of equation (4), we applied equation (5) because in this parameter, ΔE* at day 0 is zero (Ibarz and others 1999). For the *k* values of the vitamin C degradation, the data collected were fitted in the equations (1), (2), (3) and (6). The best kinetic model was chosen after comparison of the coefficient of determination (R^2), root mean square error (RMSE) and visual examination of distribution of residuals and plot of the data. The E_a was obtained from a second regression using the Arrhenius model showed in equation (7).

Zero order (eq. 1):

$$C = C_0 + k_0 t$$

First order (eq. 2):

$$C = C_0 \exp{(k_1 t)}$$

Second order (eq. 3):

$$c = \frac{1}{\left(\frac{1}{c_0}\right) + (k_2 t)}$$

Combined kinetics model (eq. 4):

$$C = \frac{k_0}{k_1} - (k_0 - C_0) \exp{(-k_1\, t)}$$

Combined kinetics model in ΔE* (eq. 5):

-

$$\Delta E = \frac{k_0}{k_1} (1 - \exp(-k_1\, t))$$

Weibull model (eq. 6):

$$C = C_0 \exp\ (-k_1 t)^{\beta}$$

Arrhenius equation (eq. 7):

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right)$$

Where:

C = Variable content studied at time t

C_0 = Variable content at time zero

k_0 = Zero order kinetic constant

k_1 = First order kinetic constant

k_2 = Second order kinetic constant

β = Shape parameter

t = time

T = Temperature (°Kelvin)

E_a = Energy of activation

R = Universal gas constant (8.314J/molK)

The fitting process for the equations (1), (2), (3) and (7) were done by linear regression in JMP 8.0 (SAS Institute, Cary, NC). Equations (4), (5) and (6) were solved by non linear techniques using Statgraphics Plus v. 5.1 (Statgraphics, Warrenton, VA).

A randomized block design was used to determine if there were significant differences among the treatments at different days. Correlation coefficients were determined between the type of ascorbic acid and each of the color parameters. Both of these procedures were solved using JMP 8.0 (SAS Institute). Tukey´s HSD was used to evaluate the differences among each treatment.

RESULTS AND DISCUSSION

Changes in dissolved oxygen, pH and •Brix during storage

The amount of dissolved oxygen at the beginning of the storage in the control and the ascorbic acid samples (AA and A2P) were 8% and 0%, respectively (Figs. 1). These differences are explained by the presence of AA. It is known that in fortified canned or bottled fruits, the

oxygen in the headspace is reduced to water due to the oxidation of AA (Liao and Seib 1988). As a consequence, we are assured that the degradation pathways in the AA and A2P treatments were entirely anaerobic. It is important to note that all vials were stored for 1 to 3 days at 4°C before time zero. Consequently, we can observe that the depletion of oxygen occurred within the 24 hours after storage under refrigeration in the vitamin C fortified samples. In a model systems prepared with glucose and AA in water, the total consumption of oxygen was after 15 hours of storage at high temperatures (70-90°C) while consumption took 25 days at lower storage temperatures (24-45°C) (Rojas and Gerschenson 1997). In orange juice packaged in Tetrabrick cartons stored at 4°C, after 60 days there was still around 1.5ppm of dissolved oxygen (at time zero there was around 4.5 ppm) (Roig and others 2000). The oxygen consumption due to the AA is catalyzed by metal ions (Fennema 1996; Liao and Seib 1988); consequently, differences among different food model systems are expected.

Table 1 shows the pH and °Brix values at the beginning and end of storage for each temperature. The pH values were always between 3 and 4 in all treatments. This range reportedly enhances nonenzymatic browning under anaerobic conditions (Fennema 1996). It is also apparent from Table 1 that the pH changes were minor at the end of storage (29 days), and not of chemical significance. The pH may change during nonenzymatic browning due to the appearance of carbonyl compounds (Roig and others 2000). In orange juice packaged in Tetrabrick cartons stored at 4°C, 20°C and 37°C for 64 days, 76°C for 9 days, and 105°C for 3 days, a pH of 3.65 was maintained (Roig and others 2000); the °Brix was also constant during storage. No variation in °Brix was observed in cashew apple (pH of 4.4) during thermal treatment at temperatures from 88 to 121°C (Damasceno and others 2007). These authors reported that the steady concentration of soluble solids, reducing and total sugars demonstrated that there was no participation of sugars in the non-enzymatic browning processes (Damasceno and others 2007). As we know, reaction of reducing sugars and amino acids (maillard reactions) is a type of non-enzymatic browning favored by a more alkaline pH (Fennema 1996).

Kinetics of color parameters

The color parameters from the CIELAB system (L*, a*, b*) and hunter Lab have been used as indicators of non-enzymatic browning in many studies (Garza and others 1999; Lozano

and Ibarz 1996; Ibarz and others 1999). These two systems are based on the same theory, and their parameters are interpreted equally (HunterLab 2008). For this reason, even though we calculated color using the CIELAB system, we will compare our data with previous research which used either scale.

In both systems, CIELAB and hunter scale, the L*, a* and b* parameters indicate how dark/light, red/green and yellow/blue the samples are, respectively (HunterLab 2008). These values, as well as the size of color difference (ΔE*), were measured and calculated throughout storage. The kinetic models which are shown in Tables 2, 3 and 4 correspond to those which were successfully applied for our data.

*Color parameter L**

Even though no significant differences at day 0 ($p>0.05$) were found, we can observe in Figs. 4-6 that the AA treatment had a consistent lighter color than the other treatments. This could be explained by the bleaching action of the hydrogen peroxide produced in the oxidation of AA (Poei-Langston and Wrolstad 1981). Research done in model systems containing anthocyanins has shown that at day 0, the L* value was already higher in those samples that did contain AA (Poei-Langston and Wrolstad 1981). Similar results were obtained when the effect of AA was evaluated in jellies (Freedman and Francis 1984). Since at day 0 the AA treatment did not have dissolved oxygen but the control treatment did, we believe that there is a high possibility of presence of hydrogen peroxide. The L* values of the control and A2P treatments were much closer.

At 40°C (Fig. 4), the L* value in the control treatment increased slightly until day 20 and decreased by day 30. On the other hand, for the treatments which had AA and A2P, the variations were more defined; in fact, the L* values increased until day 10, and then they were maintained relatively stable after that. Significant differences ($p<0.05$) were observed between control and AA treatment at day 10; significant differences ($p<0.05$) were also seen between control and the AA and A2P treatments at day 30. At 50°C (Fig. 5) in the control treatment, the maximum L* value was seen at day 20 while the maximum was seen at day 10 for AA and A2P treatments. The AA treatment was significant different ($p<0.05$) at day 20. Finally, at 60°C

(Fig. 6), all treatments had a parabolic tendency, and the maximum was seen at day 10. At 60 °C, the highest value was seen in the treatment with AA. Significant differences ($p<0.05$) were found at day 10 between the control sample and the other treatments.

Studies conducted on non-enzymatic browning in peaches have identified a tendency of L* value to decrease during heating and storage due to the darkening process (Garza and others 1999; Lozano and Ibarz 1996; Ibarz and others 1999; Chutintrasri and Noomhorm 2007). The different tendency that we observed can be explained by the presence of anthocyanins in our peach puree. We used Flavorich peaches, which are a hybrid of a Friar plum and an unnamed Pluot® (hybrid of apricot and plum) (Dave Wilson nursery, 2010), so our puree sample likely contained cyanidin and peonidin (anthocyanins). In fact, the red color in plums is due to cyanidin and peonidin (Ahmed and others 2004). The color changes of our peach samples are more complex than peaches that do not contain anthocyanins. For example, in reference to the lightness, it has been reported that anthocyanin pigments can react with AA producing color loss, and consequently, higher values of L* (Poei-Langston and Wrolstad 1981). These reactions would explain the increases in L* values at the early stages of storage. The lower values obtained at the end of the storage are explained by the presence of brown polymers produced in non-enzymatic browning (Ibarz and others 1999). In strawberry, blackberry and apple jellies, increases then decreases of L* values have been reported (Freedman and Francis 1984).

As a result of the increases and then decreases of L* values during storage, none of the treatments fitted either the zero, first, second and combined order kinetic models. First order kinetic models have been successfully applied in L* data during heating at high temperatures (80-98°C) in peach (Ibarz and others 1999) and pear puree (Garza and others 1999). The same model was applied for heating of apple, peach and plum pulp at 56-94°C (Lozano and Ibarz 1996), and for heating of pineapple puree at 70-110°C. In fact, during heating at 56°C and at 122.5°C, a k of 16.34×10^{-5}/min (Lozano and Ibarz 1996) and of 2.9×10^{-3}/min (Avila and Silva 1999) were obtained in peach puree. In pineapple juice, data obtained during heating at 55°C-95°C did not fit either zero, first or combined models (Rattanathanalerk and others 2005).

The variable tendency of the L* value during storage is the reason also of the low *r* obtained in reference to degradation of vitamin C (Table 5). Consequently, we do not consider that L* value is a good indicator of the degradation of ascorbic acid in Flavorich peach puree systems.

*Color parameter a**

The a* values had no significant treatment effects at day 0 ($p>0.05$). Consequently, both AA and A2P did not affect the natural reddish values at the beginning of the storage. In AA-anthocyanin model systems, at day 0 the samples with AA had lower hunter a values than samples with no AA (Poei-Langston and Wrolstad 1981).

At 40°C, the a* values in all treatments decreased throughout storage. The AA treatment reached the lowest values, followed by the A2P treatment (Fig. 7). The rate of decrease was faster during the first 10 days of storage. There were significant differences ($p<0.05$) among all treatments at day 10, and between control and the rest at days 20 and 30. At 50°C, (Fig. 8) the same tendencies were also seen for the control and A2P samples; however, the values reached were lower than observed at 40°C. The a* values of AA treatment slightly increased after 20 days of storage. Significant differences were reported at day 10 ($p<0.05$) between the control and the other treatments. At 60°C (Fig. 9), all treatments showed a parabolic behavior. As observed with the L* value, the lowest values were seen at day 10 for the AA and A2P treatments, but at day 20 for the control. At day 10, each sample was different from the others ($p<0.05$).

In summary, the decrease in a* seen at 40°C and 50°C indicates decrease in the redness of the samples. In contrast, the increase in the a* values observed in the samples stored at 60°C at 20 days corresponded to a more reddish color. It has been explained that in anthocyanin-AA model systems, a reduction in a* value during the first weeks of storage at 20°C indicates loss of redness, which is explained by the loss of anthocyanin pigments (Poei-Langston and Wrolstad 1981). It has been observed that at acidic conditions and at room temperature, anthocyanins are discolored (Lozano and Ibarz 1996). This explains the a* decrease in our results. The increase in a* value observed at 60°C in all samples and at 50°C in the AA treatment are explained by the

presence of brown polymers due to the nonenzymatic browning reactions. Research conducted in peach, pineapple and pear purees, showed that the tendency of a* values is to increase (Avila and Silva 1999; Chutintrasri and Noomhorm 2007; Ibarz and others 1999). Due to the yellow and white color of these samples, we believe that the brown pigments due to melanoidins are more obvious from the beginning of the heating period. Also, discoloration due to degradation of pigments must not be probable in these studies, especially since AA has a stabilizing effect on carotenoids (Steskova and others 2006).

Color data could not be fit to either zero, first or second order reaction models. After applying the combined model, only the control treatment at all temperatures of storage and the AA and A2P samples at 40°C were successfully fit (Table 4). The k_0 and k_1 are the rate constants for the color formation and color destruction of the nonenzymatic browning, respectively (Ibarz and others 1999). As explained earlier, it seems that the reactions involved in the color changes are more complex due to the probably presence of anthocyanins and other pigments, making it hard to interpret k_0 and k_1. However, the higher values of k_0 indicate that the rate constant of the reactions at the beginning of the storage were much faster. The irregular tendencies observed during storage at 50°C and 60°C (Fig. 10 and 11) resulted in data that did not fit any of models examined.

One published study reported that during heating of peach puree at 122.5°C, a* values fit a combined kinetic model (Avila and Silva 1999). In this study, the k_0 and k_1 obtained were 0.03 and 2.9×10^{-3}/min, respectively. These values are much lower than those obtained in our research (Table 4). In pineapple juice and puree, during heating at 55-95°C and 70-110°C, respectively, first and zero order reaction models were successfully applied (Rattanathanalerk and others 2005; Chutintrasri and Noomhorm 2007). The k obtained in these studies were between 0.5 to 2.4×10^{-3}/min, and 0.34 to 4.25×10^{-3}/min for the juice and puree, respectively. During heating of pear puree at 80-98°C, the a* values followed a combined kinetic model; however, the k_1 (pigment destruction rate) did not reportedly follow a clear pattern since at 80°C, the k_1 was higher than at 85°C (Ibarz and others 1999). Similar results were obtained in our study, but for both rate constants (k_0 and k_1). In fact, as we can see in Table 2, the k_0 value at 40°C in the control treatment was higher than at 50°C and 60°C; also, the k_1 obtained at 50°C was higher

than the one obtained at 60°C. These patterns prevent us from calculating the energy of activation (E_a) in the control treatment.

In Table 2 we can observe that a* value was the color parameter which best correlated with vitamin C degradation at all temperatures. This result seems to be logical due to the initial red color of the puree.

*Color parameter b**

In Figs. 10 to 12, the b* values at day 0 of the storage were similar among the three treatments ($p>0.05$). These results agree with other studies which show that fortified samples with AA did not cause any effect on b* values at the beginning of the storage (Freedman and Francis 1984).

At 40°C (Fig. 10), the control samples had a linear tendency with slope 0 during storage while the samples with A2P had a tendency to increase. The b* values of the AA treatment increased until day 10, and then decreased until the end of the storage. Significant differences ($p<0.05$) at day 10, 20 and 29 were observed between control and the AA and A2P treatments. At 50°C (Fig. 11), the AA and A2P treatments had similar behavior. They increased by day 10, and then the values were relatively stable. The control treatment at this temperature had little change during time. The only significant difference ($p<0.05$) was observed at day 10, and it was between the control and the two treatments. Finally, at 60°C (Fig. 12), the b* values increased at day 10 in both AA and A2P treatments, and then decreased. The control treatment had a slight increase at days 20 and 30 of storage. At this temperature, there were significant differences ($p<0.05$) between the control and the rest of the treatments, and between control and AA treatment at day 10 and 20, respectively. It has been discussed that anthocyanins can experience condensation reactions which produce yellow pigments (Lozano and Ibarz 1996). Also, it has been demonstrated in strawberry, blackberry and apple jelly that different levels of AA resulted in color changes towards yellow, due to a breakdown of anthocyanin pigments (Freedman and Francis 1984). This can explain the increases in b* values that were seen in our fortified samples. The subsequent decreases in b* values can be explained by the presence of brown pigments which make the samples more opaque. In more clear food products such as pear and

peach puree, tomato paste and pineapple juice, the tendency was to see a decrease in b* during storage (Garza and others 1999; Barreiro and others 1997; Rattanathanalerk and others 2005). In orange jelly, decreases in hue were reported. In systems with no anthocyaninin pigments, the color changes are simply produced by the appearance of brown polymers (Freedman and Francis 1984).

None of the studied model kinetics (zero, first, second and combined models) were successfully applied in the b*values. The same was obtained during heating of pear puree (Ibarz and others 1999). In tomato paste, pineapple juice and peach puree, first order kinetics was reported (Barreiro and others 1997; Rattanathanalerk and others 2005; Garza and others 1999).

Total color difference (ΔE)*

At 40°C (Fig. 13), the control, AA and A2P treatments had similar tendencies. The ΔE* of these samples increased until day 10, and then they were relatively stable. However, both AA and A2P fortified samples reached higher values than the control sample. There were significant differences ($p<0.05$) between the control and the other treatments at day 10, 20 and 29 of the storage. At 50°C (Fig. 14), the tendency was similar to that observed at 40°C; nevertheless, a drop in the AA treatment was observed at day 20 of storage. At this temperature at day 10, the control sample was significant different from the AA and A2P treatments ($p<0.05$). At 60°C (Fig. 15), both AA and A2P had a drop in ΔE* after 20 days of storage. There were not significant differences ($p>0.05$) during storage among treatments. Research conducted on non-enzymatic browning reported a tendency to increase during storage (Ibarz and others 1999; Garza and others 1999; Barreiro and others 1997; Rattanathanalerk and others 2005).

The Δ*E** data did not fit either zero, first or second order kinetic models. However, the control treatment at all temperatures as well as the AA and A2P at 40°C fitted in the combined model (Table 5). We did not calculate the Ea in the control treatment though we had rate constants at all temperatures. The reason of this was that the data did not follow a clear trend. In other words, as we observed for a* values, the k_0 and k_1 at 50°C were higher than at 60°C.

In Table 3, when we compare the k_0 and k_1 values among all treatments at 40°C, we can observe that the AA treatment had the highest values. The combined model has been successfully applied in many studies. For example, in the heating of pineapple juice at 55°C, k_0=28.7x10^{-3}/min and k_1=25.3x10^{-3} were reported (Rattanathanalerk and others 2005); in heating of peach puree at 122.5°C, k_0=0.0085x10^{-3}/min, k_1= 3x10^{-7}/min (Avila and Silva 1999); and in pear puree at 80°C, k_0=1.71x10^{-2}/min, k_1=1.96x10^{-3}/min (Ibarz and others 1999).

Ascorbic acid:

During storage of puree samples fortified with A2P, we observed the presence of two peaks in the chromatograms (Fig. 16), instead of the one peak which appeared in the ascorbic acid puree samples and the A2P standard curve samples. After we analyzed the spectra absorbance of both peaks, and we compared them with the spectra of AA and A2P of the samples used in the standard curve, we determined that the first and second peak were due to the presence of A2P and AA, respectively. In fact, as we can see in Fig. 17 and 18, absorbance spectra of AA and A2P have similar shapes; however, the maximum absorbances were at 242 nm and 238 nm, respectively. The reason why these two peaks appeared in the A2P puree samples since the beginning storage (day 0) must be a result of rapid hydrolysis. Indeed, it is known that acid pH can provoke hydrolysis of the ester in A2P samples (Gonçalves and others 2009).

Figs. 19 to 21 show the A2P and AA concentrations, as % of total ascorbic acid species based on calculation of concentrations using external standard calculations, during storage at all temperatures of the A2P fortified puree samples. At 40°C, the amount of A2P hydrolyzed was relatively constant. At 50°C, at day 10 and 20, the relative percentages of AA (35.3 and 36.9%) were much higher than at day 0 (15.8%), but by day 29, the percentage of AA decreased. We believe that this was due to a faster degradation of AA relative to hydrolysis of A2P. Finally, at 60°C, the hydrolysis was much faster than at lower temperatures. Fig. 21 shows that at day 10 and 20 there were more AA than A2P. By day 29, both AA and A2P were fully degraded.

In summary, the hydrolysis of A2P is greatly influenced by the temperature of storage. In food systems, research evaluating A2P has been conducted in carbonated beverages, white pan bread, bran flakes, peanut, potato flakes (Wang and others 1995; Park and others 1994), mashed

potatoes (Wang and others 1992), and in meat (Craig and others 1996; Craig and others 1991). In these systems, only the study conducted in bread has reported hydrolysis of A2P due to the presence of phosphatase in wheat flour (Park and others 1994; Wang and others 1995). In the carbonated beverage research (Wang and others 1995), no hydrolysis was reported even though the authors imply that they would be able to detect this. If we consider the low pH of the carbonated beverages, we believe that some hydrolysis must have occurred. The release of the phosphate groups can provide a secondary mode of protection in case that there is oxygen present, since these ions are able to chelate metals (Wang and others 1995). In our work, due to our anaerobic environment (Fig. 1 to 3), we do not believe that this secondary mode of protection is an advantage.

Fig. 22 to 24 shows the variation of AA and A2P throughout the storage time at 40°C, 50°C and 60°C, respectively. For the A2P samples, we considered the total vitamin C retained (both peaks in the chromatograms with concentrations calculated using separate standard curves). The variation of the control treatment was not plotted since, under the chromatographic conditions used, AA was not detected. As we expected, the different forms of vitamin C decreased during storage. Figs. 23 and 24 show that, after 29 days of storage at 50°C and 60°C, AA and A2P were completely lost. At day 20 in the samples stored at 40°C, significant differences ($p<0.05$) were observed with higher retention in A2P than AA.

As we can see in Table 4, first order kinetic models as well as the Weibull model were successfully fit to the AA/A2P data. Since the residual mean square obtained (RMSE) with the Weibull model were lower and the R^2 were higher, we chose this model for the Arrhenius analysis. In addition, as we observed in Table 4, the *k* constants obtained with the first order and Weibull model were similar, especially for the 50°C and 60°C. This is due to the fact that the Weibull model is equivalent to a first order model when the β value (shape factor) is 1 (Hui 2005). Table 4 shows that, for 50 and 60 °C, the β value was slightly higher than 1. As discussed in some studies, the AA degradation in food system has been reportedly fit to zero, first, and second orders of reaction (Roig and others 2000; Burdurlu and others 2006; Manso and others 2001; Damasceno and others 2007). The Weibull model has also been successfully applied in the thermal degradation of orange juice under aerobic conditions (Manso and others

2001), storage of fresh cut watermelon at 5-20°C (Oms-Oliu and others 2009), and during the high-intensity pulsed electric field treatment of tomato juice (Odriozola-Serrano and others 2008). We did not find kinetic studies on stability of A2P in the literature.

After we compare the *k* values of A2P and AA at all temperatures (Table 4), we can observe that the values at 60°C are practically the same. In fact, the ascorbic acid losses for A2P and AA respectively at 40°C were 41% and 92%; at 50°C, they were 90% and 95%; and at 60°C, they were 100% for both. As we have observed, the differences in stability between both types of vitamin C is more apparent at lower temperatures. In a solution of water at pH 3 stored for 1 month at 43°C, almost 30% of the total sodium ascorbyl phosphate was retained (Fermann and Gripp 2004). In carbonated beverages (Pepsi^{TM}), after 6 weeks in darkness at 35°C, 8% and almost 60% of the total A2P and AA were lost, respectively. In the same study, but in bread treatments, after 3 days of storage at 25°C, the fortified samples with either A2P or ascorbyl polyphosphate were retained at 10-15% higher levels of vitamin C than samples fortified with AA (Wang and others 1995).

Effects of temperature on the rate constant

Due to the fact that *k* values could not be determined at all temperatures in the color parameters, the effect of temperature thorough the Arrhenius equation was not evaluated. In reference to the control samples, the *k* values at the three temperatures were determined; however, as we mentioned before, they did not follow a defined tendency according to the temperatures.

The data of AA and A2P did not fit successfully in the Arrhenius equation. As we can see in Table 6, even though the R^2 were not low (0.85 and 0.95 for AA and A2P, respectively); the p-values were higher than 0.05. In a buffer solution with pH of 3.5, the temperature dependence could not be explained by the Arrhenius equation in some treatments (Assiry and others 2006). In spite of this lack of fit, we report Ea obtained in order to evaluate the differences in AA and A2P. As reported in Table 6, the Ea of A2P destruction was almost three times higher than AA. These results show that A2P concentration is more stable and also more dependent on temperature than AA. The value obtained for AA is much lower than those

reported for orange, lemon, grapefruit and tangerine juices stored at 28-45°C (Burdurlu and others 2006). As mentioned earlier, no kinetics studies have been reported in food systems using A2P.

CONCLUSION

Treatments made of peach puree fortified with ascorbic acid, ascorbyl-2-phosphate and a control were stored at 40°C, 50°C and 60°C for 29 days. The stability of the samples was evaluated through the color (CIELAB system) and vitamin C levels. Also, dissolved oxygen was measured. Constant rates were calculated and used in order to compare the different treatments. Samples with no added vitamin C showed the best stability. Kinetic models could not be applied successfully in all color parameters at all temperatures due to data variability. The unclear trends can be attributed to the content of anthocyanins in the variety of peaches that were used (Flavorich). The Weibull model best described the changes in vitamin C. At all temperatures, the rate constants of vitamin C degradation were higher in the ascorbic acid containing samples than in the ascorbyl-2-phosphate treatments. The differences in vitamin C losses between the fortified samples increased at lower temperatures.

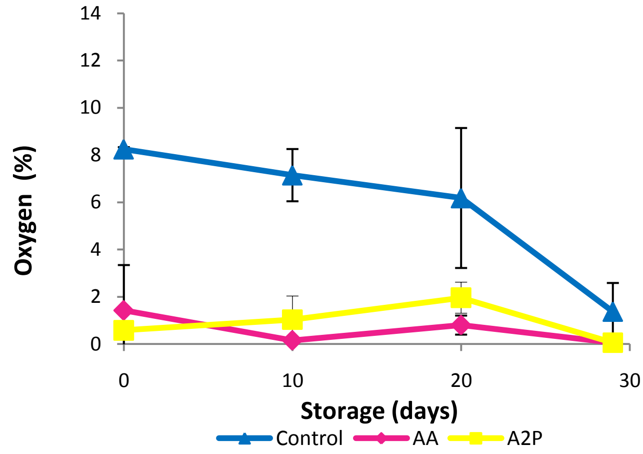

Fig. 1: Dissolved oxygen of peach purees during storage at 40°C

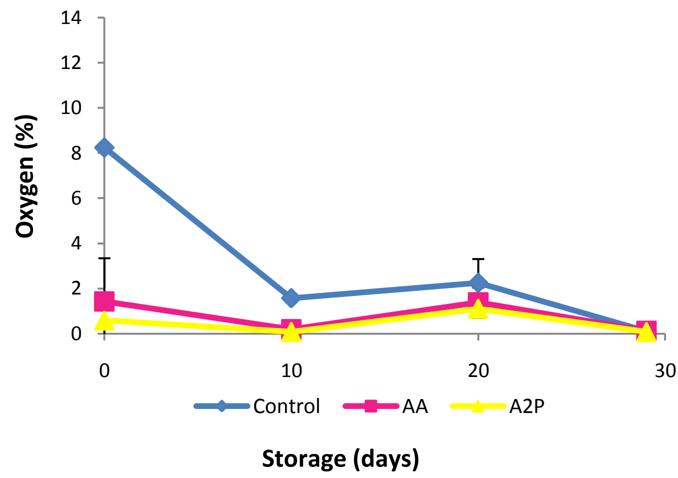

Fig. 2: Dissolved oxygen of peach purees during storage at 50°C

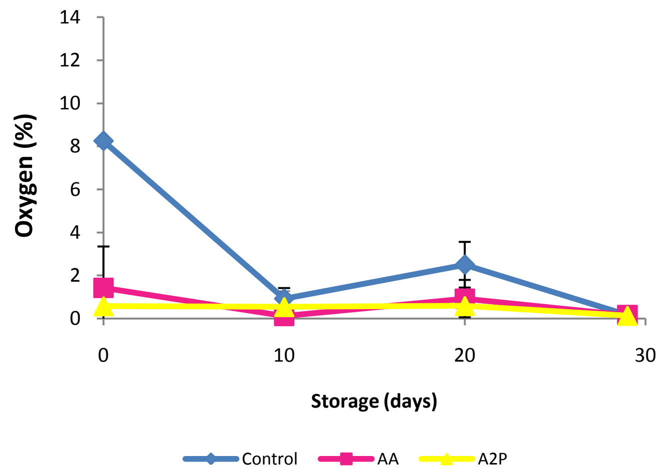

Fig. 3: Dissolved oxygen of peach purees during storage at 60°C

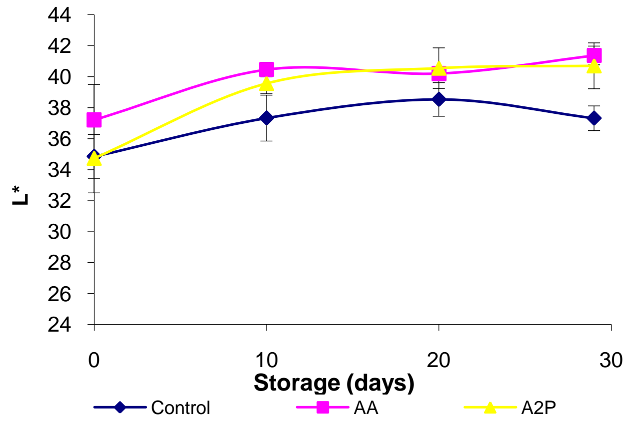

Fig. 4: Variation of the L* value during storage at 40°C

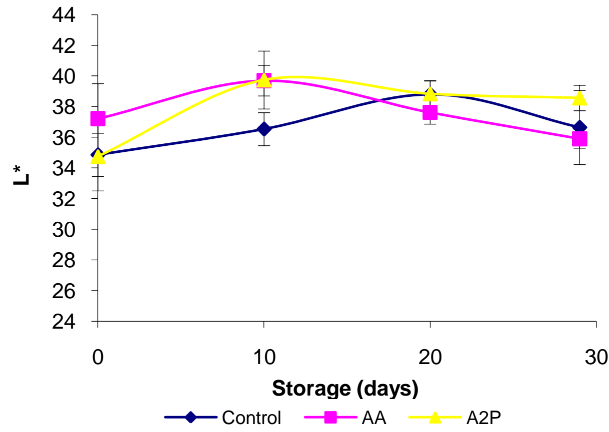

Fig. 5: Variation of the L* value during storage at 50°C

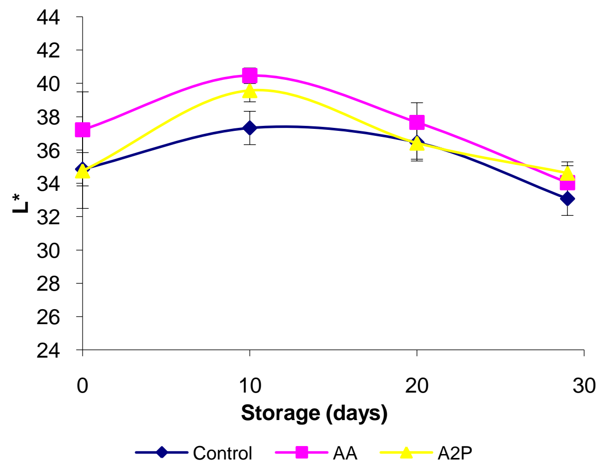

Fig. 6: Variation of the L* value during storage at 60°C

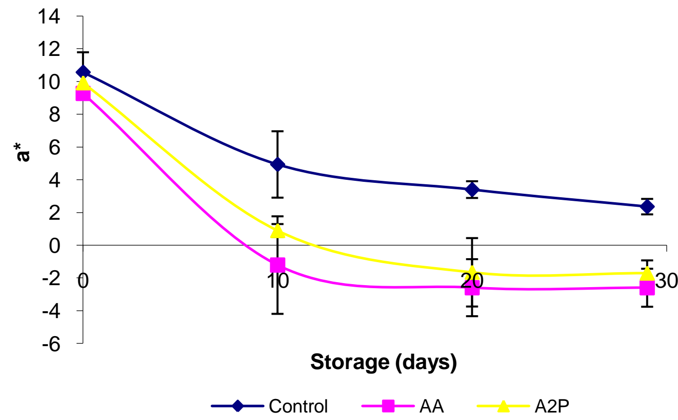

Fig. 7: Variation of the a* value during storage at 40°C

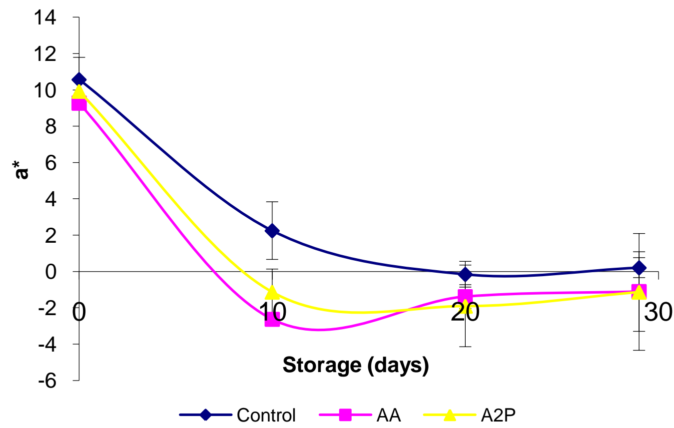

Fig. 8: Variation of the a* value during storage at 50°C

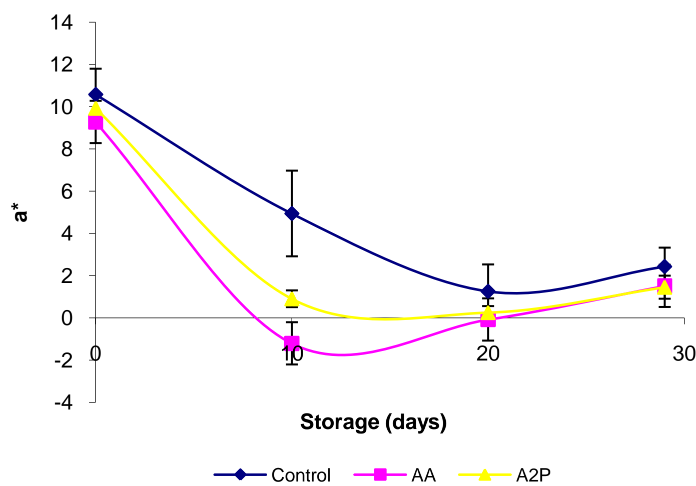

Fig. 9: Variation of the a* value during storage at 60°C

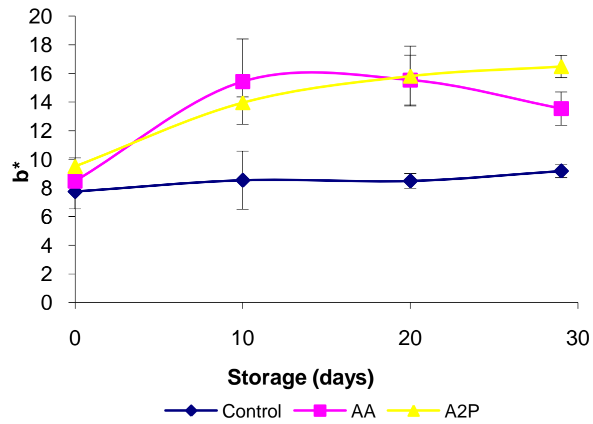

Fig. 10: Variation of the b* value during storage at 40°C

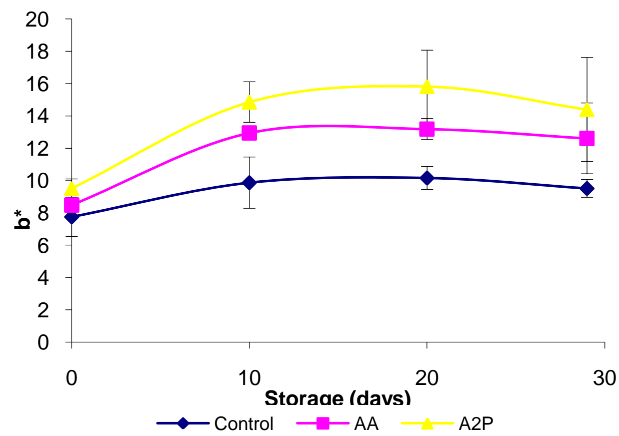

Fig. 11: Variation of the b* value during storage at 50°C

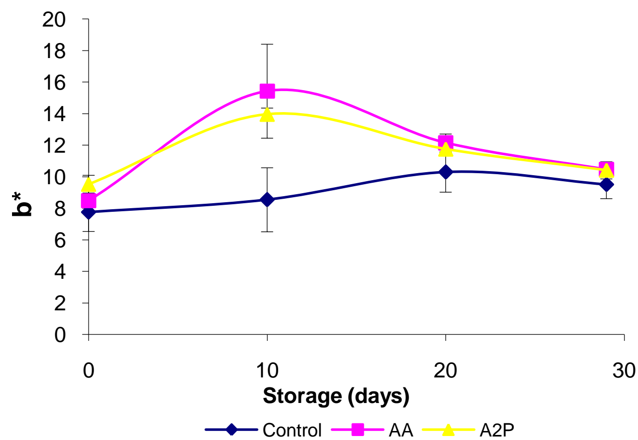

Fig. 12: Variation of the b* value during storage at 60°C

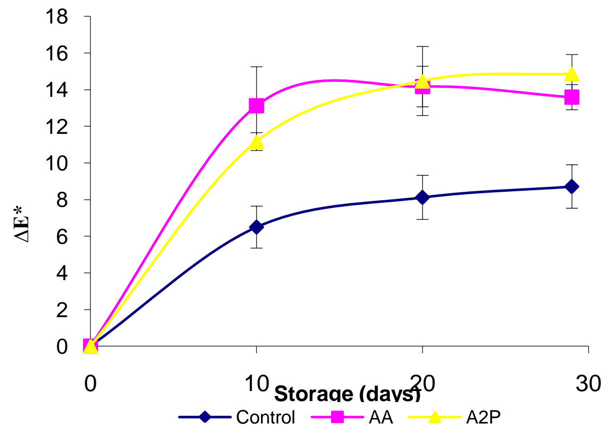

Fig. 13: Variation of ΔE* during storage at 40°C

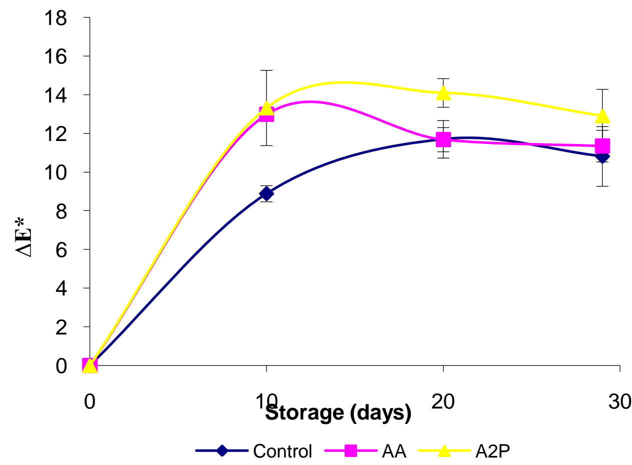

Fig. 14: Variation of ΔE* during storage at 50°C

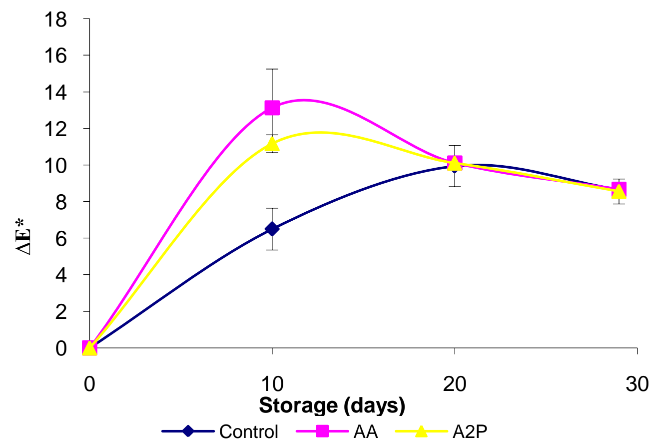

Fig. 15: Variation of ΔE* during storage at 60°C

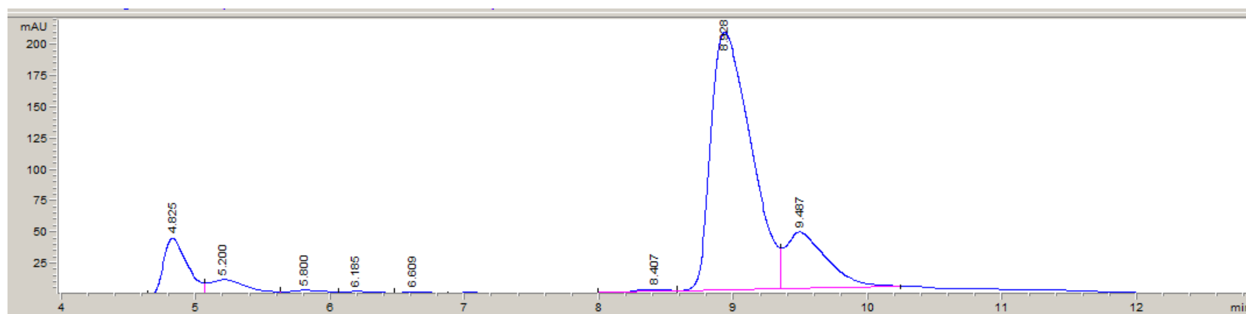

Fig. 16: Chromatogram of a peach puree sample fortified with ascorbyl 2 phosphate at day 0

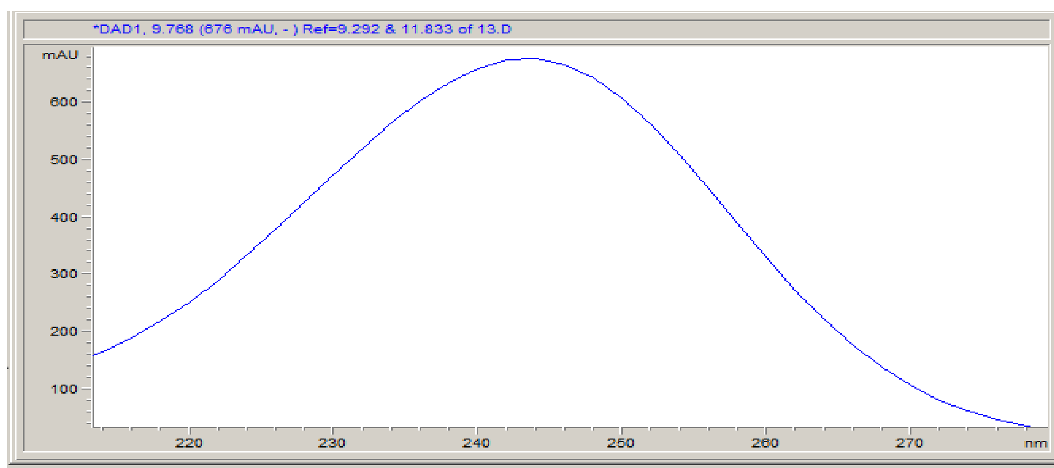

Fig. 17: Absorption spectra of ascorbic acid (0.0025M)

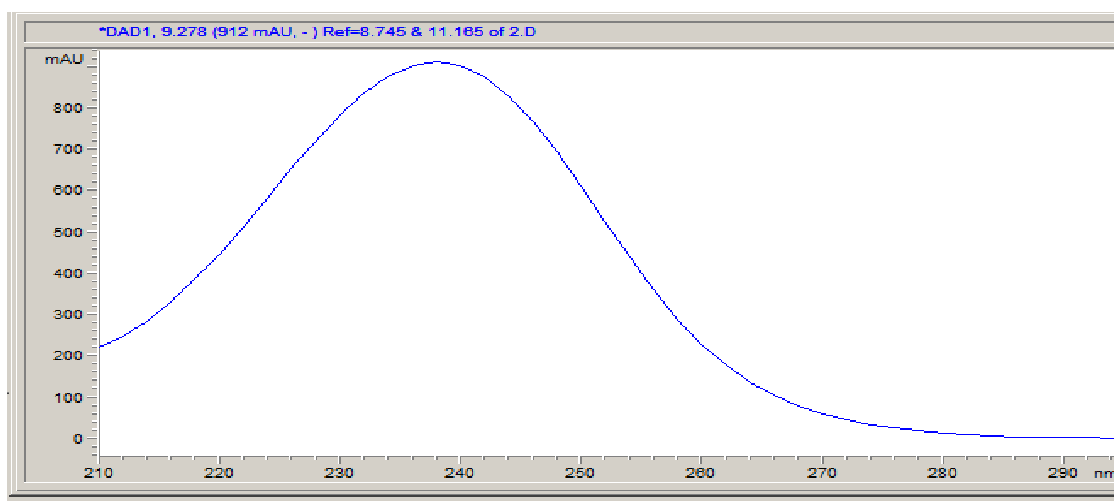

Fig. 18: Absorption spectra of ascorbyl 2 phosphate (0.0025M)

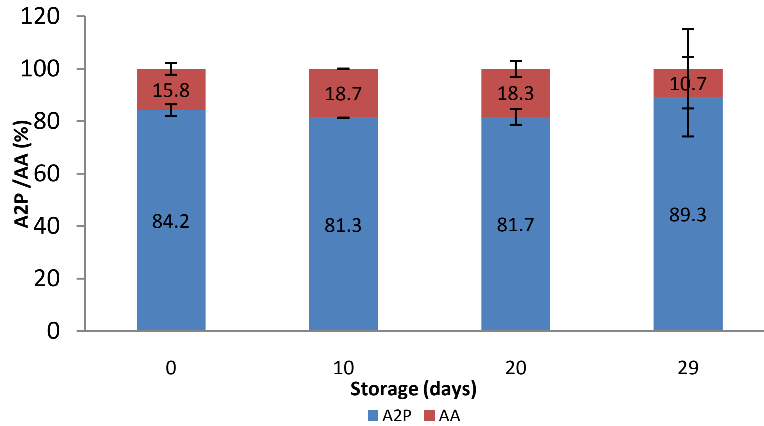

Fig. 19: Relative ascorbyl 2 phosphate and ascorbic acid concentrations (%) in the fortified ascorbyl 2 phosphate treatment during storage at 40°C

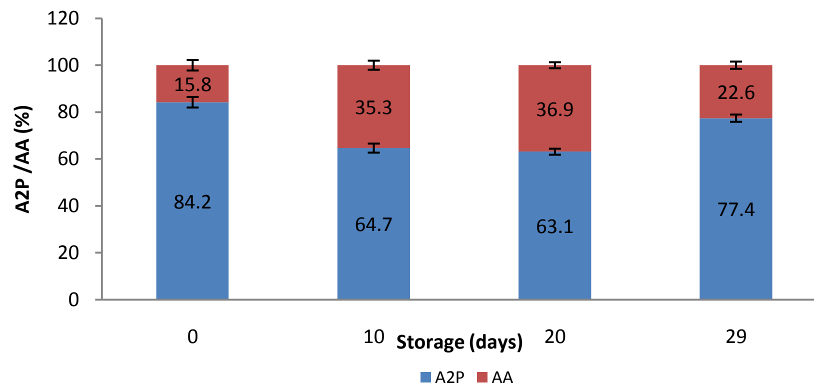

Fig. 20: Relative ascorbyl 2 phosphate and ascorbic acid concentrations (%) in the fortified ascorbyl 2 phosphate treatment during storage at 50°C

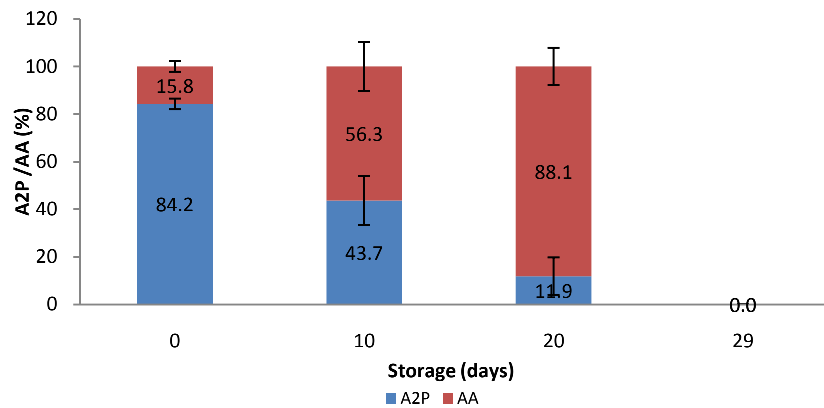

Fig. 21: relative ascorbyl 2 phosphate and ascorbic acid concentrations (%) in the fortified ascorbyl 2 phosphate treatment during storage at 60°C

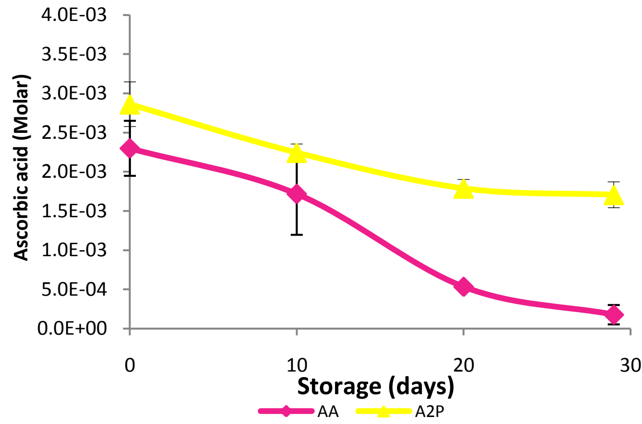

Fig. 22: Ascorbic acid concentration in peach puree during storage at 40°C

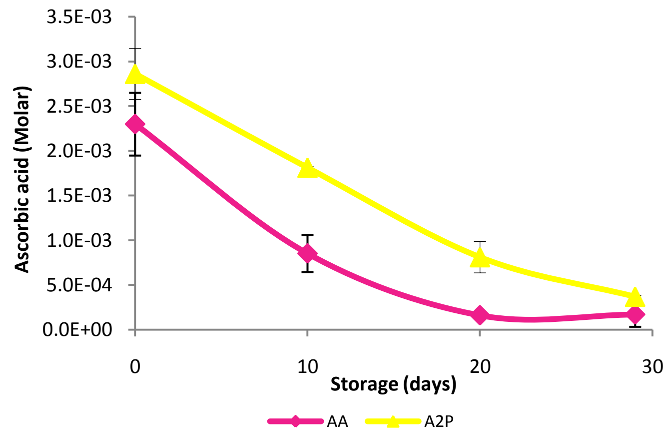

Fig.23: Ascorbic acid concentration in peach puree during storage at 50°C

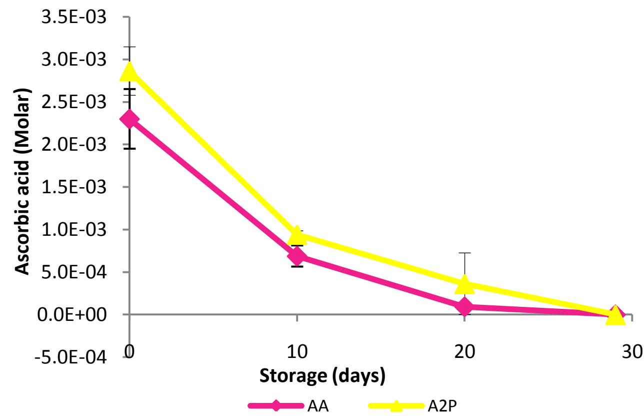

Fig.24: Ascorbic acid concentration in peach puree during storage at 60°C

Table 1: pH and °Brix values at the beginning and at the end of storage

Temperature	Treatment	pH Storage (days)		Brix Storage (days)	
		0	29	0	29
40°C	Control	3.48 ± 0.08	3.49 ± 0.01	10.4 ± 0.53	11.6 ± 0.36
	AA[1]	3.46 ± 0.02	3.42 ± 0.02	10.5 ± 0.52	10.6 ± 0.81
	A2P[2]	3.55 ± 0.01	3.65 ± 0.02	10.8 ± 0.93	11.4 ± 0.96
50°C	Control	3.48 ± 0.08	3.52 ± 0.05	10.8 ± 0.53	11.1 ± 0.66
	AA[1]	3.46 ± 0.02	3.53 ± 0.03	10.5 ± 0.52	11.1 ± 0.35
	A2P[2]	3.55 ± 0.01	3.62 ± 0.01	11.3 ± 0.93	10.9 ± 0.40
60°C	Control	3.48 ± 0.08	3.62 ± 0.04	10.4 ± 0.53	11.7 ± 1.44
	AA[1]	3.46 ± 0.02	3.52 ± 0.03	10.5 ± 0.52	11.3 ± 0.87
	A2P[2]	3.55 ± 0.01	3.64 ± 0.05	10.8 ± 0.93	10.6 ± 0.36

[1]AA, Ascorbic acid
[2]A2P, Ascorbyl 2 phosphate

Table 2: Rate constants of a* value

Treatment	Temperature (°C)	k_0 (day^{-1})	k_1 (day^{-1})	R^2	RMSE
Control	40	0.22 ± 0.12	0.78 ± 0.026	0.95	0.78
	50	-0.05 ± 0.06	0.72 ± 0.026	0.97	0.72
	60	0.15 ± 0.15	1.18 ± 0.037	0.92	1.18
AA[1]	40	-0.57 ± 0.056	0.54 ± 0.029	0.99	0.54
A2P[2]	40	-0.30 ± 0.08	1.47 ± 0.044	0.93	1.47

[1]AA, Ascorbic acid
[2]A2P, Ascorbyl 2 phosphate
R^2, Determination coefficient; RMSE, root mean square error

Table 3: Rate constants of ΔE value

Treatment	Temperature (°C)	k_0 (day^{-1})	k_1 (day^{-1})	R^2	RMSE
Control	40	1.16 ± 0.24	0.13 ± 0.03	0.94	0.92
	50	1.81 ± 0.33	0.16 ± 0.03	0.96	0.98
	60	1.22 ± 0.29	0.13 ± 0.04	0.93	1.17
AA[1]	40	4.09 ± 1.50	0.29 ± 0.11	0.96	1.14
A2P[2]	40	2.03 ± 0.26	0.13 ± 0.02	0.97	1.00

[1]AA, Ascorbic acid
[2]A2P, Ascorbyl 2 phosphate
R^2, Determination coefficient; RMSE, root mean square error

Table 4: Constants rates of the ascorbic acid and ascorbyl 2 phosphate degradation at the different studied temperatures

Type of vitamin C	Temperature (°C)	Model	*k* (day^{-1})	β	R^2	RMSE
AA	40	First-order	0.09 ± 0.01	-	0.75	$4.99x10^{-4}$
	50		0.11 ± 0.01	-	0.94	2.3610^{-4}
	60		0.16 ± 0.02	-	0.95	3.2810^{-4}
	40	Weibull	0.06 ± 0.01	2.1 ± 0.53	0.87	2.6710^{-4}
	50		0.10 ± 0.01	1.1 ± 0.4	0.95	2.3510^{-4}
	60		0.11 ± 0.01	1.4 ± 0.7	0.97	1.8510^{-4}
A2P	40	First-order	0.019 ± 0.002	-	0.87	1.8910^{-4}
	50		0.070 ± 0.005	-	0.94	2.7110^{-4}
	60		0.120 ± 0.002	-	0.97	2.6410^{-4}
	40	Weibull	0.016 ± 0.006	0.7 ± 0.2	0.88	1.8310^{-4}
	50		0.059 ± 0.004	1.3 ± 0.3	0.96	1.7910^{-4}
	60		0.085 ± 0.008	1.1 ± 0.3	0.97	2.1710^{-4}

[1]AA, Ascorbic acid
[2]A2P, Ascorbyl 2 phosphate
R^2, Determination coefficient; RMSE, root mean square error

Table 5: Correlation coefficients (R) between each color parameter and type of ascorbic acid

Color parameter	40°C		50°C		60°C	
	AA[1]	A2P[2]	AA[1]	A2P[2]	AA[1]	A2P[2]
L*	-0.75	-0.91	-0.04	-0.55	0.15	-0.17
a*	0.80	0.96	0.89	0.83	0.85	0.91
b*	-0.52	-0.89	-0.89	-0.72	-0.44	-0.36
ΔE	-0.74	-0.93	-0.89	-0.81	-0.78	-0.85

[1]AA, Ascorbic acid
[2]A2P, Ascorbyl 2 phosphate

Table 6: Energy of activation of ascorbic acid and ascorbyl-2-phosphate destruction in peach puree

Treatment	Ea (kJ/mol.K)	r^2	p-value
Ascorbic acid	28.95±12.25	0.86	0.25
Ascorbyl 2 phosphate	85.15±20.64	0.95	0.15

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CHAPTER 4: EFFECT OF L-ASCORBYL-2-PHOSPHATE AND PYCNOGENOL ON THE QUALITY OF DICED PEACHES IN FLEXIBLE RETORTABLE POUCHES

ABSTRACT

Diced peaches in flexible retortable pouches have been identified as an area of concern due to the darkening produced by ascorbic acid degradation during storage. In order to delay these reactions, L-ascorbyl-2-phosphate was studied as replacement of ascorbic acid; also, different concentrations of Pycnogenol were added to peaches due to its antioxidant properties. L-ascorbyl-2-phosphate is a more stable form of vitamin C while Pycnogenol is a blend of flavonoids that have been suggested as an inhibitor of ascorbic acid degradation. Samples were stored at 51°C and 40°C for six weeks and 12 weeks, respectively. During storage, vitamin C concentrations, color (CIELAB system) and browning index were measured at different periods of time. Rate constants were calculated in order to compare the different treatments. A complete factorial design was analyzed to determine any effect due to the Pycnogenol concentrations or Vitamin C type on the food systems. At 51°C, the rate constants were much higher than at 40°C. At each temperature, all treatments presented similar rate constants. Even though the complete factorial design presented some significant differences, these were not so defined to conclude that one treatment was better than the rest. In conclusion, neither the use of ascorbyl-2-phosphate nor Pycnogenol, under the studied conditions, showed an improvement in AA stability or color retention during the storage.

KEYWORDS

Ascorbic acid; Ascorbyl-2-phosphate; Non-enzymatic browning; Retortable pouches; Peaches;

INTRODUCTION

Ascorbic acid (AA) is frequently added to food products because of the importance of this vitamin in human health. However, due to the low stability of this compound, its lifetime is short. Not only is AA degradation a concern in terms of loss of nutrients, but also in terms of appearance. In fact, non-enzymatic degradation involves reactions in which brown pigments are developed. As a consequence, this type of browning has been studied in many food systems such as citrus juices, pears, fruit puree, fruit pulps, among others (Roig and others 2000; Chutintrasri and Noomhorm 2007; Ibarz and others 1999; Garza and others 1999; Lozano and Ibarz 1996; Buglione and Lozano 2002). One of the products that has been identified as an area of concern is diced peaches in syrup packaged in retortable pouches (Mount and others 2007).

The retort pouch package was invented and introduced by the US Army Natick Laboratory. This type of package was developed as a replacement for the metal can due to the advantages that pouches offer. For example, retort pouches are soft containers that do not injure soldiers when the food is carried in pockets. Also, since retort pouches are thin packages, the length of heat treatment is reduced; consequently, the sensory quality of food is not affected as much as in metal cans (Clark 2009). As a result, nowadays the US army uses these containers to send rations in different parts of the world. Even though retort pouches solve some of the problems in soldiers' rations, there are several problems that are still under study. In fact, the stability of food products is still a challenge for food scientists, especially when refrigeration is not available. For instance, diced peaches in syrup were once part of soldiers' rations; however, due to the darkening developed during storage, this item was removed. Just a few studies involving non-enzymatic browning of food products manufactured in retort pouches have been published until now (Clark and others 2002; Kluter and others 1996; Kluter and others 1994; Olivas and others 2002; Rodriguez and others 2003).

One method to avoid or delay non-enzymatic browning due to AA degradation is the use of plant-derived flavonoids. Flavonoids are secondary plant metabolites and have been shown to have good antioxidant properties, due to their free radical scavenging and metal chelation activities. In fact, their protective role against ascorbic acid degradation has been known since 1936, when Szent-Györgyi and coworkers found that flavonoids, at that time called Vitamin P,

had an ascorbate protective role (Rice-Evans and Packer 1998). Subsequently, research *in-vitro* has confirmed this protective role (Bors and others 1995; Rice-Evans and Packer 1998; Sivonova and others 2006; Cossins and others 1998). Among the flavonoids, a blend of procyanidns and phenolic acid glycosylation products, which is sold under the name of Pycnogenol, has been demonstrated to have a better ascorbate protective activity than other flavonoids. Pycnogenol is a tradename for an extract of pine bark developed originally by French scientist, Jack Masquelieer (Masquelier 1987). In fact, there is evidence which suggest that Pycnogenol regenerates ascorbate radical from dehydroascorbic acid (Cossins and others 1998):

$$FlOH + DHA \leftrightarrow FlO^{\cdot -} + Asc^{\cdot -} + 2H^{+}$$

Most research on Pycnogenol has focused on its antimicrobial and antioxidant properties (Ahn and others 2006; Torras and others 2005). One study in yogurt demonstrated the stability of Pycnogenol during storage in this acid product (Ruggeri and others 2008). Even though the ascorbate protective role of Pycnogenol was noted more than one decade ago (Cossins and others 1998), Pycnogenol has not been evaluated as a potential additive to avoid ascorbic acid degradation in food products.

Another way to avoid or delay AA degradation is to use more stable ascorbic acid derivatives. It is known that AA derivatives with substituted groups at carbon 2 and carbon 3 have improved stability (Iacobucci and Sweeny 1980). One of the derivatives which has shown good results is ascorbyl-2-phosphate (A2P) (O'Keefe 2001). For example, in carbonated beverages, A2P and ascorbyl-2-polyphosphate (A2PP) had better stability than AA at 15°C, 25°C and 35°C (Wang and others 1992). In mashed potatoes after processing and storage, the losses of AA and A2P were 50% and 20%, respectively (Wang and others 1995). In addition, higher stability of A2P has been shown in cosmetics and aquatic feeds (O'Keefe 2001; Austria and others 1997). Furthermore, there are studies in animals which show that A2P and AA have the same bioavailability (O'Keefe 2001). Even though this AA derivative still does not have approval for food use, A2P has reportedly higher stability in different model systems and is, thus, of great interest, as a replacement for AA in foods.

The objective of this research was to study the effect of Pycnogenol (0%, 0.01% and 0.1%) and form of vitamin C (AA and A2P) on the quality of diced peaches in syrup packaged in retortable pouches during storage at 51°C and 40°C. To achieve this goal, we measured vitamin C content, color parameters (CIELAB system) and browning index at different periods of time during 6 weeks and 12 weeks at 51 and 40°C, respectively. We applied different kinetic models to obtain accurate kinetic parameters and to make comparisons among treatments.

MATERIALS AND METHODS

Freestone yellow peaches produced in California (USA) were used. The peaches were cut into halves, lye peeled (82°C/ 3% w/v NaOH aq. for 5 min), rinsed and pitted. The peaches were diced into 0.5cm cubes. To avoid enzymatic browning, as soon as the peaches were diced, they were dipped in a citric acid (1% w/v)-ascorbic acid (58ppm)-sucrose (11% w/v) solution for no more than 5 min. The effectiveness of this solution as enzymatic browning inhibitor was validated in preliminary work (this solution delayed the browning for one hour at room temperature).

In each retort pouch, 105 grams of diced peaches with 35 grams of syrup were added (ratio peaches:syrup was 3:1, w/w). Six syrup treatments were prepared. The first was composed of calcium chloride (0.5% w/v aq.), sugar (47° Brix), and AA (1.5×10^{-2} M). The second and third had the same composition with in addition 0.04% and 0.4% w/v Pycnogenol (Horphag Research, Hoboken, NJ), respectively. Finally, the last three syrups were as the same as the first three except that instead of AA (Fisher Scientific, Pittsburgh, PA), A2P trisodium salt (Sigma-Aldrich, St. Louis, MO) was used. The amounts of sugar and vitamin C in the syrups were calculated in order to achieve final concentrations after equilibrium in each pouch of 18°Brix and 4×10^{-3} M, respectively. The Pycnogenol concentrations in syrups (0.04% and 0.4%) were calculated to obtain final concentrations in the pouches of 0.01% and 0.1%, respectively. The retort pouches (Cadillac products, Paris, Illinois) were sealed using a Multivac Sealing Machine (Kansas City, MO) adjusted at 80 mbar of vacuum. After sealing, the pouches were retorted until reaching an F-value of 6.3 min (T=100°C, z=10°C). The pouches were stored for 1 week at 4°C. After this time, they were divided in two groups: the first group was stored at 51°C for 6 weeks, and the second was stored at 40°C for 12 weeks. Pouches stored at 51°C were

analyzed at 0, 3 and 6 week, while pouches stored at 40°C were analyzed at 0, 6 and 12 weeks. Two repetitions were analyzed per each treatment at each time and temperature.

Ascorbic acid determination

A high performance liquid chromatography (HPLC) procedure based on an earlier report (Gokmen and others 2000) was used to quantify ascorbic acid in the samples. Diced peaches and syrup, in the same ratio as they were found in each pouch (3:1, w/w), were combined with a methaphosphoric acid (5%w/v aq.) solution in a ratio of 3:1 (product:methaposphoric acid solution). This mixture was homogenized for 90 seconds using a Virtis homogenizer-Model TP 18/10 (Wilmington, NC) at medium speed. The homogenized samples were filtered through Whatman No. 4 (Maidstone, UK) and 0.45 µm filters (Millipore, Massachusetts, USA). The samples were protected from light during filtration. The clarified samples were analyzed by high performance liquid chromatography (HPLC). A model 1200 HPLC consisting of degasser, pump, refrigerated autosampler, column oven, and diode array ultraviolet-visible (UV-VIS) detector was obtained from Agilent (Santa Clara CA). The mobile phase was pH 2.4 20mM aqueous phosphate buffer. The column (Luna 5µ C18(2) 100Ǻ, 250mm x 4.6mm, i.d.) was obtained from Phenomenex (Torrance CA) and was used at a flow rate was 0.5 ml/min. The detector was set to 245nm and UV spectra from 190-300 NM were recorded.

Colorimetric Analysis

A Chroma Meter CR-200 tristimulus color analyzer (Minolta, Osaka, Japan) was calibrated with a standard plate (L*: 97.20, a*: -0.18, b*: 3.75) before each use. The diced peaches (70$\pm$5 g) were placed inside a glass petri dish (9 cm diameter), and the color was measured from the bottom of the dish. Before each measurement, the measuring head of the colorimeter was covered with a cardboard box to block stray light. All readings were obtained in the CIELAB scale (L*, a*, b*). The ΔE* was calculated in order to quantify the variation of color throughout storage. ΔE*was defined by the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*) + (\Delta a^*) + (\Delta b^*)}.$$

Browning index

Diced peaches were blended with water in a 1:1 (w/w) ratio. The mixture was centrifuged at 1000xg for 15 minutes in a Damon/IEC centrifuge (Needham Heights, MA). The supernatant was combined with ethanol in a ratio of 1:1 v/v. This second mixture was centrifuged at 1000xg for 15 minutes at 2°C in a Sorvall RC-5B refrigerated Superspeed Centrifuge (Du Pont instruments, Wilmington, DE). The browning index was measured by its absorbance at 420 nm in a Thermo Electron Genesys 10 UV Scanning Spectrophotometer with 1 cm path cuvette (Waltham, MA). This method is based on a previously published technique (Klim and Nagy 1988).

pH and °Brix

The pH and °Brix were measured after the homogenization of diced peaches and syrup (in the same ratio as they were found in each pouch). The pH and °Brix were measured using a model XL-20 potentiometer (Fisher Scientific) and an Abbe Mark II refractometer (Reichertai Inc,. Buffalo, NY), respectively.

Statistical analysis

The rate constants (k) were obtained by linear regression analysis (Arabshahi and Lund 1985). The color parameters (L*, a*, b* and ΔE*) were evaluated through equations (1), (2), (3) and (4). In the case of ΔE*, instead of equation (4), we applied equation (5) because this parameter, ΔE*, is zero at day 0 (Ibarz and others 1999). For the k values of vitamin C degradation, the data collected were fitted using the equations (1), (2), (3) and (6). The best kinetic model was chosen after comparison of the coefficients of determination (R^2), root mean square error (RMSE) and visual examination of distribution of residuals of plotted data.

Zero order (eq. 1): $$C = C_0 + k_0 t$$

First order (eq. 2): $$C = C_0 \exp{(k_1 t)}$$

Second order (eq. 3): $c = \frac{1}{\left(\frac{1}{c_0}\right)+(k_2 t)}$

Combined kinetics model (eq. 4): $C = \frac{k_0}{k_1} - (k_0 - C_0)\exp(-k_1 t)$

Combined kinetics model in ΔE* (eq. 5): $\Delta E = \frac{k_0}{k_1}(1 - \exp(-k_1 t))$

Weibull model (eq. 6):

$$C = C_0 \exp\ (-k_1 t)^{\beta}$$

Where:

C = Variable content studied at time t

C_0 = Variable content at time zero

k_0 = Zero order kinetic constant

k_1 = First order kinetic constant

k_2 = Second order kinetic constant

β = Shape parameter

t = time

The fitting process for the equations (1), (2) and (3) was done by using linear regression in JMP 8.0 (SAS Institute, Cary, NC). Equations (4), (5) and (6) were solved by non linear techniques using Statgraphics Plus v. 5.1 (Statgraphics, Warrenton, VA).

A complete factorial design was used to determine if there were significant effects due to the type of vitamin C (AA, A2P), Pycnogenol concentration (0, 0.01%, 0.1% w/v), temperature and interactions. Correlation coefficients were determined separately for vitamin C types and each of the color parameters with time and temperature. These calculations were performed using JMP 8.0 (SAS Institute). Tukey´s HSD was used in order to evaluate the differences among each treatment.

RESULTS AND DISCUSSIONS

Syrup characteristics

There were some obvious differences between syrups containing AA and A2P. We could not dissolve A2P in solutions that already contained sugar (47 °Brix) and $CaCl_2$ (0.5% w/v), although AA was soluble under these conditions. For this reason, we first had to dissolve the A2P and $CaCl_2$ in water, and then we added the sugar. Looking at the chemical structures of AA and A2P (Fig. 1), we can understand why the A2P trisodium salt had lower solubility than AA. The A2P molecule is more neutral due to the presence of three sodium ions (Na^+). In previous research, during the preparation of fortified potato flakes with ascorbyl-2-phosphate magnesium salt, a non-uniform vitamin distribution was reported. The researchers in this paper reported that somehow the vitamin became insoluble in the base mix. They also prepared a treatment with AA which did not have this problem (Wang and others 1992).

Syrups were prepared ~5 hours before thermal processing. During this time, they were stored at refrigeration temperature (4 °C) and protected from light. Just before they were used, we realized that the A2P-control (0% Pycnogenol) syrup jelled at this temperature. A new solution was then prepared 30 minutes before it was used; during this time, the solution remained liquid.

In order to understand why this syrup jelled, experiments were conducted on 4 syrups which were composed of sucrose and A2P salt in concentrations of 47°Brix and $1.5x10^{-2}$ M, respectively. These concentrations corresponded to those used in our retort pouches treatments. Four syrups were prepared with $CaCl_2$ at 0%, 0.1%, 0.3% and 0.5% w/v. Syrups were prepared at pH 7 and at pH 3.8. Two repetitions were conducted per treatment at each pH. In order to acidify the solutions, citric acid was added. None of the solutions at acid pH jelled; however, at neutral pH, the syrups that had the highest concentrations of $CaCl_2$ jelled (0.3% and 0.5%) within 24 hours of storage at room temperature (~24 °C). Hardness was measured in these gels. These measurements were made using a TA.XT.plus Texture analyzer (Stable Micro Systems, Godalming, UK) and cylindrical probe dimensions 13mm diameter and 35 mm height. The gel of the sample which contained 0.5% of $CaCl_2$ was harder than the sample which contained 0.3% of $CaCl_2$ (Fig. 2). We believe that at neutral pH, the Ca^{2+} added reacts with the phosphate

groups forming a polymer which entraps the sugar solution. It appears that the same principle which explains the jellification due to carbohydrates, such as pectin, can be observed in our systems. For example, low methoxyl pectin solutions need Ca^{2+} ions to gel since these divalent cations form crossbridges between pectins. If we increase the Ca^{2+} content, the gel strength is increased (Fennema 1996). Going back to our retort pouches experiments, the other two treatments which had A2P (A2P-0.01% Pycnogenol and A2P-0.1%Pycnogenol) during the 5 hours of storage at refrigeration did not gel. In these last syrups, we believe that the Pycnogenol provided H^+ which prevented the reactions between Ca^{2+} and A2P. As we can see in Table 1, the syrups which contained the highest concentrations of Pycnogenol had the lowest pH.

We could have added an acidulant such as citric acid to the A2P syrups in order to lower the pH; however, we did not do this since we wanted to maintain a balanced experimental design. Also, it has been suggested that citric acid participates in the formation of some of the brown pigments in non-enzymatic reactions (Clegg 1964); consequently the addition of citric acid could have caused extraneous effects. Finally, since the pH in the mixture of diced peaches and syrup were already between 3 and 4, the syrups did not form jells inside the pouches.

pH and •Brix during storage

Tables 2 and 3 show the pH and °Brix values at the beginning and end of storage for each temperature. No significant changes in pH or °Brix were observed at either temperature. Some reports have shown that pH may decrease during non-enzymatic browning, due to the appearance of carbonyl compounds (Roig and others 2000). On the other hand, it has also been reported that pH increases during the storage of retort pouch wet-pack pears (Olivas and others 2002). In orange juice packaged in tetrabrix cartons, a pH of 3.65 was maintained during storage (Roig and others 2000). No changes in °Brix were observed in cashew apple (pH 4.4) during storage. In this last paper, the constant concentration of soluble solids, reducing and total sugars suggested that that there was no participation of sugars in the non-enzymatic browning reactions (Damasceno and others 2007). As we know, reaction of reducing sugars and amino acids cause maillard reactions, which is a type of non-enzymatic browning favored by a more alkaline pH environment (Fennema 1996).

Color parameters (CIELAB System)

The L*, a* and b* parameters indicate how dark/light, red/green and yellow/blue the samples are, respectively (HunterLab 2008). These values, as well as the size of color difference (ΔE^*), were measured or calculated throughout storage (Figures 2-10). The results of the complete factorial design to determine the effect of each factor and the interactions between them (vitamin type and Pycnogenol concentration) are presented in Tables 4-7. The kinetic models presented in Table 8 correspond to those that were successfully applied for our data. Those models which presented a high root mean square error (RMSE) and a low R^2 were not included.

Color parameter L*

At the beginning of the storage (Table 4), the type of vitamin C did not have an effect on the lightness of the samples ($p>0.05$). In the case of Pycnogenol, even though we observe that this had an effect ($p<0.05$), the differences are not of chemical importance. During storage at both temperatures, the L* values had tendencies to decrease (Figs. 3-4), however, the slopes obtained at 51°C were higher than at 40°C. These trends are similar to those reported in other studies on non-enzymatic browning of peaches (Garza and others 1999; Lozano and Ibarz 1996; Ibarz and others 1999; Chutintrasri and Noomhorm 2007). Also, during storage of peaches (Kluter and others 1994) and pears (Kluter and others 1996) in retortable pouches, the hunter L values decreased, and this was higher at 38°C than at 21°C. In our results, at the end of 40°C storage, we observed significant differences ($p<0.05$) in L* value between samples prepared with AA or A2P; however, these differences were small (A2P was higher than AA by 2 units).

The kinetic parameters for L* values are shown in Table 8. Both zero and the combined model successfully fitted our data at both storage temperatures. In fact, both models had very similar R^2 and RMSE values. In these cases, it is recommended to choose the model which has the lowest number of parameters but that still offers a good fit (van Boekel 2008). Based on this principle, and on the linear regressions seen in Figs. 3-4, we used the k obtained in the zero order model. Most studies done either in heated or stored fruit systems report first order changes in L* values (Ibarz and others 1999; Garza and others 1999; Lozano and Ibarz 1996; Avila and Silva 1999). On the other hand, it has been reported that for maillard browning, the formation of

brown pigments from sugars and amino acids, follows a zero order reaction (van Boekel 2008). In a study conducted in pears packaged in retortable pouches, a zero order reaction was identified for browning index. In this study, browning index was calculated from a formula which includes the L*, a* and b* parameters (Olivas and others 2002).

As we can predict from Figs. 3 and 4, the *k* values obtained at 51°C were higher than at 40°C. At 51°C, the *k* values were similar in all treatments with the exception of the slightly lower values observed in the A2P-Control and the A2P-0.1% Pycnogenol samples. However, at the end of the storage, there were not significant differences ($p<0.05$) in these treatments (Table 4). At 40°C, the *k* values observed among the treatments were nearly identical.

Color parameter a*

Figures 5 and 6 show higher a* values at day 0 for the treatments that contained 0.1% Pycnogenol. This difference was statistically significant ($p<0.05$) in comparison with the other concentrations, as we can see in Table 5. This more reddish color is explained by the brownish color of the Pycnogenol extract (Rohdewald 2002). There was also a significant difference ($p<0.05$) between types of vitamin C. Indeed, the samples with A2P were more opaque.

Fig. 5 and 6 show that a* values increase in all treatments during storage. This is explained by the formation of brown pigments which increase the reddish color of the samples. The browning observed agrees with other reports. For example, research conducted in peach, pineapple and pear puree, show a similar tendency (Avila and Silva 1999; Chutintrasri and Noomhorm 2007; Ibarz and others 1999). In retortable pouches with diced pears, after 60 days of storage at 37.8°C, the a* value increased by less than 2 units (Clark and others 2002). This change is much lower than what we observed in our results (Fig. 6).

After 42 days of storage at 51°C, the treatment which contained Pycnogenol at 0.1% was still significantly more reddish ($p<0.05$) than the other treatments. After 84 days of storage at 40°C (Table 5), there were significant interactions between Pycnogenol and type of vitamin C ($p<0.05$); however, the differences among treatments were so small that we consider them of not chemical importance.

For the kinetic models, as we also observed with the L* values, both zero and combined models successfully fit our data, as can be seen in Table 8. However, following the principle of choosing the model with lower number of parameters (van Boekel 2008), we used the zero order reaction model. Other studies report combined, zero or first order kinetic models for a* values during storage (Chutintrasri and Noomhorm 2007; Rattanathanalerk and others 2005; Ibarz and others 1999).

Table 8 shows that at 40°C, all *k* values were much lower than at 51°C. At 51°C, the A2P-0.1% Pycnogenol treatment had a slightly lower rate compared to the other five treatments. This same treatment and the one composed by the A2P-control had the lowest *k* values at 40°C. However, these values did not show significant differences at the end of the storage (Table 5). In pineapple puree during heating at 70-110°C, zero order reaction models were successfully applied (Chutintrasri and Noomhorm 2007). The *k* obtained in this last research was $0.34x10^{-3}$/min at 70°C. Our results at 51°C and 40°C ranged between 0.15-0.22/day^{-1} and 0.02-0.06/day^{-1}.

Color parameter b*

At the beginning of storage, there were no significant differences ($p>0.05$) due to the effects of vitamin C form nor Pycnogenol concentration. During storage at 51°C, even though the tendency was always to decrease over the storage period (Fig. 7), this was not clear for all treatments. At 40°C, we observed an increase at day 84 for the A2P-0.01% and A2P-0.1% Pycnogenol treatments. The tendency to decrease is explained by the loss of yellowness due to the appearance of browning pigments. In another study, during heating of peach puree at 80°C, there was no defined trends in a* and b* parameters, although trends were clearer at higher temperatures (Garza and others 1999). Also, during heating of pear puree, the changes in b* were as apparent as for a* values such, as we also observed (Ibarz and others 1999). A general tendency for b* values to decrease during storage or heating has been reported in other research (Avila and Silva 1999; Garza and others 1999; Chutintrasri and Noomhorm 2007; Rattanathanalerk and others 2005). At 51°C, by the end of the storage period, there were no significant effects due to type of vitamin C nor Pycnogenol concentrations ($p>0.05$). On the

other hand, at 40°C after 84 days of storage, a higher b* value ($p<0.05$) was observed in the treatments with A2P (Table 5).

We could not calculate kinetic constants for b* because of the low R^2 and high RMSE obtained using either combined, zero, first or second order reaction models. During heating of peach puree and pineapple juice, b* values followed first-order reaction models (Avila and Silva 1999; Rattanathanalerk and others 2005).

Color parameter ΔE*

At 51°C and 40°C storage, the tendency of ΔE* values was always to increase. Also, the slopes observed at 51°C (Fig. 9) were higher than those at 40°C (Fig. 10). At 51°C, at the end of the storage, there was a significant effect ($p<0.05$) due to the type of vitamin C and Pycnogenol concentration. In fact, the AA treatments had a higher ΔE*, and the 0.1% Pycnogenol treatment had the lowest ΔE* ($p<0.05$). At 40°C, the same significant differences ($p<0.05$) were seen (Table 7).

As we observed for L* and a* values, ΔE* successfully fit zero order and combined model kinetics. For the reasons explained earlier (van Boekel 2008), we chose the zero order model. The lowest k value was obtained by the treatment which contained A2P and 0.1% Pycnogenol. These values were 0.24/day and 0.04/day, for storage at 51°C and 40°C, respectively. In fruit systems, usually combined models have been reported for ΔE* values (Rattanathanalerk and others 2005; Avila and Silva 1999; Ibarz and others 1999; Damasceno and others 2007; Garza and others 1999). In the case of thermal processing of pineapple puree, zero order reactions were identified; at 70°C, a k value of 0.87×10^{-2}/min was reported (Chutintrasri and Noomhorm 2007).

Browning index (Spectrophotometric assay)

At day 0, the browning index (BI) did not equal zero (Figs 11-12). This absorbance is likely due to components of the peaches such as carotenoids (Roig and others 2000). In fact, as we can see in Table 9, the treatments with higher concentration of Pycnogenol had a higher browning index. This might be due to the brownish color of the Pycnogenol extract (Rohdewald

2002). Statistically, there was no effect ($p>0.05$) of Pycnogenol concentration nor vitamin C form on browning index.

At both storage temperatures, the pattern of BI change was to increase with time; nevertheless, the slopes were higher at 51°C than at 40°C (Fig. 11 and 12). In contrast with the color parameters (L*, a*, ΔE*) which better fit a zero order model, the browning index at 51°C had a better fit for a first order model. Table 10 shows that at 51°C, the R^2 and RMSE for both first order and combined models were very close, with the exception of the A2P-control. In this last treatment, the combined model had an R^2 of 0.99, while the first order model had an R^2 of 0.87. Following the principle of choosing the model which offer the lowest number of parameters (van Boekel 2008), and that we believe that an R^2 of 0.87 is still acceptable, we chose first order over the combined model. Finally, the treatment composed of A2P and 0.01% Pycnogenol had the lowest R^2 with both models, as we can see in Table 10. At 40°C, the zero order model fit the data much better than the first order model. For this reason, we included the zero order model in Table 10 at 40°C. At this temperature, the combined model fit data better than the zero order model for the treatments which had AA. The reverse occurred for the A2P treatments. This difference is explained by the fact that at week 6, the AA treatments presented a higher BI than the A2P treatments. Other studies conducted with non-enzymatic browning show that BI fits either a zero (Chutintrasri and Noomhorm 2007; Rattanathanalerk and others 2005; Ibarz and others 1999) or first order model (Garza and others 1999). Some reports suggest that the data adjusted to both zero and first models, but while temperature increases, the data fits better the first order model (Buedo and others 2000). The results obtained in our research are much lower than the obtained by others at 55°C or higher temperatures (Garza and others 1999; Rattanathanalerk and others 2005).

At the end of the storage (Table 9), we can observe that at 51°C there was a significant effect ($p<0.05$) of type of vitamin C on BI. In fact, the treatments with AA had higher BI than the samples with A2P. At 40°C, no significant effects ($p>0.05$) were observed for vitamin C form or Pycnogenol concentration.

Ascorbic acid content

From day 0, we noticed the presence of two peaks in the A2P chromatograms (Fig. 13) instead of the single peak that appeared in the AA treatments and A2P standard curves. After analyzing the UV absorbance spectra of both peaks and comparing them with the retention times and spectra of AA and A2P standards, we determined that the peaks were due to the presence of A2P and AA. In fact, as we can see in Figs. 14 and 15, AA and A2P UV absorbances have similar shapes; however, their maximum absorbances are at 242 nm and 238 nm, respectively. The reason why these two peaks appeared in the A2P from day 0 must be result of hydrolysis. Indeed, it is known that acidic pH can cause ester hydrolysis in A2P (Gonçalves and others 2009). In comparison to the results reported in the previous chapter of this thesis (puree samples), we noticed that the hydrolysis in the retort pouches was much higher (around 50%) than in the puree samples (around 16%). The reason of this difference is most likely due to the higher thermal process which the pouches received.

From Figs. 16-21, we can observe the A2P and AA concentrations (% based on total vitamin C content) during storage at 51°C and 40°C in all treatments which had A2P. At both temperatures, the tendency was the same: the percentage of A2P increased throughout storage. Even though we believe that the hydrolysis of the phosphate group was still occurring, the rate of degradation of the AA molecules was likely higher than the rate of hydrolysis. A2P is known and advertised for its greater stability than AA at high temperatures (Fermann and Gripp 2004).

In food systems, research evaluating A2P has been conducted in carbonated beverages, white pan bread, bran flakes, peanut, potato flakes (Wang and others 1995; Park and others 1994), mashed potatoes (Wang and others 1992), and in meat (Craig and others 1996; Craig and others 1991). In these food systems, the only study conducted in bread has reported hydrolysis of A2P due to the presence of phosphatase in wheat flour (Park and others 1994; Wang and others 1995). In the carbonated beverage research (Wang and others 1995), no hydrolysis was reported, even though the authors imply that they would be able to detect hydrolysis if it occurred. Because of the low pH of the carbonated beverages, we expect that some hydrolysis must have occurred. The release of the phosphate groups can provide a secondary mode of

antioxidant protection since phosphoric acid ions are able to chelate metal ions (Wang and others 1995).

For the analysis of variance (Table 11) and the graphs of the trends during storage (Figs. 22-23) we considered the total vitamin C present in the samples. In other words, for the A2P treatments, we considered the sum of both concentrations presented in the chromatograms (measured separately with external standard curves). At day 0, there were no significant differences ($p>0.05$) between treatments. At the end of the storage at 51°C, there was a significant effect due to the vitamin C type. In fact, as we can see in Table 11, the treatments with AA had higher vitamin C contents. Even though the difference was not large, this contradicts what we were expecting. As mentioned earlier, research conducted with A2P has demonstrated a higher stability of this compound compared to AA (Wang and others 1995; O'Keefe 2001). At 40°C, no significant effects due to either vitamin C type or Pycnogenol concentration were observed.

Both the Weibull and the zero order models successfully fit the data at each storage temperatures. Due to the principle that it is better to choose the model with the lowest number of parameters (van Boekel 2008), we decided to use the zero order model. As discussed in some studies, AA degradation in food systems has been fit to zero, first, and second orders of reaction (Roig and others 2000; Burdurlu and others 2006; Manso and others 2001; Damasceno and others 2007).

From Table 12, we can see that the rate constants at 51°C were much higher than at 40°C. At 51°C, the AA-control showed a slightly lower rate than the other treatments. At 40°C, we observed the same trend. These results indicate that under the conditions used, neither A2P nor Pycnogenol offered any protection for AA retention. The AA-control treatment stored at 40°C had a low R^2 with all models. In citrus juice stored at 28-45°C, the loss of ascorbic acid followed a first-order kinetic model (Burdurlu and others 2006). On the other hand, in single-strength orange juice aseptically processed in tetrabrik cartons stored at 37°C, a zero order model was applied. In this last result a k of 3.8 mg/day was obtained.

CONCLUSION

Ascorbyl-2-phosphate, as replacement of ascorbic acid, and different concentrations of Pycnogenol (0%, 0.01% and 0.1%) were added into the food systems composed of diced peaches and syrup packaged in retortable pouches. Samples were stored at 51°C and 40°C for six weeks and 12 weeks, respectively. During these storages, vitamin C concentrations, color (CIELAB system) and browning index were measured at different periods of time. Rate constants and complete factorial designs were analyzed to determine any effect due to the Pycnogenol concentrations or Vitamin C type on the food systems. At each temperature, all treatments had similar rate constants. Even though the complete factorial design had some significant differences, they were not so clear to conclude that one treatment was better than the rest.

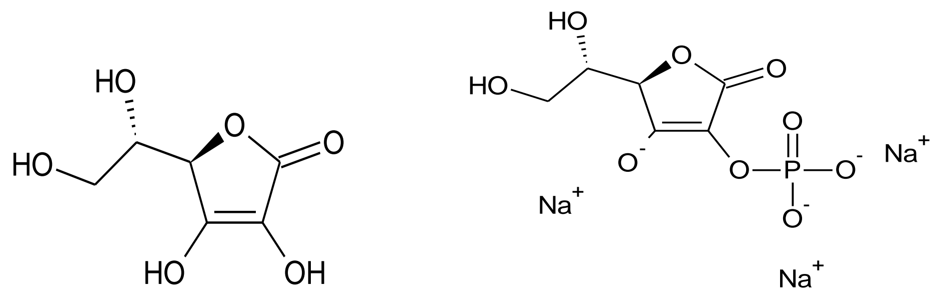

Fig. 1: L-ascorbic acid and 2-phospho-L-ascorbic acid trisodium salt

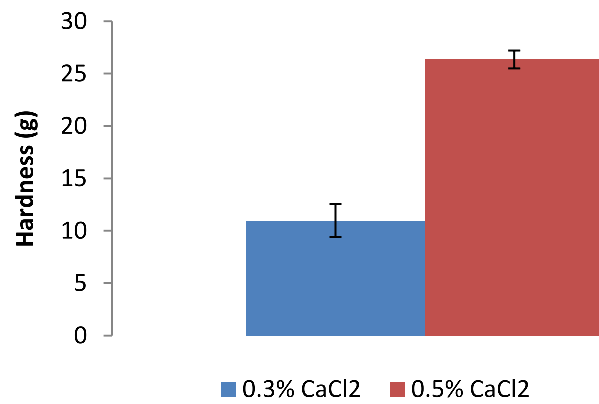

Fig. 2: Influence of Calcium Chloride in the gel formation in syrups at neutral pH

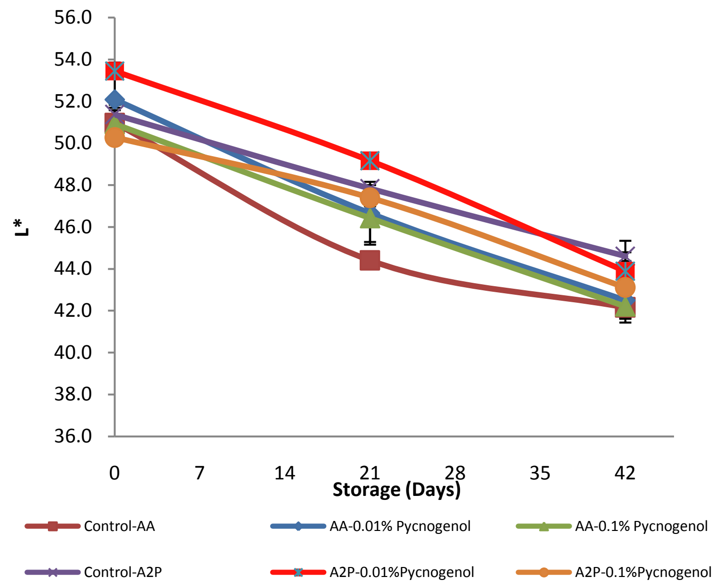

Fig. 3: Variation of the L*value during storage at 51°C

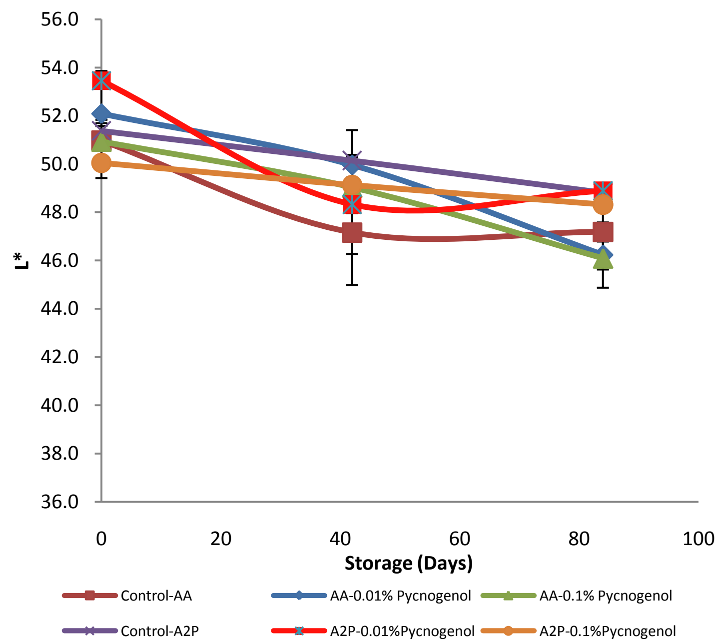

Fig. 4: Variation of the L*value during storage at 40°C

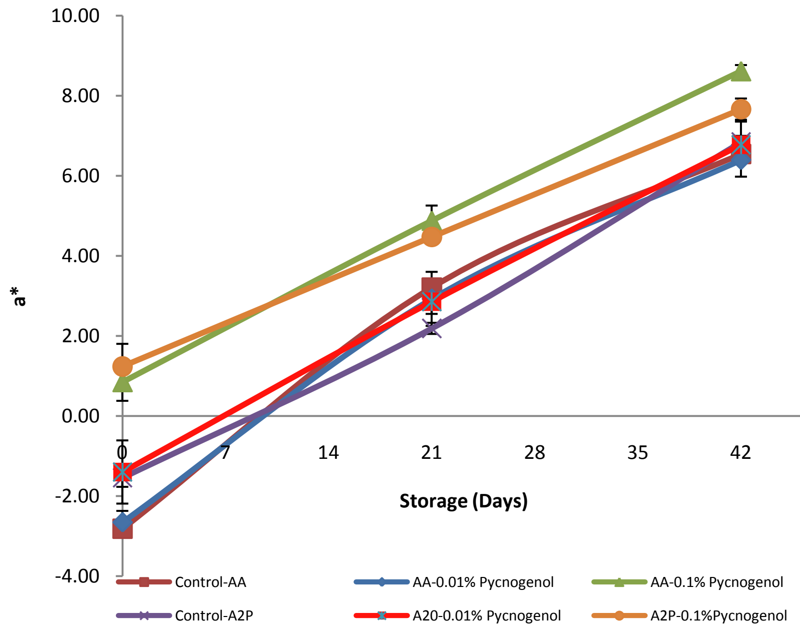

Fig. 5: Variation of the a* value during storage at 51°C

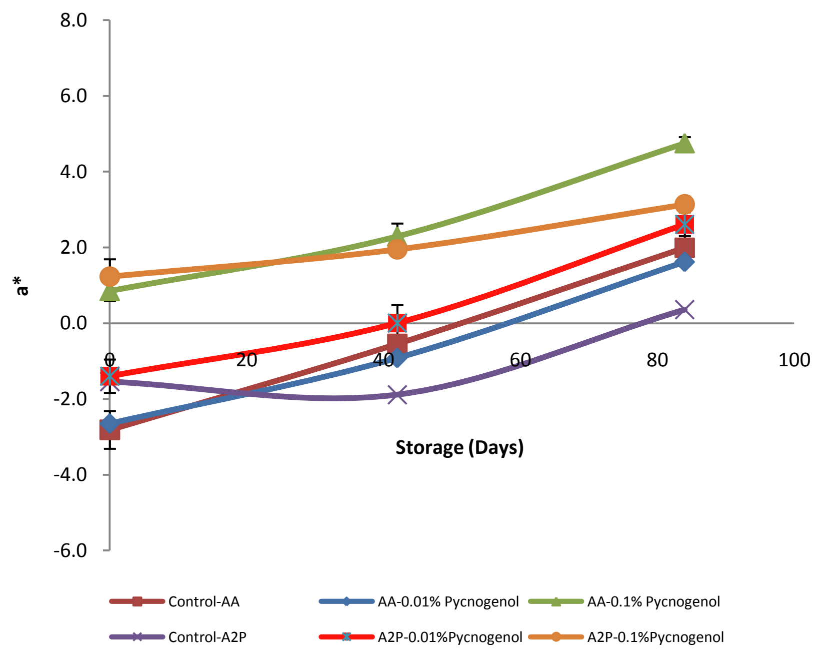

Fig. 6: Variation of the a* value during storage at 40°C

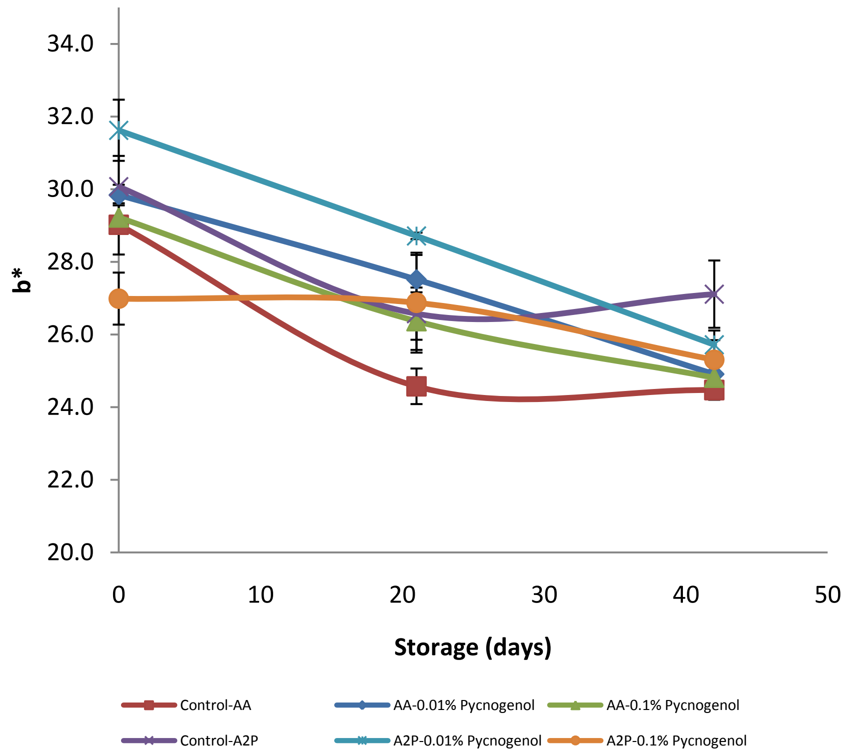

Fig. 7: Variation of the b* value during storage at 51°C

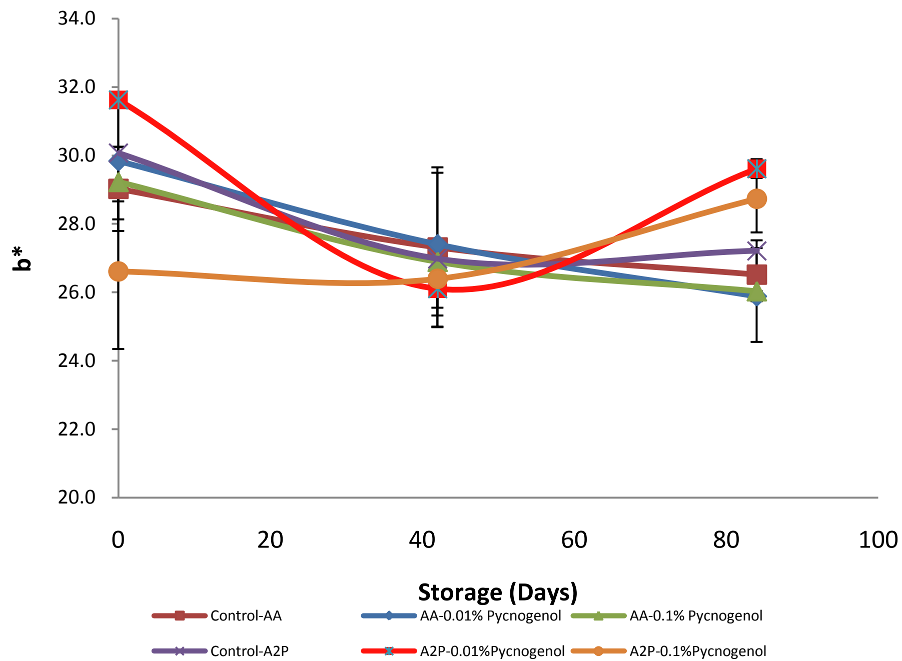

Fig. 8: Variation of the b* value during storage at 40°C

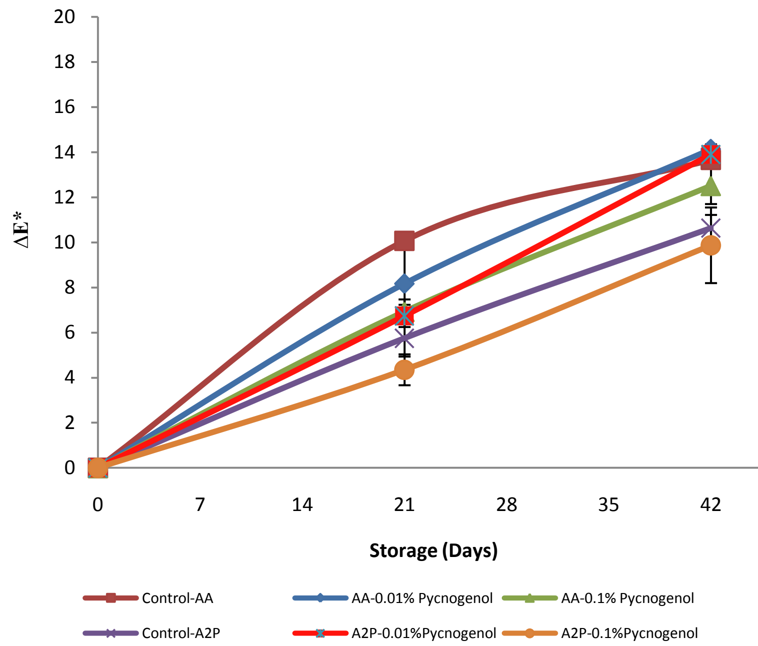

Fig. 9: Variation of the ΔE*value during storage at 51°C

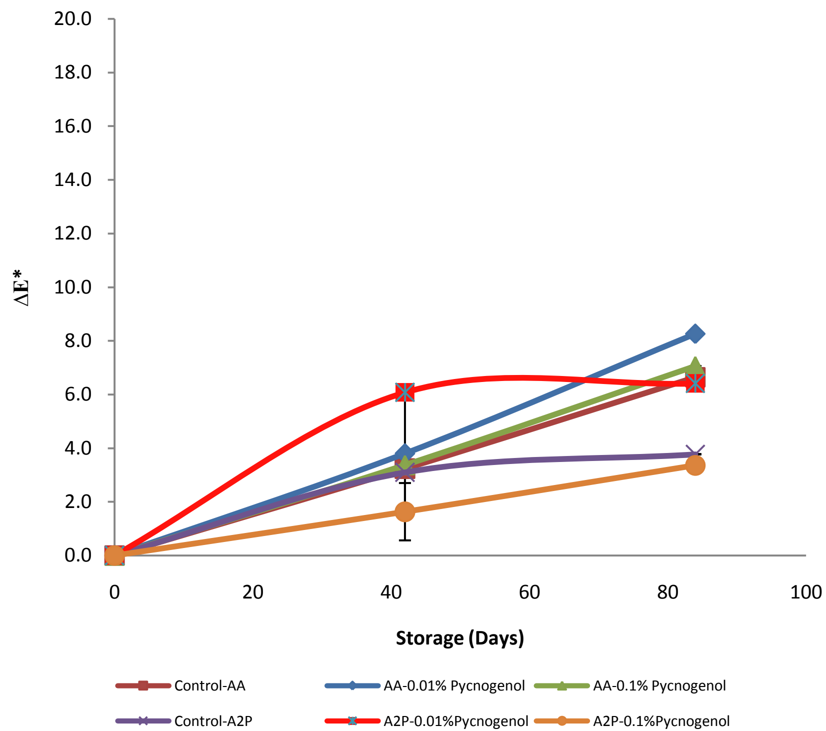

Fig. 10: Variation of the ΔE*value during storage at 40°C

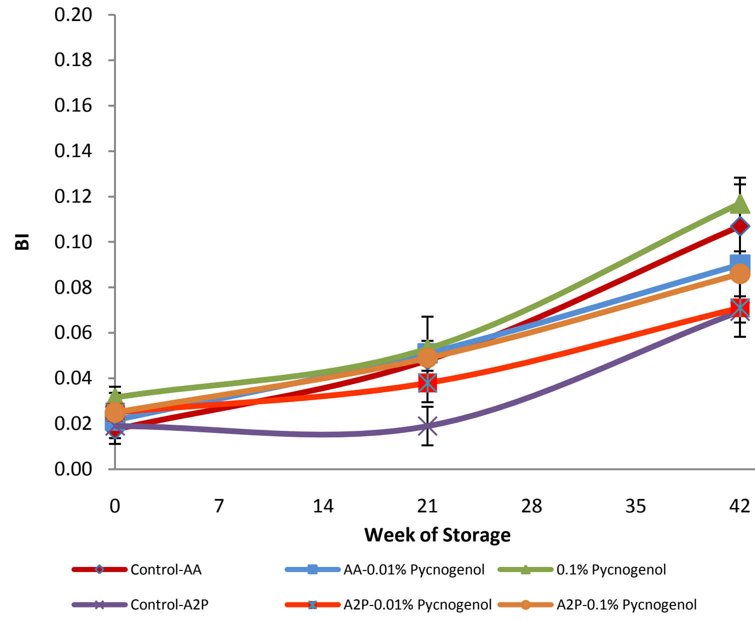

Fig. 11: Variation of the browning index during storage at 51°C

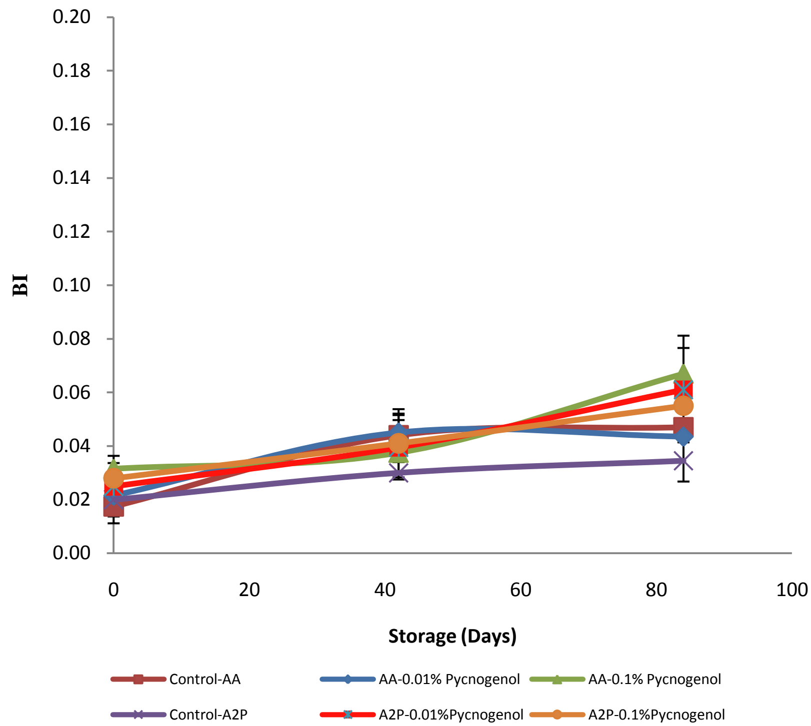

Fig. 12: Variation of the browning index during storage at 40°C

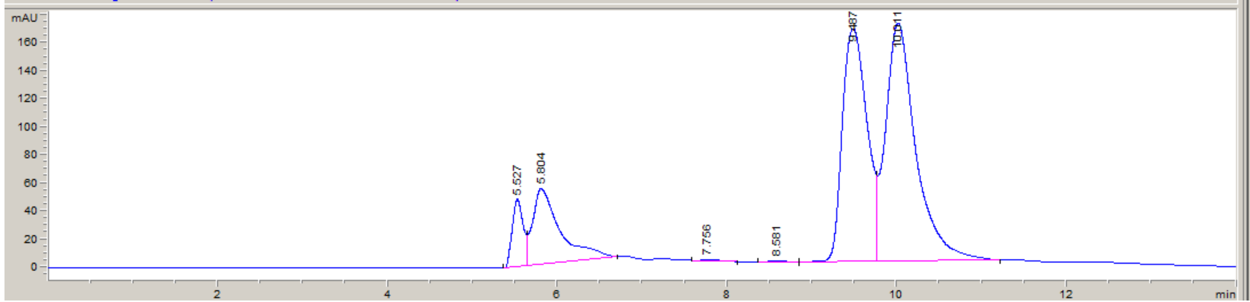

Fig. 13: Chromatogram of one of the samples fortified with ascorbyl 2 phosphate at day 0

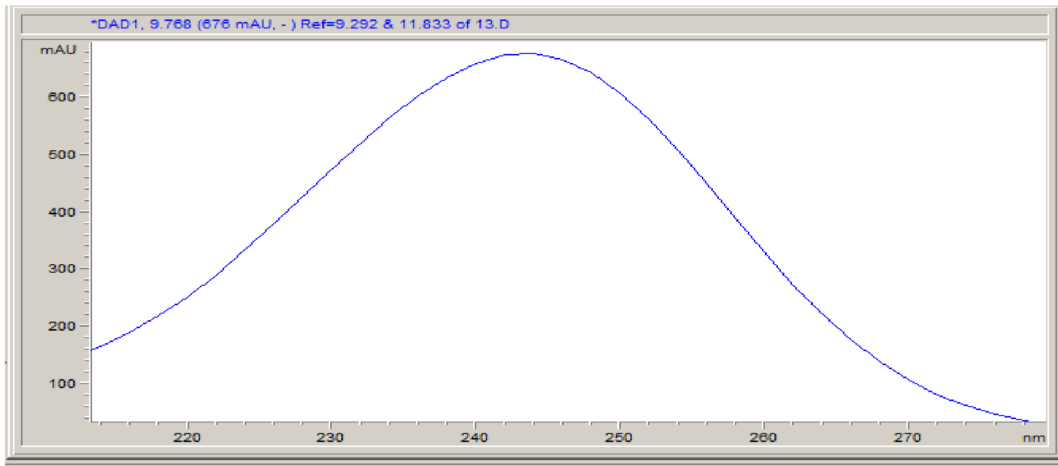

Fig. 14: Absorption spectra of ascorbic acid (0.0025M)

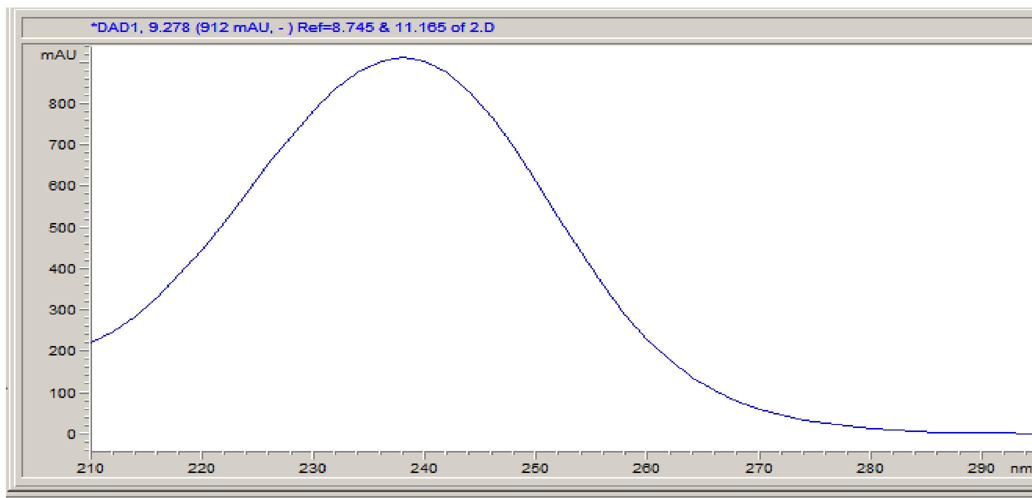

Fig. 15: Absorption spectra of ascorbyl 2 phosphate (0.0025M)

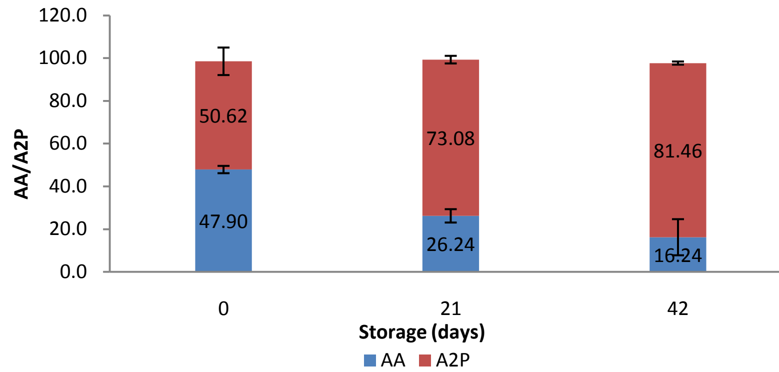

Fig. 16: Relative ascorbyl 2 phosphate and ascorbic acid concentrations (%) in the A2P-Control treatment during storage at 51°C

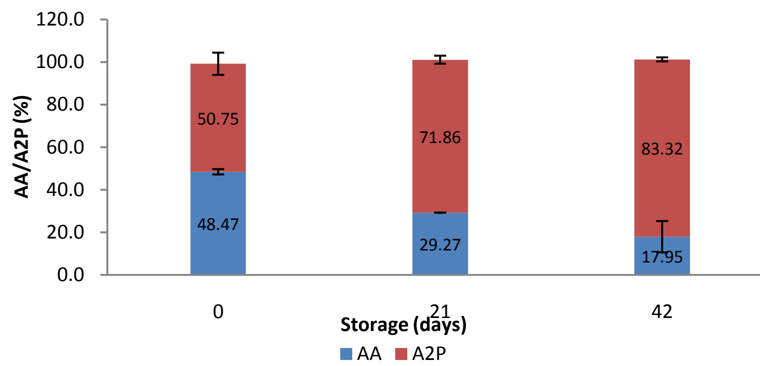

Fig. 17: Relative ascorbyl 2 phosphate and ascorbic acid concentrations (%) in the A2P-0.01%Pycnogenol during storage at 51°C

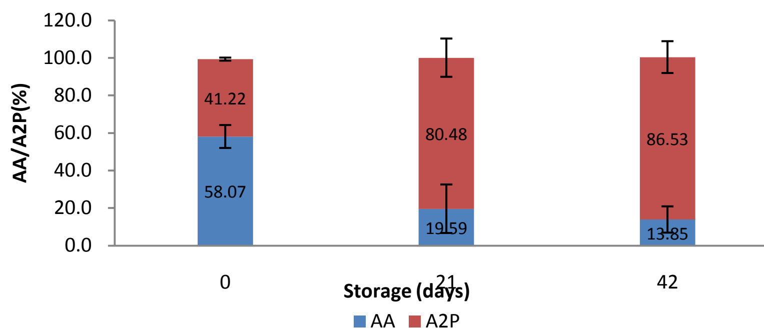

Fig. 18: Relative ascorbyl 2 phosphate and ascorbic acid concentrations (%) in the A2P-0.1%Pycnogenol during storage at 51°C

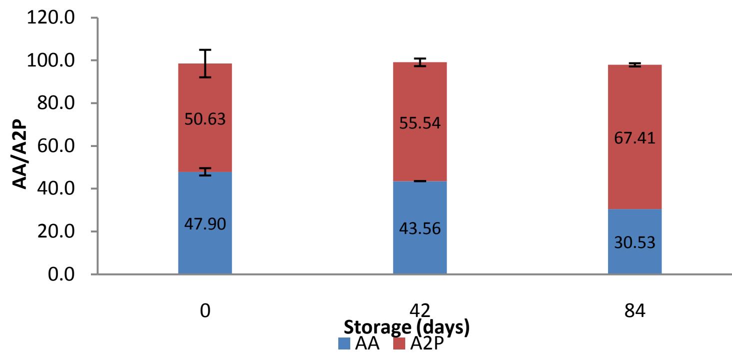

Fig. 19: Ascorbyl 2 phosphate and ascorbic acid concentrations (%) in the A2P-Control treatment during storage at 40°C

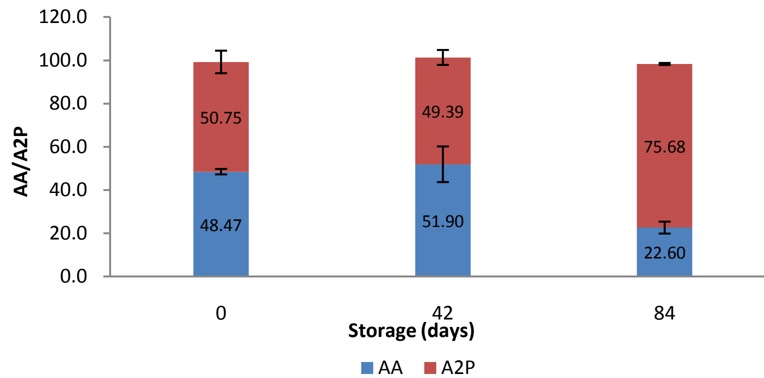

Fig. 20: Ascorbyl 2 phosphate and ascorbic acid concentrations (%) in the A2P-0.01%Pycnogenol during storage at 40°C

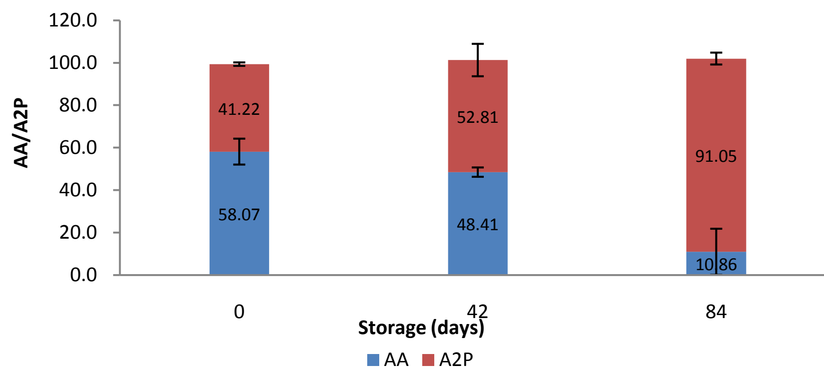

Fig. 21: Ascorbyl 2 phosphate and ascorbic acid concentrations (%) in the A2P-0.01%Pycnogenol during storage at 40°C

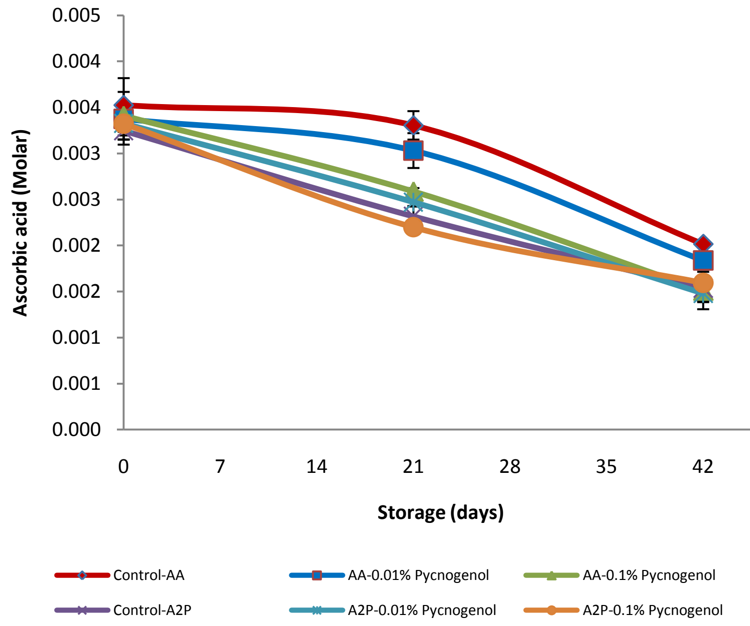

Fig. 22: Variation of the ascorbic acid concentration during storage at 51°C

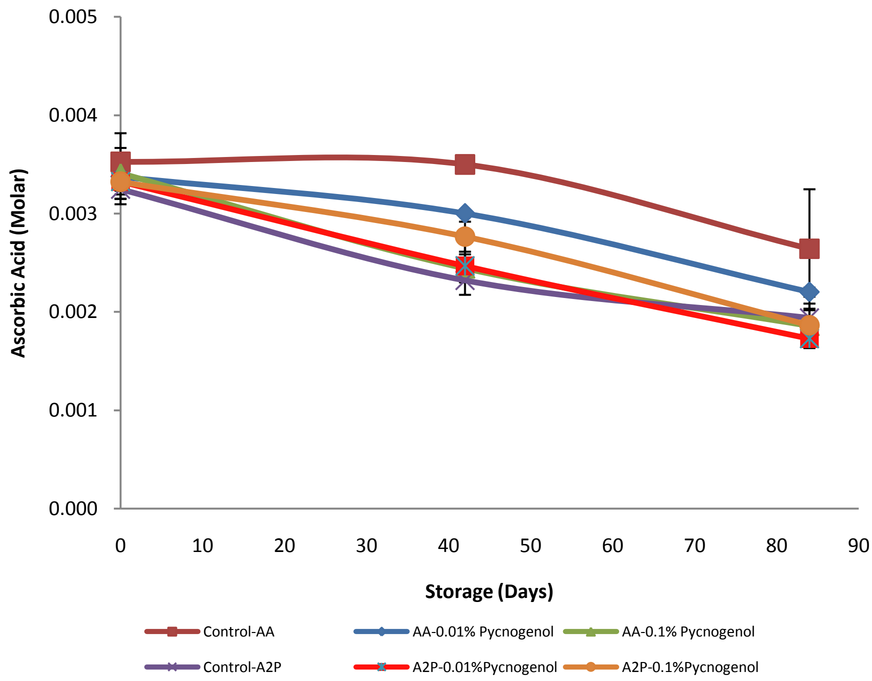

Fig. 23: Variation of the ascorbic acid concentration during storage at 40°C

Table 1: Characterizations of syrups

Syrup	pH	°Brix
AA-Control	2.53	47.5
AA-001% Pycnogenol	2.47	47.4
AA-0.1% Pycnogenol	2.40	47.8
A2P-Control	6.63	47.2
A2P-0.01% Pycnogenol	6.00	47.8
A2P-0.1% Pycnogenol	5.68	47.0

Table 2: Changes of pH during storage at 51°C and 40°C in retortable pouches

	Storage (days)		
Treatment	0	42 at 51°C	84 at 40°C
Control-AA	3.32 ± 0.04	3.32 ± 0.03	3.4 ± 0.02
AA-0.01%Pycnogenol	3.33 ± 0.04	3.38 ± 0.03	3.37 ± 0.01
AA-0.1%Pycnognenol	3.37 ± 0.03	3.40 ± 0.1	3.33 ± 0.03
Control-A2P	3.46 ± 0.08	3.42 ± 0.16	3.44 ± 0.02
A2P-0.01%Pycnogenol	3.48 ± 0.01	3.51 ± 0.01	3.45 ± 0.03
A2P-0.1%Pycnogenol	3.53 ± 0.01	3.47 ± 0.09	3.48 ± 0.04

Table 3: Changes of °Brix during storage at 51°C and 40°C in retortable pouches

	Storage (days)		
Treatment	0	42 at 51°C	84 at 40°C
Control-AA	18.2 ± 0.2	18.05 ± 0.07	18.0 ± 0.1
AA-0.01%Pycnogenol	18.4 ± 0.5	17.95 ± 0.07	18.10 ± 0.02
AA-0.1%Pycnognenol	18.4 ± 0.5	18.1 ± 0.4	18.20 ± 0.05
Control-A2P	18.8 ± 0.9	18.3 ± 0.4	18.30 ± 0.02
A2P-0.01%Pycnogenol	17.6 ± 0.5	18.1 ± 0.1	18.01 ± 0.03
A2P-0.1%Pycnogenol	18.5 ± 0.7	18.2 ± 0.2	18.2 ± 0.1

Table 4: Vitamin C and Pycnogenol concentration effect on L* values at the beginning and at the end of the storage

Factor	Levels	Day 0	Day 42 (Storage at 51°C)	Day 84 (Storage at 40°C)
Vitamin C	A2P	51.70[a]	43.87[a]	48.65[a]
	AA	51.33[a]	42.29[b]	46.60[b]
Pycnogel concentration	0%	51.17[a,b]	43.39[a]	47.92 [a]
	0.01%	52.77[a]	43.19 [a]	47.56 [a]
	0.10%	50.60[b]	42.66 [a]	47.20 [a]
Vitamin C*Pycnogenol	Interaction	n.s.	n.s.	n.s.

Different letters indicate significant differences ($p<0.05$) exist between means of the levels within each factor.
n.s. and * correspond to non-significant ($p>0.05$) and significant ($p<0.05$) effects due to interaction of $CaCl_2$ and flavonoid, respectively.

Table 5: Vitamin C and Pycnogenol concentration effect on a* values at the beginning and at the end of the storage

Factor	Levels	Day 0	Day 42 (Storage at 51°C)	Day 84 (Storage at 40°C)
Vitamin C	A2P	-0.57[a]	7.10[a]	2.37[a]
	AA	-1.54[b]	7.19[a]	2.79[a]
Pycnogel concentration	0%	-2.18[b]	6.70[b]	30.73[a]
	0.01%	-2.03[b]	6.59[b]	28.11[b]
	0.10%	1.04[a]	8.14[a]	29.55[c]
Vitamin C*Pycnogenol	Interaction	n.s.	n.s.	*

Different letters indicate significant differences ($p<0.05$) exist between means of the levels within each factor.
n.s. and * correspond to non-significant ($p>0.05$) and significant ($p<0.05$) effects due to interaction of $CaCl_2$ and flavonoid, respectively.

Table 6: Vitamin C and Pycnogenol concentration effect on b* values at the beginning and end of the storage

Factor	Levels	Day 0	Day 42 (Storage at 51°C)	Day 84 (Storage at 40°C)
Vitamin C	A2P	29.56[a]	26.05[a]	28.78[a]
	AA	29.36[a]	24.73[a]	26.15[b]
Pycnogel concentration	0%	29.55[a]	25.79[a]	26.75[a]
	0.01%	30.73[a]	25.32[a]	27.75[a]
	0.10%	28.12[a]	25.06[a]	27.38[a]
Vitamin C*Pycnogenol	Interaction	n.s.	n.s.	n.s.

Different letters indicate significant differences ($p<0.05$) exist between means of the levels within each factor.
n.s. and * correspond to non-significant ($p>0.05$) and significant ($p<0.05$) effects due to interaction of $CaCl_2$ and flavonoid, respectively.

Table 7: Vitamin C and Pycnogenol concentration effect on ΔE* values at the end of the storage

Factor	Levels	Day 42 (Storage at 51°C)	Day 84 (Storage at 40°C)
Vitamin C	A2P	11.47[a]	4.66[a]
	AA	13.43[b]	7.32[b]
Pycnogel concentration	0%	12.15[b]	5.69[b]
	0.01%	14.02[b]	7.34[a]
	0.10%	11.19[a]	5.21[b]
Vitamin C*Pycnogenol	Interaction	n.s.	n.s.

Different letters indicate significant differences ($p<0.05$) exist between means of the levels within each factor.
n.s. and * correspond to non-significant ($p>0.05$) and significant ($p<0.05$) effects due to interaction of $CaCl_2$ and flavonoid, respectively.

Table 8: Rate constants of L*, a* and ΔE* parameters

Treat.[a]	Temp.[b]	Model	Para.[c]	L*			a*			ΔE*		
				Estimate (day^{-1})	R^2	RMSE[d]	Estimate (day^{-1})	R^2	RMSE[d]	Estimate (day^{-1})	R^2	RMSE[d]
A	50	Zero-order	k	-0.21 ± 0.03	0.92	1.34	0.22 ± 0.02	0.96	0.63	0.33 ± 0.05	0.93	1.89
		Combined model	k_0	2.1 ± 0.62	0.99	0.56	0.30 ± 0.03	0.99	0.42	0.77 ± 0.04	0.99	0.26
			k_1	0.05 ± 0.01			0.03 ± 0.01			0.05 ± 0.01		
	40	Zero-order	k	-0.04 ± 0.01	0.9	0.68	0.06 ± 0.004	0.98	0.30	0.08 ± 0.02	0.82	1.56
		Combined model	k_0	0.40 ± 0.63	0.96	0.76	0.06 ± 0.005	0.99	0.34	0.19 ± 0.09	0.89	1.24
			k_1	0.01 ± 0.01			0.001 ± 0.006			0.03 ± 0.02		
B	50	Zero-order	k	-0.23 ± 0.03	0.93	1.28	0.22 ± 0.02	0.97	0.85	0.34 ± 0.03	0.98	1.04
		Combined model	k_0	0.37 ± 1.17	0.94	1.42	0.27 ± 0.04	0.98	0.69	0.45 ± 0.07	0.99	0.83
			k_1	0.01 ± 0.03			0.02 ± 0.01			0.01 ± 0.01		
	40	Zero-order	k	-0.07 ± 0.02	0.84	0.80	0.05 ± 0.003	0.99	0.24	0.10 ± 0.01	0.94	1.09
		Combined model	k_0	-0.73 ± 1.05	0.86	1.37	0.06 ± 0.001	0.99	0.07	0.08 ± 0.03	0.94	1.07
			k_1	-0.01 ± 0.02			-0.01 ± 0.001			0.00 ± 0.01		
C	50	Zero-order	k	-0.21 ± 0.02	0.97	0.72	0.18 ± 0.02	0.96	0.79	0.30 ± 0.02	0.99	0.62
		Combined model	k_0	-0.07 ± 0.73	0.97	0.83	0.20 ± 0.10	0.96	0.90	0.37 ± 0.04	0.99	0.49
			k_1	0.001 ± 0.016			0.001 ± 0.019			0.01 0.01		
	40	Zero-order	k	-0.06 ± 0.01	0.91	0.78	0.05 ± 0.004	0.96	2.63	0.08 ± 0.01	0.96	0.72
		Combined model	k_0	-0.56 ± 0.73	0.92	0.83	0.02 ± 0.02	0.99	0.27	0.08 ± 0.02	0.96	0.71
			k_1	-0.01 ± 0.02			-0.01 ± 0.01			0.001 ± 0.008		
D	50	Zero-order	k	-0.16 ± 0.01	0.98	0.45	0.20 ± 0.01	0.99	0.29	0.25 ± 0.01	0.99	0.56
		Combined model	k_0	0.04 ± 0.60	0.98	0.51	0.18 ± 0.01	0.99	0.15	0.30 ± 0.03	0.99	0.50
			k_1	0.001 ± 0.010			-0.01 ± 0.003			0.010 ± 0.007		
	40	Zero-order	k	-0.03 ± 0.01	0.95	0.33	0.02 ± 0.01	0.73	0.61	0.05 ± 0.01	0.91	0.74
		Combined model	k_0	-0.12 ± 1.01	0.95	0.46	0.14 ± 0.02	0.99	0.98	0.15 ± 0.00	100	0.00
			k_1	0.001 ± 0.02			-0.09 ± 0.01			0.04 ± 0.00		
E	50	Zero-order	k	-0.23 ± 0.01	0.99	0.43	0.19 ± 0.01	0.99	0.30	0.33 ± 0.01	0.99	0.29
		Combined model	k_0	-0.71 ± 0.32	0.99	0.38	0.21 ± 0.02	0.99	0.33	0.31 ± 0.02	0.99	0.26
			k_1	-0.01 ± 0.01			0.001 ± 0.010			0.001 ± 0.003		
	40	Zero-order	k	-0.05 ± 0.01	0.93	0.74	0.05 ± 0.01	0.92	0.59	0.08 ± 0.02	0.86	1.49
		Combined model	k_0	1.64 ± 0.39	0.99	0.20	0.04 ± 0.01	0.95	0.54	6.78 ± 0.49	0.99	1.00
			k_1	0.03 ± 0.01			-0.02 ± 0.01			0.98 ± 0.00		
F	50	Zero-order	k	-0.17 ± 0.02	0.93	0.97	0.15 ± 0.01	0.99	0.34	0.24 ± 0.02	0.96	0.97
		Combined model	k_0	-1.07 ± 1.16	0.94	1.01	0.16 ± 0.05	0.99	0.43	0.18 ± 0.05	0.97	0.91
			k_1	-0.02 ± 0.02			0.0 0.01			-0.01 ± 0.01		
	40	Zero-order	k	-0.02 ± 0.01	0.56	0.76	0.02 ± 0.003	0.91	0.29	0.04 ± 0.01	0.91	0.54
		Combined model	k_0	0.13 ± 2.07	0.56	0.88	0.001 ± 0.028	0.93	0.30	0.04 ± 0.02	0.91	0.44
			k_1	0.001 ± 0.042			-0.01 ± 0.014			0.00 ± 0.01		

[a]Treat.: Treatment; [b]Temp.: Temperature (°C); [c]Para.: Parameter; [D]RMSE: Root Mean Square

Table 9: Vitamin C and Pycnogenol concentration effect on browning index values at the beginning and end of the storage

Factor	Levels	Day 0	Day 42 (Storage at 51°C)	Day 84 (Storage at 40°C)
Vitamin C	A2P	0.023[a]	0.076[a]	0.050[a]
	AA	0.024[a]	0.104[b]	0.053[a]
Pycnogenol concentration	0%	0.018[a]	0.088[a]	0.041[a]
	0.01%	0.023[a]	0.081[a]	0.052[a]
	0.10%	0.028[a]	0.102[a]	0.061[a]
Vitamin C*Pycnogenol	Interaction	n.s.	n.s.	n.s.

Different letters indicate significant differences ($p<0.05$) exist between means of the levels within each factor.
n.s. and * correspond to non-significant ($p>0.05$) and significant ($p<0.05$) effects due to interaction of $CaCl_2$ and flavonoid, respectively.

Table 10: Rate constants of browning index

Treat.[a]	Temp.[b]	Model	Para.[c]	Browning index: Estimate (day^{-1})	R^2	RMSE[d]
A	50	First-order	k	0.04 ± 0.01	0.94	0.11
		Combined model	k0	0.001 ± 0.001	0.95	0.01
			k1	-0.03 ± 0.02		
	40	Zero-order	k	0.0004 ± 0.0001	0.75	0.01
		Combined model	k0	0.003 ± 0.002	0.91	0.01
			k1	0.051 ± 0.049		
B	50	First-order	k	0.034 ± 0.002	0.98	0.01
		Combined model	k0	0.0009 ± 0.0001	0.99	0.01
			k1	-0.013 ± 0.002		
	40	Zero-order	k	0.0003 ± 0.0001	0.65	0.01
		Combined model	k0	0.023 ± 0.001	0.93	0.01
			k1	0.54 ± 0.00		
C	50	First-order	k	0.031 ± 0.004	0.95	0.01
		Combined model	k0	-0.001 ± 0.002	0.96	0.01
			k1	-0.052 ± 0.028		
	40	Zero-order	k	0.0004 ± 0.0001	0.76	0.01
		Combined model	k0	-0.001 ± 0.002	0.87	0.01
			k1	-0.04 ± 0.03		
D	50	First-order	k	0.031 ± 0.007	0.87	0.01
		Combined model	k0	-0.002 ± 0.0009	0.99	0.01
			k1	-0.12 ± 0.029		
	40	Zero-order	k	0.0002 ± 0.0001	0.67	0.01
		Combined model	k0	0.0007 ± 0.0021	0.70	0.01
			k1	0.019 ± 0.06		
E	50	First-order	k	0.026 ± 0.007	0.85	0.01
		Combined model	k0	-0.0007 ± 0.0020	0.86	0.01
			k1	-0.04 ± 0.05		
	40	Zero-order	k	0.0004 ± 0.0001	0.71	0.01
		Combined model	k0	0.0000 ± 0.0013	0.72	0.01
			k1	-0.009 ± 0.031		
F	50	First-order	k	0.029 ± 0.002	0.98	0.01
		Combined model	k0	0.0004 ± 0.001	0.97	0.01
			k1	-0.02 ± 0.019		
	40	Zero-order	k	0.0003 ± 0.0001	0.77	0.01
		Combined model	k0	0.0002 ± 0.001	0.77	0.01
			k1	-0.002 ± 0.026		

[a]Treat.: Treatment; [b]Temp.: Temperature; [c]Para.: Parameter; [D]RMSE: Root Mean Square

Table 11: Vitamin C and Pycnogenol concentration effect on vitamin C concentration at the beginning and end of the storage

Factor	Levels	Day 0 (Molar)	Day 42 at 51°C (Molar)	Day 84 at 40°C (Molar)
Vitamin C	**A2P**	0.0033[a]	0.0015[a]	0.0022[a]
	AA	0.0034[a]	0.0018[b]	0.0018[a]
Pycnogel concentration	**0%**	0.0034[a]	0.0018[a]	0.0024[a]
	0.01%	0.0034[a]	0.0016[a]	0.0020[a]
	0.10%	0.0034[a]	0.0016[a]	0.0019[a]
Vitamin C*Pycnogenol	**Interaction**	n.s.	n.s.	n.s.

Different letters indicate significant differences ($p<0.05$) exist between means of the levels within each factor.
n.s. and * correspond to non-significant ($p>0.05$) and significant ($p<0.05$) effects due to interaction of $CaCl_2$ and flavonoid, respectively.

Table 12: Rate constants vitamin C

Treat.	Temp.	Model	Para.	Vitamin C		
				Estimate (day^{-1})	R^2	RMSE
A	50	Zero-order	k	$-3.60x10^{-5} \pm 0.0$	0.82	0.0003
		Weibull model	k	0.020 ± 0.002	0.90	0.0002
			β	3.1 ± 1.2		
	40	Zero-order	k	$-1.02x10^{-5} \pm 0.0$	0.55	0.0004
		Weibull model	k	0.01 ± 0.19	0.60	0.0004
			β	11 ± 1.7		
B	50	Zero-order	k	$-3.67x10^{-5} \pm 0.0$	0.90	0.0003
		Weibull model	k	0.020 ± 0.001	0.90	0.0001
			β	2.5 ± 0.4		
	40	Zero-order	k	$-1.40x10^{-5} \pm 0.0$	0.96	0.0001
		Weibull model	k	0.008 ± 0.0003	0.99	0.0000
			β	1.86 ± 0.13		
C	50	Zero-order	k	$-4.52x10^{-5} \pm 0.0$	0.97	0.0002
		Weibull model	k	0.021 ± 0.009	0.98	0.0002
			β	1.6 ± 0.34		
	40	Zero-order	k	$-1.85x10^{-5} \pm 0.0$	0.93	0.0002
		Weibull model	k	0.006 ± 0.002	0.92	0.0002
			β	0.8 ± 0.34		
D	50	Zero-order	k	$-4.17x10^{-5} \pm 0.0$	0.97	0.0002
		Weibull model	k	0.019 ± 0.002	0.97	0.0002
			β	1.2 ± 0.28		
	40	Zero-order	k	$-1.62x10^{-5} \pm 0.0$	0.93	0.0002
		Weibull model	k	0.004 ± 0.005	0.93	0.0002
			β	0.62 ± 0.50		
E	50	Zero-order	k	$-4.40 x10^{-5} \pm 0.0$	0.99	0.0001
		Weibull model	k	0.02 ± 0.001	0.99	0.0001
			β	1.5 ± 0.1		
	40	Zero-order	k	$-1.90x10^{-5} \pm 0.0$	0.96	0.0002
		Weibull model	k	0.008 ± 0.002	0.97	0.0002
			β	1.13 ± 0.33		
F	50	Zero-order	k	$-4.05x10^{-5} \pm 0.0$	0.97	0.0002
		Weibull model	k	0.016 ± 0.002	0.95	0.0001
			β	0.84 ± 0.12		
	40	Zero-order	k	$-1.74x10^{-5} \pm 0.0$	0.94	0.0002
		Weibull model	k	0.008 ± 0.001	0.94	0.0002
			β	1.7 ± 0.40		

[a]Treat.: Treatment; [b]Temp.: Temperature (°C); [c]Para.: Parameter; [D]RMSE: Root Mean Square

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CHAPTER 5: EFFECT OF α-GLUCOSYL RUTIN, CALCIUM CHLORIDE AND FRUIT SOURCE ON THE QUALITY OF DICED PEACHES IN FLEXIBLE RETORTABLE POUCHES

ABSTRACT

Diced peaches in flexible retortable pouches have been identified as an area of concern due to the darkening produced by ascorbic acid degradation during storage. Experiments were conducted to determine if α-glucosylrutin (α-GR) would delay browning reactions. α-GR is a flavonoid manufactured through the addition of a molecule of glucose to rutin to improve its solubility. The use of $CaCl_2$ and different fruit sources (fresh, individually quick frozen and canned peaches) were also evaluated. Samples were stored at 51°C and 40°C for 42 and 270 days, respectively. Vitamin C concentrations, color (CIELAB), pH and °Brix were measured during storage. Rate constants were calculated to compare the different treatments. A complete factorial design was used to determine effects due to the flavonoid or $CaCl_2$. At 51°C, the treatments (control, $CaCl_2$, α-GR and $CaCl_2$-α-GR) did not slow the rate of AA or color deterioration. At 40°C, the treatments that had α-GR and were made using individually quick frozen or canned peaches had a lower rate of browning and loss of ascorbic acid than the rest of treatments.

KEYWORDS: Ascorbic acid; α-glucosylrutin; non-enzymatic browning; retortable pouches; peaches.

INTRODUCTION

Shelf stable fruits have been identified as a problem due to the appearance of brown pigments resulting from non-enzymatic reactions. In fact, in acid food products, non-enzymatic browning due to degradation of ascorbic acid (AA) is a major pathway leading to browning (Fennema 1996). This type of browning as well as methods of delaying its appearance have been studied in many food systems such as citrus juices, pears, fruit puree, fruit pulps, among others (Roig and others 2000; Chutintrasri and Noomhorm 2007; Ibarz and others 1999; Garza and others 1999; Lozano and Ibarz 1996; Buglione and Lozano 2002; Kacem and others 1987).

Non-enzymatic browning due to AA degradation has been identified as the principal reason of the short shelf life of diced peaches packaged in retortable pouches. In fact, this product used to be included by the US army in the Individual Meals Ready-to-Eat (MRE) military rations; however, due to the browning that developed during storage, this item was removed. Improvement of the shelf life of this product has become a challenge, especially because this item is delivered to areas where refrigeration is not available and ambient temperatures are high. Currently, there are just a few studies that have focused on non-enzymatic browning of food products packaged in retortable pouches (Clark and others 2002; Kluter and others 1996; Kluter and others 1994; Olivas and others 2002; Rodriguez and others 2003).

The use of flavonoids in food systems to delay AA browning has not been studied. Flavonoids are secondary plant metabolites that have been shown to have good antioxidant properties, due to their free radical scavenging and metal chelation activities. Their protective role against ascorbic acid degradation has been known since 1936, when Szent-Györgyi and coworkers found that flavonoids, at that time called Vitamin P, had ascorbate protective properties (Rice-Evans and Packer 1998). Subsequently, research *in-vitro* has confirmed this protective role (Bors and others 1995; Rice-Evans and Packer 1998; Sivonova and others 2006; Cossins and others 1998). Also, there is evidence that shows that juices with high levels of phenolic compounds have good AA stability (Clegg and Morton 1968; Rice-Evans and Packer 1998). Unfortunately, the study of flavonoids in food products is hampered by their low solubility. In order to counteract this disadvantage, some flavonoids, such as rutin (quercetin-3-

rutinoside), have been modified by the addition of glucose groups (Schonrock and Kruse 2004; Anonymous 2006); however, the antioxidant and anti-browning effects of modified flavonoids in actual food systems have not yet been studied.

The objective of this research was to evaluate the effect of a water soluble flavonoid, α-glucosylrutin, as an antibrowning agent in diced peaches packaged in retortable pouches. In addition, we investigated the effects of calcium chloride ($CaCl_2$) and fruit source. $CaCl_2$ is usually added to processed fruits to form insoluble pectates, which increases the firmmess of food products (Fellows 2009). Besides browning, texture deterioration is a problem in MRE peach products during storage. The browning rates of MRE packaged fresh and processed peaches (IQF and canned) are of interest since because the US army has contemplated the production of MREs from these different raw materials.

MATERIALS AND METHODS

Peaches of a clingstone variety were obtained and processed at McCall Farm (Effingham, SC) in August, 2009. The processing consisted on lye peeling, pitting, washing and dicing. Approximately 1.9 cm chunks were prepared using a commercial dicer (Urschel Dicer, Valparaiso, IN). The operations after these steps were slightly different for each type of source (fresh, IQF and canned peaches).

Dices for IQF treatment were dipped in an ascorbic acid solution (approximately 1000pm) to inhibit browning. The dipped dices were drained and conveyed in a blast freezer tunnel (18.2 meters long x 5.5 meters wide x 5.5 meters tall) at -40°C. After freezing, the diced peaches were packaged in PET bags in cartoon boxes and stored for 40 days at normal commercial freezing temperatures (-18°C). Dices for canning treatment were placed in N°10 cans coated with enamel. The diced peaches inside the cans were mixed with syrup composed of sucrose (21°Brix) and $CaCl_2$ (0.5%). The cans were exhausted, sealed, and retorted using an F-value (z= 10°C, T=100°C) of 6.3 min. The canned peaches were stored for 40 days at room temperature before processing into retort pouches. For the fresh source, the peaches, after dicing, were placed immediately into of the pouches.

The fresh diced peaches in pouches were retorted the same day that the peaches were obtained. The IQF and canned peaches were processed 40 days later. This was designed to mimic the processes that have been used commercially in the past for MRE peach products. Canned peaches were drained and placed in the pouches. The syrup of the cans were retained and modified to be used in the pouches of this source. IQF diced peaches were placed in the pouches immediately (no thawing). In all the sources, the ratio of diced peaches and syrup was 3:1, w/w, and the net pouch content weight was 140g. Four types of syrups corresponding to the four different types of treatments were prepared. Syrup 1 was composed of water, sucrose and ascorbic acid while syrup 2 had in addition $CaCl_2$ (0.5%). Syrups 3 and 4 were composed as 1 and 2 respectively but had in addition the flavonoid α-glucosyl rutin (0.3% w/w). The amounts of sucrose and ascorbic acid were added to the syrup to reach final concentrations of 18°Brix and 700 ppm of ascorbic acid in each pouch. After sealing, the pouches were retorted using an F-value (z= 10°C, T=100°C) of 6.3 min.

After processing, samples were stored at refrigeration (4°C) for 1 month for the fresh and IQF sources, and for 3 months for the canned source. The samples were then stored at three different temperatures (51°C, 40°C and 24°C). At 51°C, the samples were analyzed at day 0, 14, 28 and 42; at 40°C, they were examined at day 0, 90, 180 and 270 for the fresh and IQF sources while at day 0, 88, 193 and 245 for the canned source. At 24°C, the analyses were conducted at day 0, 180 and 240 for the fresh source, at day 0, 180 and 295 for the IQF source, and at 0 and 180 days of the canned source. Three repetitions were prepared for each temperature and treatment.

Ascorbic acid determination

A high performance liquid chromatography (HPLC) procedure based on an earlier report (Gokmen and others 2000) was used to quantify ascorbic acid in the samples. Diced peaches and syrup, in the same ratio as they were found in each pouch (3:1, w/w), were combined with a methaphosphoric acid (5%w/v aq.) solution in a ratio of 3:1 (product:methaposphoric acid solution, w/v). This mixture was homogenized for 90 seconds using a Virtis homogenizer-Model TP 18/10 (Wilmington, NC) at medium speed. The homogenized samples were filtered through Whatman No. 4 (Maidstone, UK) and 0.45 μm filters (Millipore, Massachusetts, USA).

The samples were protected from light during filtration. The clarified samples were analyzed by high performance liquid chromatography (HPLC). A model 1200 HPLC consisting of degasser, pump, refrigerated autosampler, column oven, and diode array ultraviolet-visible (UV-VIS) detector was obtained from Agilent (Santa Clara CA). The mobile phase was pH 2.4 20mM aqueous phosphate buffer. The column (Luna 5μ C18(2) 100Å, 250mm x 4.6mm, i.d.) was obtained from Phenomenex (Torrance CA) and was used at a flow rate was 0.5 ml/min. The detector was set to 245nm and UV spectra from 190-300 NM were recorded.

Colorimetric Analysis

A Chroma Meter CR-200 tristimulus color analyzer (Minolta, Osaka, Japan) was calibrated with a standard plate (L*: 97.20, a*: -0.18, b*: 3.75) before each use. The diced peaches (70±5 g) were placed inside a glass petri dish (9 cm diameter), and the color was measured from the bottom of the dish. Before each measurement, the measuring head of the colorimeter was covered with a cardboard box to block stray light. All readings were obtained in the CIELAB scale (L*, a*, b*). The ΔE* was calculated in order to quantify the variation of color throughout storage. ΔE*was defined by the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*) + (\Delta a^*) + (\Delta b^*)}$$

pH and °Brix

The pH and °Brix were measured directly from the puree after opening the vials. The pH and °Brix were measured using a potentiometer XL-20 (Fisher Scientific) and an Abbe Mark II (Reichertai Inc,. Buffalo, NY), respectively.

Statistical analysis

The rate constants (*k*) were obtained by linear regression analysis (Arabshahi and Lund 1985). The color parameters (L*, a*, b* and ΔE*) were evaluated through equations (1), (2), (3) and (4). In the case of ΔE*, instead of equation (4), we applied equation (5) because this parameter, ΔE*, is zero at at day 0 (Ibarz and others 1999). For the *k* values of vitamin C degradation, the data collected were fitted using the equations (1), (2), (3) and (6). The best

kinetic model was chosen after comparison of the coefficients of determination (R^2), root mean square error (RMSE) and visual examination of distribution of residuals of plotted data.

Zero order (eq. 1): $$C = C_0 + k_0 t$$

First order (eq. 2): $$C = C_0 \exp(k_1 t)$$

Second order (eq. 3): $$c = \frac{1}{\left(\frac{1}{c_0}\right) + (k_2 t)}$$

Combined kinetics model (eq. 4): $$C = \frac{k_0}{k_1} - (k_0 - C_0)\exp(-k_1 t)$$

Combined kinetics model in ΔE* (eq. 5): $$\Delta E = \frac{k_0}{k_1}(1 - \exp(-k_1 t))$$

Weibull model (eq. 6):

$$C = C_0 \exp(-k_1 t)^{\beta}$$

Where:

C = Variable content studied at time t

C_0 = Variable content at time zero

k_0 = Zero order kinetic constant

k_1 = First order kinetic constant

k_2 = Second order kinetic constant

β = Shape parameter

t = time

The fitting process for the equations (1), (2) and (3) was done by using linear regression in JMP 8.0 (SAS Institute, Cary, NC). Equations (4), (5) and (6) were solved by non linear techniques using Statgraphics Plus v. 5.1 (Statgraphics, Warrenton, VA). A complete factorial design using JMP 8.0 (SAS Institute, Cary, NC) was conducted to determine if there were

significant effects due to the $CaCl_2$ or α-glucosylrutin. Tukey´s HSD was used to evaluate the differences among each treatment.

RESULTS AND DISCUSSION

The L*, a* and b* parameters indicate how dark/light, red/green and yellow/blue the samples are, respectively (HunterLab 2008). ΔE* indicates the size of the color difference in reference to the color measured at day 0. The variation of these parameters throughout the storage are shown from Figs. 1 to 8 for the three types of pouches (A: pouches made of fresh peaches; B: pouches made of IQF peaches; C: pouches made of canned peaches) and for each treatment (control, $CaCl_2$, flavonoid, $CaCl_2$-flavonoid). Table 1 shows the pH and °Brix values at the beginning and end of the 51°C and 40°C storage. Table 2 shows the factorial analysis design at the beginning and end of the storage at 51°C and 40°C. This design was conducted to determine if there were effects due to the addition of $CaCl_2$ and flavonoid or interaction between these factors. In addition, Table 3 shows the rate constants *(k)* of each of the parameters measured. These *k* values were calculated from the zero order models. As noted earlier, different models were applied to the data (zero, first, second and combined order models); however, the zero order was the one that had the highest R^2 and lowest root mean square error (RMSE). Results from samples stored at 24°C are not included since there were no changes of chemical importance during the storage period examined.

pH and •Brix during storage

Table 1 shows the pH and °Brix values at the beginning and end of storage for each temperature. No significant changes of chemical importance in pH and °Brix were observed at either temperature. It has been suggested that the pH may decrease during non-enzymatic browning, due to the appearance of carbonyl compounds (Roig and others 2000). On the other hand, it has also been reported that pH may increase during storage of retort pouch wet-pack pears (Olivas and others 2002). In orange juice packed in tetrabrick cartons stored at temperatures between 4°C-105°C, a pH of 3.65 was maintained (Roig and others 2000). No changes in °Brix were observed in cashew apple (pH 4.4) during heating at temperatures from 88°C-121°C; in fact, this paper also shows constant levels of reducing and total sugars during storage, which suggests there was no significant participation of sugars in the non-enzymatic

reactions (Damasceno and others 2007). Reactions of reducing sugars and amino acids cause maillard reactions, which is a type of non-enzymatic browning favored by a more alkaline pH environment (Fennema 1996).

Effect of the source of fruit

At day 0, Fig. 1 shows that treatments of the canned source peaches have lower L* values than the other sources. This can be attributed to the double heat treatment of the diced peaches in this group. It is known that high temperatures have an effect on the pigments in fruits; indeed, carotenoids are converted from 5,6-expoxides to 5,8-epoxides, and 5,8-epoxide is less intensely colored than the original carotenoid (Fellows 2009). Also, it is known that heat sterilization produces cis/trans isomerization of carotenoids (Fennema 1996). In a study comparing fresh and canned peaches, it was reported that canned peaches had higher amounts of 9-cis β-carotene (Chandler and Schwartz 1987). In addition to the effect of canning, at day 0 we observed that L* values from treatments made with fresh peaches are less precise than those of other treatments. This can be attributed to the high variability on the initial color of fresh-diced peaches. In fact, during the filling of retort pouches with fresh peaches, some enzymatic browning may have occurred since no treatment was used to avoid phenoloxidase (PO) activity. This could also have caused the higher a* value of the treatment composed of flavonoid alone in the fresh source (Fig. 4). In contrast, before freezing the IQF dices were dipped in an ascorbic solution that may have inhibited the action of PO. Ascorbic acid was used as an inhibitor of PO because this compound can regenerate the phenol compounds avoiding the enzymatic browning (Cheftel and Cheftel 1992). Finally, the peaches from the can source were likely not affected by PO due to the much faster filling operation because the number of cans to be filled was lower than the number of pouches for the fresh source. In fact, each can contained approximately 850 g. of diced peaches, which corresponds approximately to the peach content of 8 pouches. This unbalanced design (IQF peaches were dipped in an AA bath after dicing while fresh and canned peaches were not) was used because each of the processes have been used commercially for the peach processing for the MRE pouches in the past.

The IQF treatments had the highest a* (more brownish) and lowest b* (more yellowness) values of the three peach sources (Fig. 3 and 5). As mentioned earlier, the color of the pouches

made of fresh and canned sources were influenced by the enzymatic browning and canning sterilization, respectively. However, for peaches processed with IQF freezing, it is possible that the frozen storage and the type of thawing may produce color changes as well. As we noted in the methods, an air-blast freezer was used. This type of freezer uses recycled air which can cause freezer burn and oxidative changes as well as dehydration losses in IQF products (Fellows 2009). Furthermore, the frozen storage can also produce degradation of pigments due to the activity of enzymes such as lipoxygenases and PO (Fellows 2009). Finally, it is important to note that thawing can produce critical changes if not done properly (Li and Sun 2002). In our research, since diced peaches were placed in the pouches while frozen, the thawing largely occurred in the retort. In general, it is recommended to use low temperatures for thawing to ensure food quality (Li and Sun 2002). A previous study on clingstone peaches packed in retort pouches found that, using a sensory panel for color assessment, the color quality was higher for IQF than for fresh source peaches at day 0 (Kluter and others 1994). This study mentions a difference on the quality between pouches made of fresh and IQF peaches since the former were probably not as ripe as the latter. Also, the authors mention that the levels of enzyme content in the fresh peaches were higher than in the IQF peaches. Finally, in comparison with our research, this published study included a blanching operation before freezing, and a partial thaw and rinse of the sliced peaches before placing them inside the pouches.

As we can see in Fig. 9, the initial level of ascorbic acid from the canned source was the highest. In fact, pouches made of fresh, IQF and canned peaches at day 0 had concentrations of 581±16, 488±20, and 875±124 ppm of AA, respectively. This difference in the canned source is most probably due to experiment error during the preparation of the syrups. The initial ascorbic acid was estimated using a test kit and ascorbic acid added to produce a final concentration of ~700ppm in the final product (syrup + peaches). The difference is important because there is evidence that AA concentration has an effect on the degree of browning. For example, in aseptically packaged orange juice stored at 24°C for 24 weeks, samples containing higher concentrations of AA developed more browning. In this research, the AA concentrations evaluated were 718, 300 and 42 ppm (Kacem and others 1987).

Even though, in general, all sources of peaches had similar trends at both temperatures (51°C and 40°C) in measured characteristics (L*, a*, b*, ΔE* and ascorbic acid concentration), differences in the values reached at the end of the storage were observed. In Table 3, the *k* values of IQF treatments were higher than fresh and canned treatments. Peaches from the IQF source tended to have higher rates of the detrimental reactions. This contradicts the result given in a previous study where, at the end of storage at 38°C for 15 months and 21°C storage for 36 months, pouches made of IQF diced peaches had higher quality than pouches made of fresh peaches (Kluter and others 1994). However, as we mentioned before, this published study mentions lower initial quality on the fresh source due to the probably lower ripeness and higher enzyme levels than the IQF peaches. In our study, all peaches had the same degree of ripeness; consequently, we believe that the differences were attributed to the process. In fact, in a study in peaches and mangoes, it was shown that air-blast freezing provoked higher cell damage than other types of freezing (cryogenic and high-pressure shift freezing) (Otero and others 2000). Also, the quick thawing realized inside of the pouches could also provoked damage in the internal cell structure.

Effect of $CaCl_2$

Table 2 shows that, at day 0 in the treatments within the fresh source, there were significant effects ($p<0.05$) due to $CaCl_2$ on the L* value. We do not believe that this difference was due to the presence of $CaCl_2$; instead, we think that this difference was due to the high variability on the color of the fresh peaches, which was discussed earlier in relation to enzymatic browning. In the other sources (IQF and canned peaches) no significant effects ($p>0.05$) due to the presence of $CaCl_2$ were observed.

At 51°C, among the treatments within each source, there were differences in the rate constants (Table 3). For example, among the L* values, the two treatments composed of IQF peaches and $CaCl_2$, and canned peaches and $CaCl_2$ alone had higher *k* values. Similar effects due to $CaCl_2$ were observed for the a* and ΔE* parameters, since among the treatments of fresh and IQF sources, those that had $CaCl_2$ alone had higher *k* values as well. Finally, in the AA analysis, Table 3 shows that within each source, all treatments had similar rate constants, with the exception of the treatments composed of $CaCl_2$ alone in the canned source and the treatment

made of IQF-α-GR-$CaCl_2$. At 40°C, similar results were obtained. In fact, Table 3 shows that for L* values, within the treatments of IQF and canned peaches, those composed of $CaCl_2$ and $CaCl_2$-α-GR had higher rates of darkening. Similar results were observed for the ΔE* parameter. Within the canned source, the treatment composed of $CaCl_2$ alone had a higher rate of AA loss.

The differences in *k* values due to $CaCl_2$ are correlated with the results shown in Table 2. Here we observe that at the end of the 51°C storage, in the IQF and canned sources, $CaCl_2$ provoked a higher darkness (lower L* values), higher reddish color (higher a* value), higher color change (higher ΔE*), and lower AA levels ($p<0.05$). For the b* values, $CaCl_2$ produced lower yellowness; however, the only significant difference ($p<0.05$) was seen in the canned source. For the fresh source, significant interactions ($p<0.05$) between $CaCl_2$ and flavonoid were observed in all parameters studied with the exception of the L* and b* values. At the end of the 40°C storage, $CaCl_2$ did not have an effect on any of the parameters for the fresh source; however, in the IQF source, significant interactions ($p<0.05$) between $CaCl_2$ and α-GR were obtained for all parameters with the exception of the a* value. Finally, in the canned source, significant interactions ($p<0.05$) were seen with the exception of a* and b* values; in fact, in these last parameters, $CaCl_2$ produced significant higher a* and lower b* values ($p<0.05$).

In the results explained above, we can observe that $CaCl_2$ accelerated the rate of non-enzymatic browning. The reported effect of $CaCl_2$ in non-enzymatic browning reaction is contradictory, according to different studies. For example, it was observed that the pre-treatment of tomato slices with 1% $CaCl_2$ (dipping solution) caused a lower degree of non-enzymatic browning after dehydration and storage of tomato powder. These authors note that it has been reported that $CaCl_2$ can either block amino groups or can chelate organic substances, resulting in a control of browning reactions (Ghavidel and Davoodi 2010). On the other hand, in dried apples, the pre-treatment of apple with a $CaCl_2$ dipping solution increased the non-enzymatic browning (in terms of L* value) when the concentrations were increased from 0.5% to 5% (Bolin and Steele 1987). It is our hypothesis that $CaCl_2$ increased the rate of darkening in our systems due to the oxidation of phenol compounds by this salt, with further reaction and polymerization of the quinones. In previous research on fresh pineapples, the application of a dipping solution (2% of $CaCl_2$) for 10 and 20 min resulted in a reduction of internal browning since not only the

PO were inhibited, but also the total content of phenolic compounds were reduced (Botrel and others 2000). In a more detailed study on the mechanisms of the inhibition of enzymatic reactions by sodium chloride, it was obtained that this salt inactivated PO and oxidized chlorogenic acid in a buffer system at pH of 4.6 (He and others 2008). These authors noted that the quinones formed after oxidation of chlorogenic acid did not polymerize; consequently, no browning occurred. We believe it likely that in our peach systems, $CaCl_2$ oxidized phenol compounds to quinones, which later reacted with other compounds, such as amino acids, increasing the rate of darkening. In fact, it is known that compounds resulting from the reaction of quinones and amino acids are colored (Bittner 2006). In peach cans stored at 37°C, the decrease in leucoanthocyanin (phenol compound) was presented at the same time as an increase in browning (Luh and Phithakpol 1972).

Effect of α-GR

Initially (day 0), the only significant effect ($p<0.05$) due to the presence of flavonoid was observed in the fresh source for the a* parameter (Table 2). As discussed earlier, we believe that this was due to the high variability on the initial color as a result of the activity of the PO enzymes before packaging.

Table 3 shows that α-GR did not have an effect on the rate constants of any of the studied parameter at 51°C of storage. However, at 40°C, effects were seen within the treatments made of IQF and canned sources. For example, within IQF pouches, the treatment which had α-GR alone had the lowest k values for L*, a* and ΔE* parameters, as well AA degradation. In pouches made of the canned source, the lowest rate of darkening was also presented by the same treatment (flavonoid alone) for ΔE* values.

Table 2 shows that at the end of the 51°C storage, α-GR caused a significantly lower browning ($p<0.05$) (lower a*) in the IQF source. In the canned source, α-GR produced a significantly ($p<0.05$) higher yellowness (higher b*) and lower change of color (lower ΔE*). At 40°C, in the presence of flavonoid, there was a significantly ($p<0.05$) lower browning (lower a* value) in the IQF and canned sources. Significant interactions ($p<0.05$) between $CaCl_2$ and

flavonoid were presented in most of the studied parameters in the IQF and canned sources, as we can see in Table 2.

The reason why the α-GR did not have an effect on the pouches made from fresh source can be attributed to the fact that PO catalyzed the flavonoid oxidation before this enzyme could be denatured in the heat sterilization. As we know, different compounds from the groups of mono, di or polyphenols can act as substrates in the enzymatic reactions (Cheftel and Cheftel 1992). Also, it has been reported that PO from two varieties of peaches were able to oxidized quercetin (Garro and Gasull 2010), which is a chemical component of rutin.

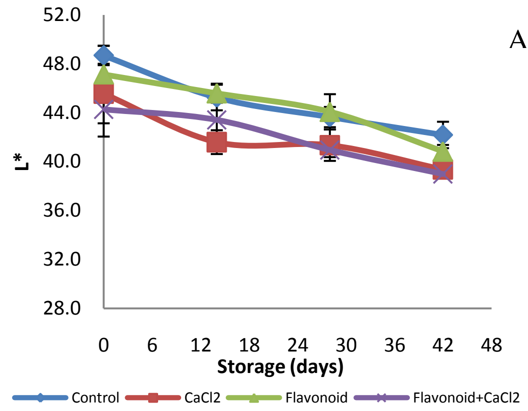
As mentioned earlier, even though in IQF and canned sources there was a desired effect due to the presence of α-GR, this was not observed in the treatment which also had $CaCl_2$. The reason can be attributed to the possible oxidation of α-GR by $CaCl_2$. As discussed earlier, $CaCl_2$ was able to reduce the total phenol content in pineapples (Botrel and others 2000) and sodium chloride was able to oxidized chlorogenic acid in a buffer at pH of 4.6 (He and others 2008). This could also explain why there were significant interactions ($p<0.05$) between flavonoids and $CaCl_2$ in some of the studied parameters.

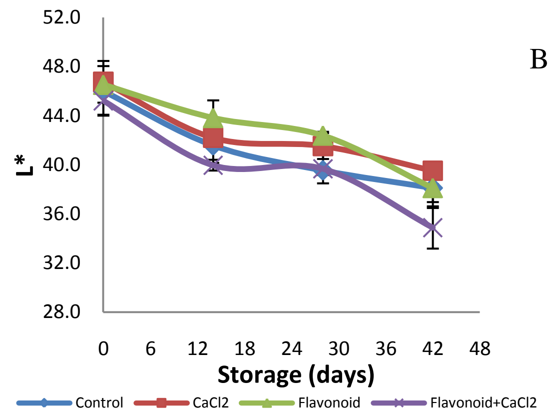
In the results presented above, we can also observe that the flavonoid exerted a stronger effect in the IQF than in the canned peaches. We can attribute this to the higher concentration of AA present in the canned treatments. In fact, the canned source had almost double the AA concentration compared to the IQF source. There is evidence that shows that higher concentrations of AA in orange drinks packaged in polyethylene bags corresponded to more browning development after 20 weeks of storage at 24°C (Kacem and others 1987). In fact the absorbance (420nm) measures were ~0.70, ~0.40, 0.15 for the 718, 300 and 42 ppm of AA, respectively. The same study done in retort pouches did not show so high differences probably due to the absence of oxygen; however, the results still show a difference between the drinks which had 42 ppm (~0.12 absorbance) and the drinks which had 300 and 718 ppm (~0.2 absorbance) after the same period of storage. A model solution of orange juice packaged in vials and stored at 50°C with headspace for 60 days also showed the contribution of the concentration

of AA in the developed browning. In fact, these results show absorbance (420 nm) of 0.19, 2.29, 3.72 and 4.62 for the 300, 7700, 10700 and 11400 ppm of AA (Shinoda and others 2004).

CONCLUSION

α-Glucosylrutin was added in systems made of diced peaches and syrup packaged in retortable pouches to prevent ascorbic acid browning. The use of $CaCl_2$ and different fruit sources (fresh, individually quick frozen and canned peaches) were also evaluated. Samples were stored at 51°C and 40°C for 42 and 270 days, respectively. Vitamin C concentrations and color (CIELAB) were measured during storage. Rate constants were calculated to compare the different treatments. A complete factorial design was used to determine effects due to the flavonoid or $CaCl_2$. At 51°C, the treatments (control, $CaCl_2$, α-GR and $CaCl_2$-α-GR) did not slow the rate of AA or color deterioration. At 40°C, the treatments that had α-GR and were made using individually quick frozen or canned peaches had a lower rate of browning and loss of ascorbic acid than the rest of treatments. There is evidence which suggests that $CaCl_2$ could promote ascorbic acid browning under the evaluated conditions.





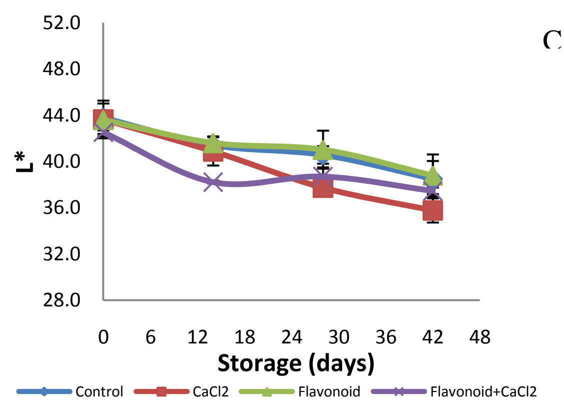


Fig. 1: Effect of treatment on L* value during storage at 51°C. A: Pouches made of fresh peaches; B: Pouches made of IQF peaches; C: Pouches made of canned peaches

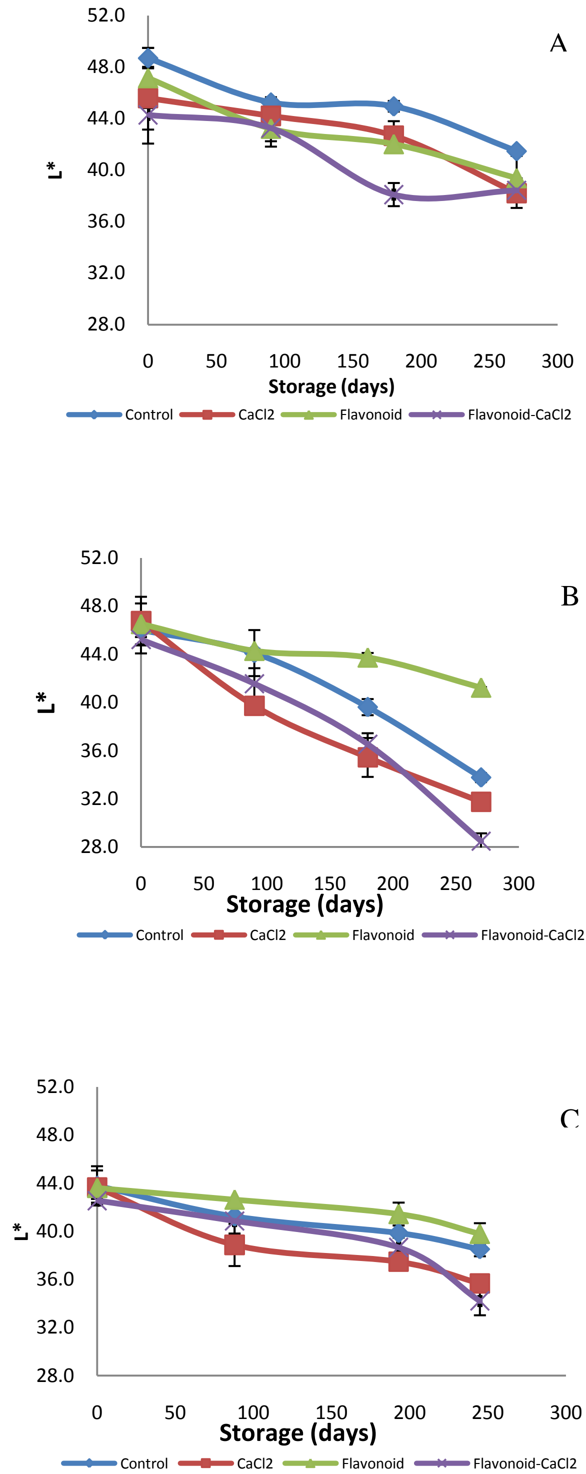


Fig. 2: Effect of treatment on L* value during storage at 40°C. A: Pouches made of fresh peaches; B: Pouches made of IQF peaches; C: Pouches made of canned peaches

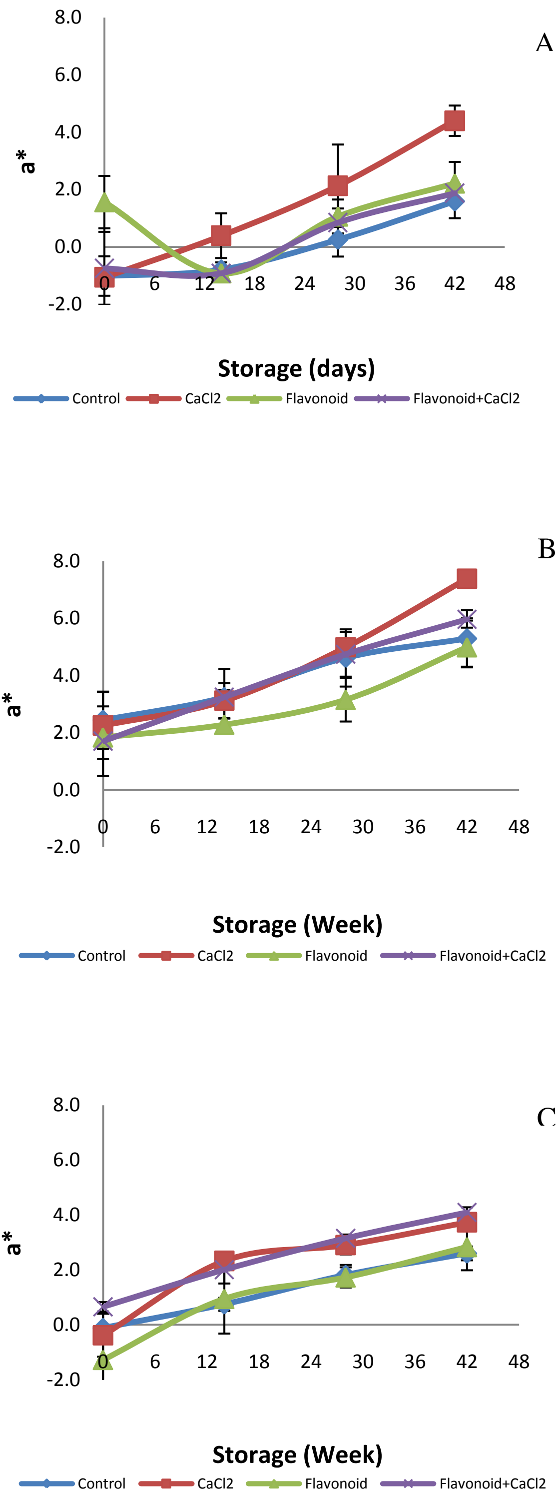


Fig. 3: Effect of treatment on a* value during storage at 51°C A: Pouches made of fresh peaches; B: Pouches made of IQF peaches; C: Pouches made of canned peaches

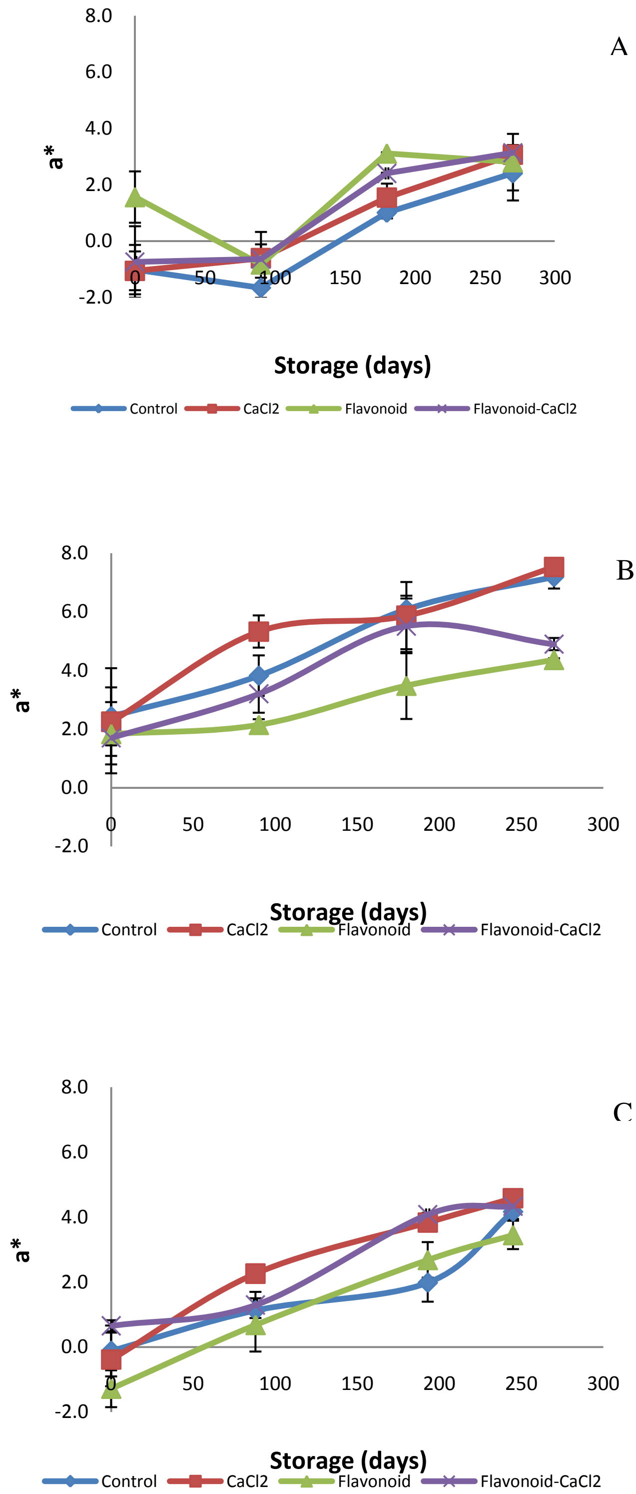
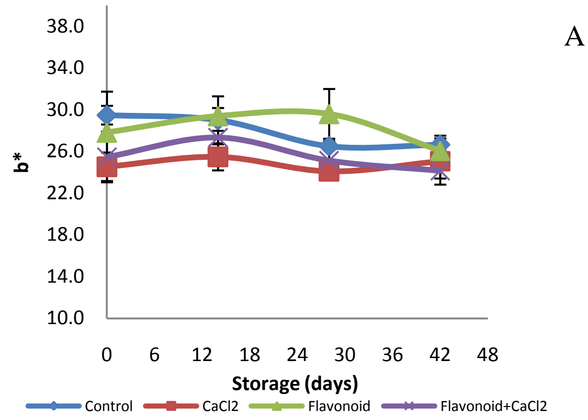
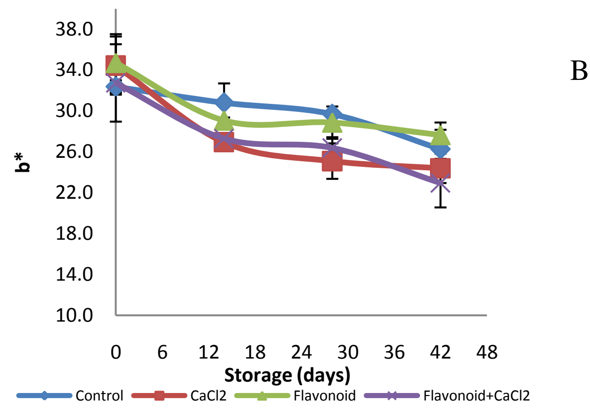


Fig. 4: Effect of treatment on a* value during storage at 40°C A: Pouches made of fresh peaches; B: Pouches made of IQF peaches; C: Pouches made of canned peaches





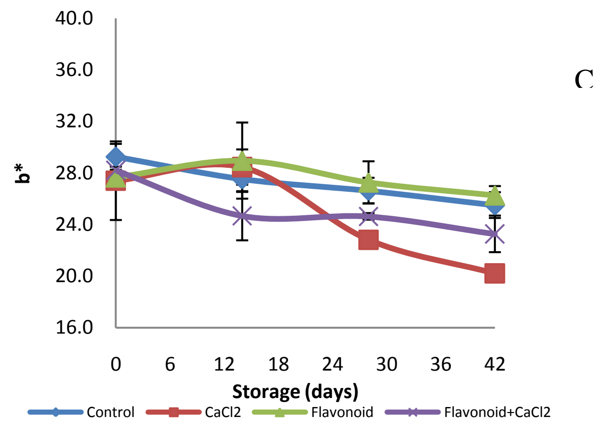


Fig. 5: Effect of treatment on b* value during storage at 51°C. A: Pouches made of fresh peaches; B: Pouches made of IQF peaches; C: Pouches made of canned peaches

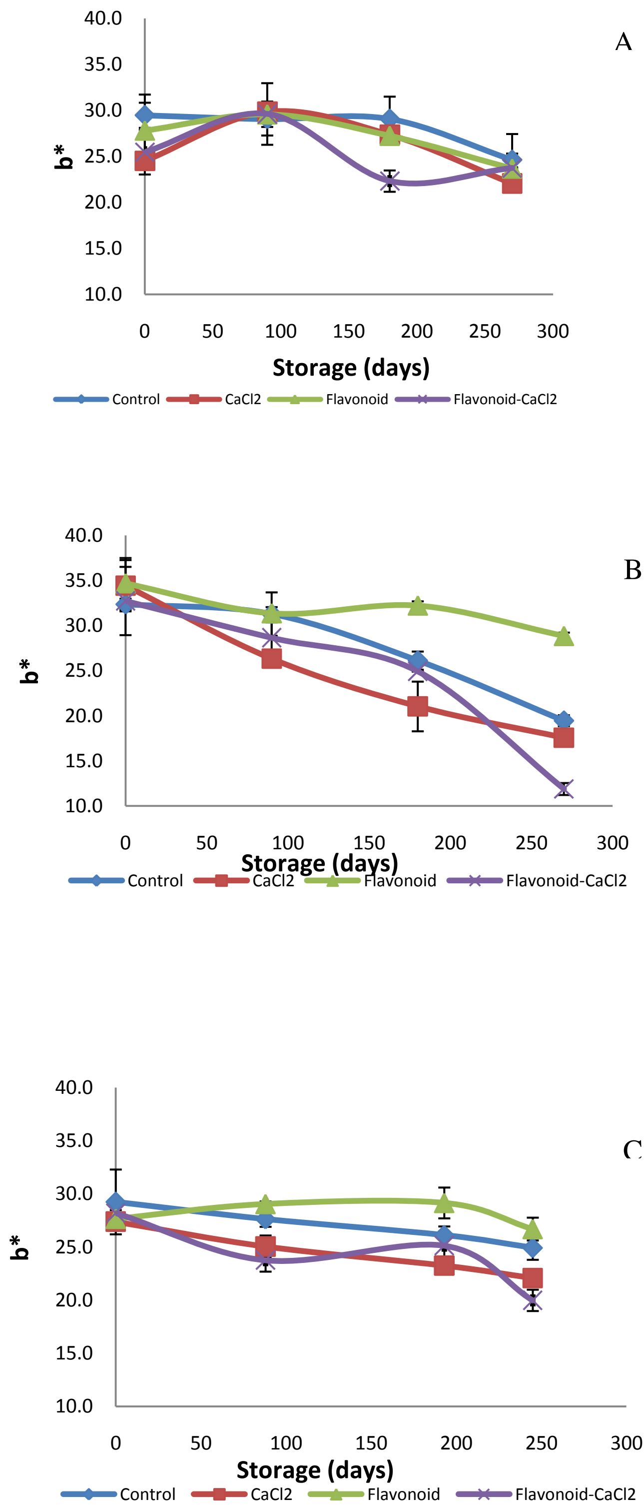
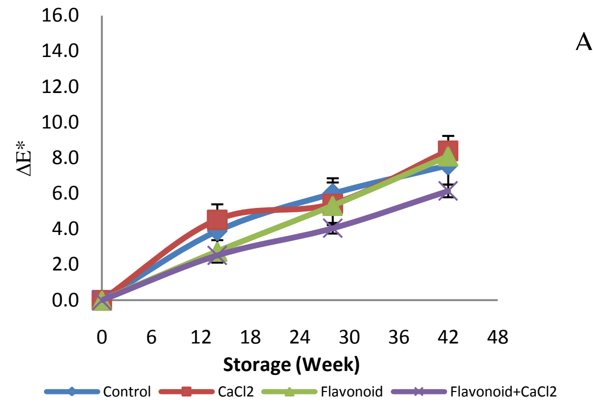
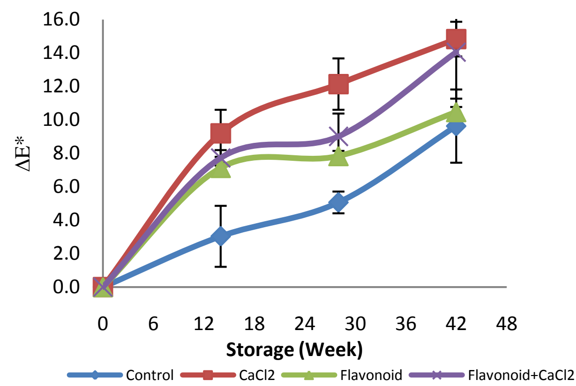


Fig. 6: Effect of treatment on b* value during storage at 40°C. A: Pouches made of fresh peaches; B: Pouches made of IQF peaches; C: Pouches made of canned peaches





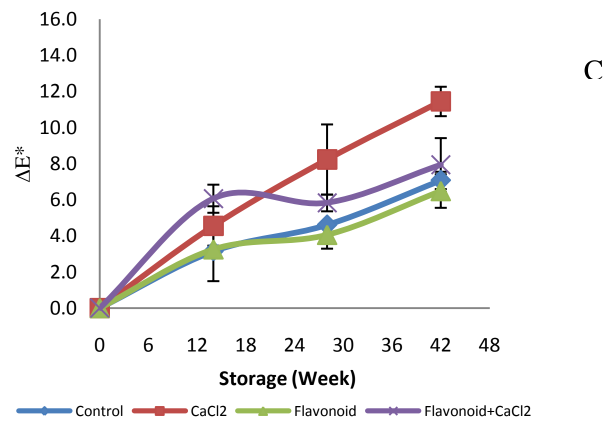
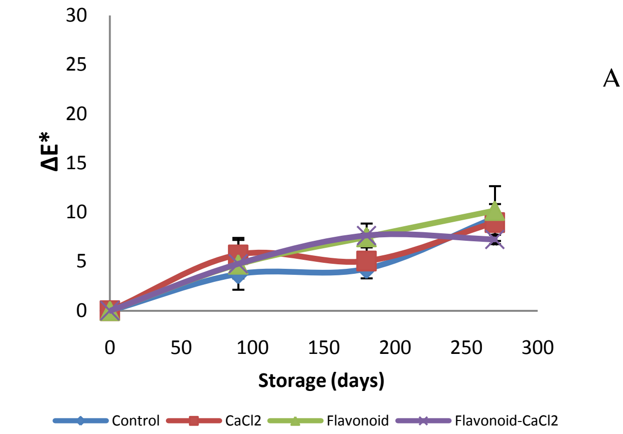
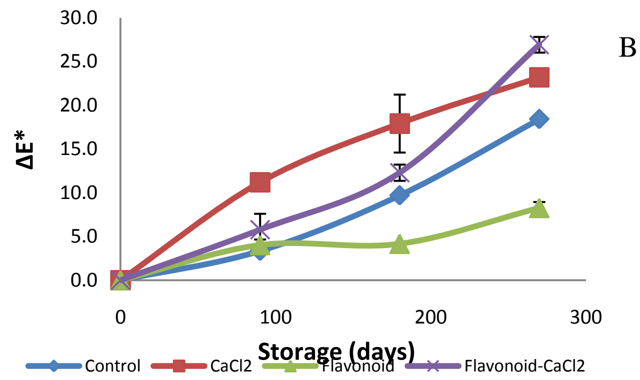


Fig. 7: Effect of treatment on ΔE* value during storage at 51°C. A: Pouches made of fresh peaches; B: Pouches made of IQF peaches; C: Pouches made of canned peaches





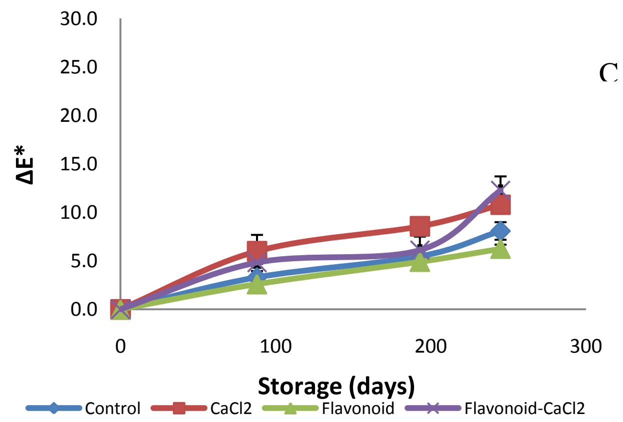


Fig. 8: Effect of treatment on ΔE* value during storage at 40°C. A: Pouches made of fresh peaches; B: Pouches made of IQF peaches; C: Pouches made of canned peaches

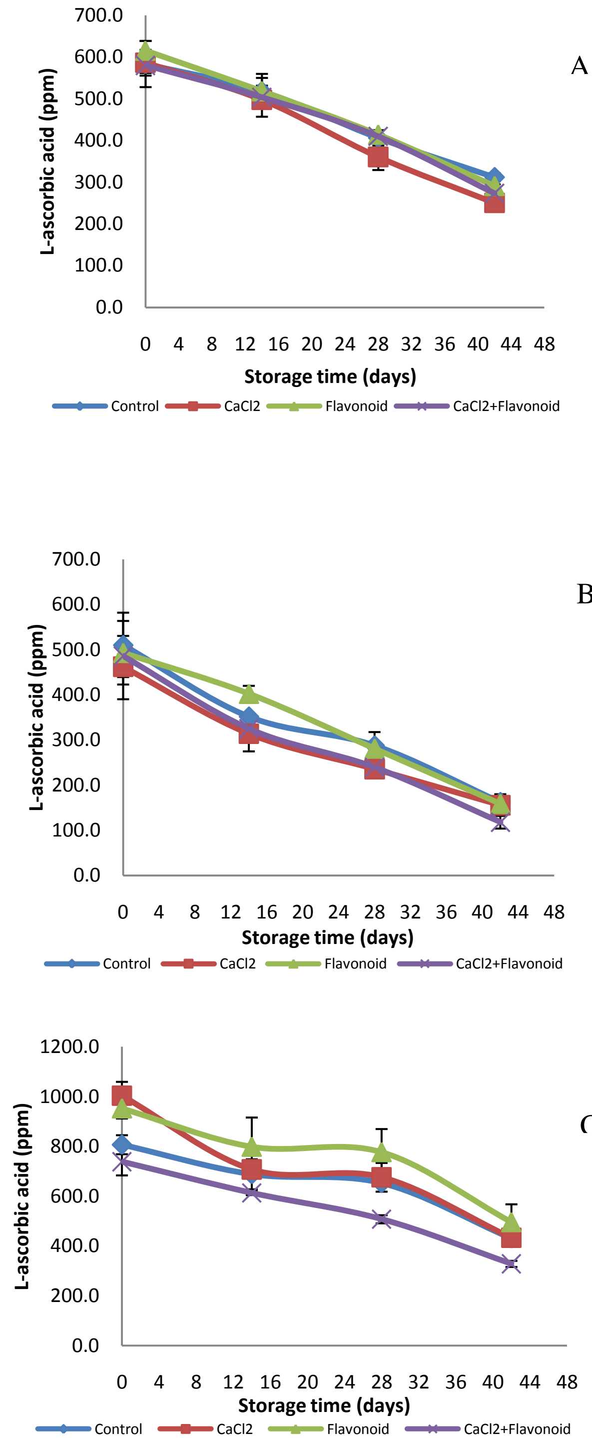


Fig. 9: Effect of treatment on ascorbic acid concentration during storage at 51°C. A: Pouches made of fresh peaches; B: Pouches made of IQF peaches; C: Pouches made of canned peaches

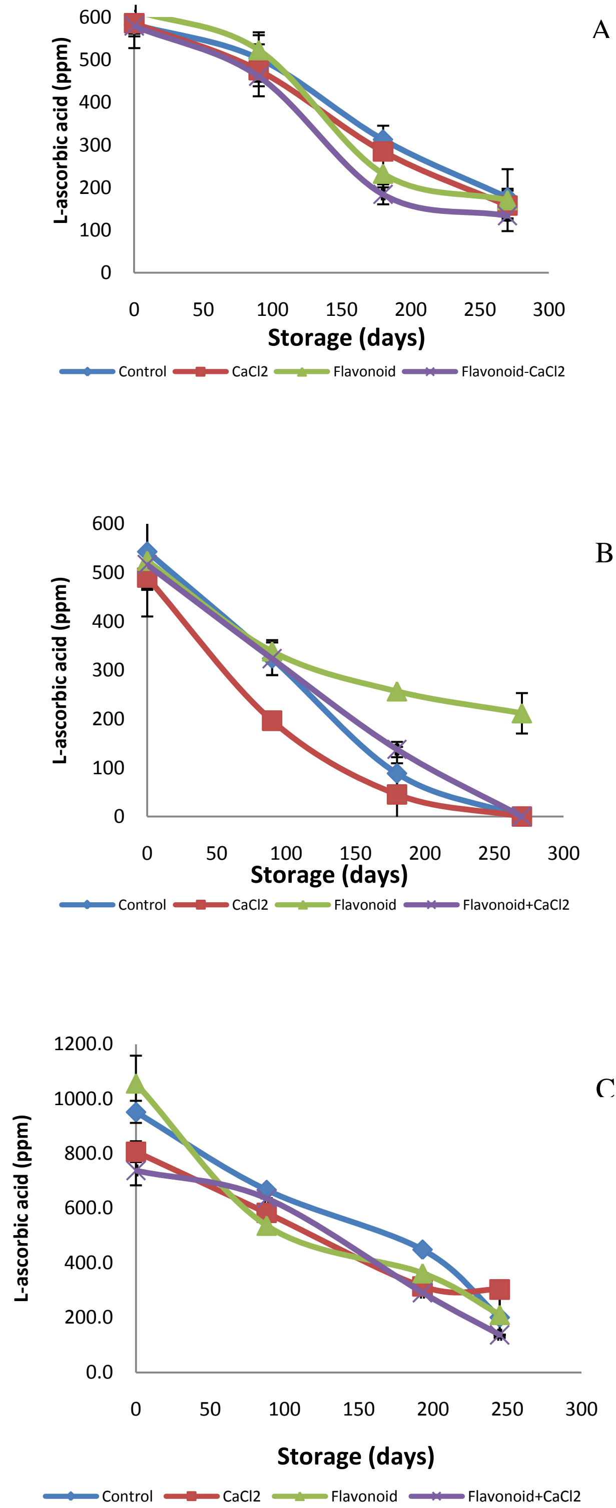


Fig. 10: Effect of treatment on the ascorbic acid concentration during storage at 40°C. A: Pouches made of fresh peaches; B: Pouches made of IQF peaches; C: Pouches made of canned peaches

Table 1: pH and °Brix values at the beginning and at the end of storage

Parameter	Source	Treatment	Period of Storage		
			Beginning	End (51°C)	End (40°C)
pH	Fresh	Control	4.05 ± 0.05	3.87 ± 0.02	3.91 ± 0.05
		$CaCl_2$	3.47 ±0.04	3.58 ± 0.05	3.50 ± 0.04
		α-GR	3.95 ± 0.09	3.95 ± 0.04	3.80 ± 0.03
		$CaCl_2$-α-GR	3.55 ± 0.07	3.71 ± 0.16	3.40 ± 0.04
	IQF	Control	3.52 ± 0.07	3.45 ± 0.1	3.35 ± 0.01
		$CaCl_2$	4.11 ± 0.06	4.08 ± 0.01	3.76 ± 0.02
		α-GR	4.00 ± 0.20	4.04 ± 0.01	3.87 ± 0.04
		$CaCl_2$-α-GR	3.40 ± 0.07	3.52 ± 0.03	3.30 ± 0.03
	Canned	Control	3.48 ± 0.14	3.52 ± 0.02	3.47 ± 0.11
		$CaCl_2$	3.92 ± 0.02	3.94 ± 0.02	3.86 ± 0.13
		α-GR	3.97 ± 0.02	3.92 ± 0.09	3.90 ± 0.04
		$CaCl_2$-α-GR	3.58 ± 0.06	3.43 ± 0.04	3.41 ± 0.17
Brix	Fresh	Control	17.47 ± 0.35	17.33 ± 0.12	18.10 ± 0.71
		$CaCl_2$	16.70 ± 0.72	17.30 ± 0.36	16.40 ± 0.6
		α-GR	17.43 ± 0.06	17.40 ± 0.2	17.30 ± 0.87
		$CaCl_2$-α-GR	17.30 ± 0.52	18.53 ± 0.71	10.00 ± 0.76
	IQF	Control	18.23 ± 0.61	19.45 ± 0.49	18.80 ± 0.31
		$CaCl_2$	18.43 ± 0.15	19.10 ± 0.71	18.70 ± 0.45
		α-GR	18.40 ± 0.36	18.70 ± 0.14	18.50 ± 0.13
		$CaCl_2$-α-GR	18.17 ± 0.64	19.60 ± 1.13	18.46 ± 0.34
	Canned	Control	21.47 ± 0.84	20.77 ± 0.9	20.56 ± 0.75
		$CaCl_2$	19.77 ± 0.67	19.87 ± 0.67	19.80 ± 0.72
		α-GR	20.00 ± 0.57	19.13 ± 0.61	19.40 ± 0.65
		$CaCl_2$-α-GR	21.17 ± 0.61	20.27 ± 0.81	21.40 ± 0.78

Table 2: $CaCl_2$ and α-GR effects on the color parameters and ascorbic acid concentration at the beginning and end of storages

Source	Factor	Levels	Beginning					End at 51°C					End at 40°C				
			L*	a*	b*	ΔE*	AA	L*	a*	b*	ΔE*	AA	L*	a*	b*	ΔE*	AA
Fresh	$CaCl_2$	0	47.9^{a}	0.28^{a}	28.7^{a}	0.0^{a}	596.4^{a}	41.5^{a}	1.91^{a}	26.4^{a}	7.9^{a}	301.8^{a}	40.4^{a}	2.6^{a}	24.2^{a}	9.8^{a}	173.9^{a}
		0.50%	44.9^{b}	-0.89^{a}	25.0^{a}	0.0^{a}	593.3^{a}	39.2^{b}	3.14^{b}	24.6^{a}	7.3^{a}	262.3^{b}	38.3^{a}	3.1^{a}	22.9^{a}	8.1^{a}	146.3^{a}
	α-GR	0%	47.1^{a}	-1.03^{a}	27.0^{a}	0.0^{a}	586.5^{a}	40.8^{a}	3.00^{a}	25.9^{a}	7.9^{a}	281.5^{a}	39.8^{a}	2.7^{a}	23.4^{a}	9.2^{a}	167.6^{a}
		0.30%	45.7^{a}	0.42^{b}	26.6^{a}	0.0^{a}	603.2^{a}	39.9^{a}	2.05^{a}	25.1^{a}	7.1^{a}	282.6^{a}	38.9^{a}	2.9^{a}	23.7^{a}	8.7^{a}	152.6^{a}
	$CaCl_2$*α-GR		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.
IQF	$CaCl_2$	0	46.2^{a}	2.14^{a}	33.5^{a}	0.0^{a}	501.7^{a}	39.4^{a}	5.14^{a}	26.9^{a}	10.1^{a}	160.5^{a}	37.5^{a}	5.8^{a}	24.2^{a}	13.3^{a}	84.8^{a}
		0.50%	45.9^{a}	1.98^{a}	33.6^{a}	0.0^{a}	471.9^{a}	36.6^{b}	6.67^{b}	23.7^{a}	13.5^{a}	136.7^{a}	30.1^{b}	6.2^{a}	14.7^{b}	25.1^{b}	0.0^{b}
	α-GR	0%	46.3^{a}	2.35^{a}	33.4^{a}	0.0^{a}	486.2^{a}	38.2^{a}	6.34^{a}	25.3^{a}	12.2^{a}	159.0^{a}	32.8^{a}	7.4^{a}	18.5^{a}	20.8^{a}	0.0^{a}
		0.30%	45.8^{a}	1.77^{a}	33.7^{a}	0.0^{a}	490.5^{a}	37.8^{a}	5.48^{b}	25.3^{a}	11.8^{a}	138.2^{a}	34.9^{b}	4.6^{b}	20.4^{b}	17.6^{b}	84.8^{b}
	$CaCl_2$*α-GR		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	*	*	*
Canned	$CaCl_2$	0	43.6^{a}	-0.70^{a}	28.5^{a}	0.0^{a}	865.1^{a}	38.7^{a}	2.74^{a}	25.9^{a}	6.7^{a}	463.0^{a}	39.1^{a}	3.8^{a}	25.8^{a}	7.2^{a}	257.6^{a}
		0.50%	43.1^{a}	0.13^{a}	27.8^{a}	0.0^{a}	844.0^{a}	36.6^{b}	3.91^{b}	21.7^{b}	9.7^{b}	380.5^{b}	34.9^{b}	4.5^{b}	21.0^{b}	11.5^{b}	144.2^{b}
	α-GR	0%	43.7^{a}	-0.25^{a}	28.3^{a}	0.0^{a}	885.3^{a}	36.9^{a}	3.28^{a}	22.3^{a}	9.7^{a}	431.6^{a}	37.1^{a}	4.4^{a}	23.5^{a}	9.5^{a}	184.8^{a}
		0.30%	43.1^{a}	-0.32^{a}	27.9^{a}	0.0^{a}	823.8^{a}	38.1^{a}	3.46^{a}	24.8^{b}	7.2^{b}	411.9^{a}	37.0^{a}	3.9^{b}	23.3^{a}	9.3^{a}	217.0^{b}
	$CaCl_2$*α-GR		n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	*

Different letters indicate significant differences ($p<0.05$) exist between means of the levels within each factor.
n.s. and * correspond to non-significant ($p>0.05$) and significant ($p<0.05$) effects due to interaction of $CaCl_2$ and flavonoid, respectively.

Table 3: Rate constants of L*, a*, ΔE* and ascorbic acid

Source	Treatment	L*				a*			ΔE*			Ascorbic Acid		
		Temp.	k (day^{-1})	R^2	RMSE	k (day^{-1})	R^2	RMSE	k (day^{-1})	R^2	RMSE	k (day^{-1})	R^2	RMSE
Fresh	Control	51	-0.15 ± 0.02	0.86	1.03	0.06 ± 0.01	0.72	0.67	0.18 ± 0.02	0.88	1.12	-6.6 ± 0.5	0.96	24.80
		40	-0.03 ± 0.003	0.90	0.92	0.014 ± 0.003	0.68	1.05	0.03 ± 0.003	0.92	1.07	-1.6 ± 0.1	0.95	38.88
	CaCl2	51	-0.14 ± 0.03	0.69	1.57	0.13 ± 0.01	0.69	1.57	0.19 ± 0.02	0.9	1.06	-8.2 ± 0.5	0.97	24.41
		40	-0.03 ± 0.01	0.76	1.67	0.016 ± 0.002	0.84	0.77	0.03 ± 0.01	0.74	1.93	-1.6 ± 0.1	0.95	40.10
	α-GR	51	-0.15 ± 0.03	0.79	1.37	0.11 ± 0.02	0.89	0.54	0.15 ± 0.01	0.92	0.77	-7.7 ± 0.5	0.97	24.47
		40	-0.03 ± 0.01	0.74	1.49	0.022 ± 0.006	0.67	1.25	0.03 ± 0.01	0.83	1.44	-1.9 ± 0.2	0.95	65.70
	α-GR+$CaCl_2$	51	-0.13 ± 0.03	0.74	1.33	0.06 ± 0.01	0.69	0.78	0.14 ± 0.01	0.98	0.32	-7.2 ± 0.5	0.95	29.45
		40	-0.03 ± 0.01	0.70	1.82	0.016 ± 0.003	0.76	0.98	0.03 ± 0.01	0.72	1.99	-1.8 ± 0.2	0.94	51.40
IQF	Control	51	-0.16 ± 0.02	0.9	0.97	0.07 ± 0.02	0.61	1.03	0.27 ± 0.03	0.93	1.35	-7.9 ± 0.8	0.91	41.62
		40	-0.05 ± 0.005	0.91	1.53	0.018 ± 0.003	0.82	0.95	0.071 ± 0.003	0.99	0.8	-1.9 ± 0.2	0.95	52.64
	$CaCl_2$	51	-0.22 ± 0.03	0.86	1.57	0.12 ± 0.02	0.87	0.79	0.35 ± 0.05	0.87	2.32	-7.3 ± 0.6	0.96	26.45
		40	-0.06 ± 0.01	0.94	1.55	0.019 ± 0.003	0.81	1.003	0.09 ± 0.01	0.97	1.5	-1.7 ± 0.2	0.9	65.44
	α-GR	51	-0.16 ± 0.02	0.80	1.38	0.07 ± 0.01	0.83	0.57	0.24 ± 0.04	0.82	1.91	-7.9 ± 0.5	0.97	25.60
		40	-0.018 ± 0.003	0.80	1	0.010 ± 0.002	0.81	0.53	0.03 ± 0.003	0.95	0.87	-1.1 ± 0.1	0.9	38.52
	α-GR+$CaCl_2$	51	-0.19 ± 0.03	0.82	1.53	0.10 ± 0.01	0.86	0.69	0.31 ± 0.04	0.88	1.91	-8.5 ± 0.9	0.92	42.27
		40	-0.06 ± 0.01	0.95	1.53	0.01 ± 0.003	0.68	1.03	0.099 ± 0.01	0.97	2.01	-1.8 ± 0.1	0.96	38.25
Canned	Control	51	-0.10 ± 0.02	0.68	1.25	0.06 ± 0.01	0.79	0.56	0.14 ± 0.02	0.83	1.16	-8.3 ± 1.0	0.87	54.16
		40	-0.021 ± 0.003	0.83	1.05	0.02 ± 0.002	0.87	0.70	0.03 ± 0.003	0.95	0.83	-2.5 ± 0.1	0.99	24.21
	$CaCl_2$	51	-0.19 ± 0.02	0.89	1.20	0.10 ± 0.02	0.81	0.82	0.27 ± 0.02	0.95	1.12	-12.9 ± 1.3	0.92	69.27
		40	-0.031 ± 0.004	0.88	1.27	0.02 ± 0.002	0.94	0.57	0.04 ± 0.003	0.95	1.11	-3.6 ± 0.4	0.93	115.21
	α-GR	51	-0.11 ± 0.01	0.87	0.74	0.10 ± 0.009	0.92	0.50	0.15 ± 0.01	0.92	0.76	-10.2 ± 1.7	0.82	92.55
		40	-0.02 ± 0.002	0.86	0.69	0.02 ± 0.002	0.95	1.81	0.02 ± 0.003	0.89	1.81	-2.7 ± 0.1	0.99	33.36
	α-GR+$CaCl_2$	51	-0.11 ± 0.03	0.66	1.38	0.08 ± 0.01	0.77	0.51	0.17 ± 0.03	0.74	1.70	-9.5 ± 0.6	0.96	29.68
		40	-0.032 ± 0.004	0.88	1.34	0.02 ± 0.001	0.95	0.44	0.05 ± 0.01	0.89	1.81	-2.5 ± 0.2	0.97	59.00

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CHAPTER 6: CONCLUSION

Three different experiments were evaluated in order to study if the replacement of ascorbic acid by ascorbyl-2-phosphate and the use of flavonoids (Pycnogenol and α-glucosylrutin) in peaches can prevent the non-enzymatic browning due to degradation of ascorbic acid.

Treatments made of peach puree fortified with ascorbic acid, ascorbyl-2-phosphate and a control were stored at 40°C, 50°C and 60°C for 29 days. The stability of the samples was evaluated through the color (CIELAB system) and vitamin C levels. Also, dissolved oxygen was measured. Constant rates were calculated and used in order to compare the different treatments. Samples with no added vitamin C showed the best stability. Kinetic models could not be applied successfully in all color parameters at all temperatures due to data variability. The unclear trends can be attributed to the content of anthocyanins in the variety of peaches that were used (Flavorich). The Weibull model best described the changes in vitamin C. At all temperatures, the rate constants of vitamin C degradation were higher in the ascorbic acid containing samples than in the ascorbyl-2-phosphate treatments. The differences in vitamin C losses between the fortified samples increased at lower temperatures.

Ascorbyl-2-phosphate, as replacement of ascorbic acid, and different concentrations of Pycnogenol (0%, 0.01% and 0.1%) were added into the food systems composed of diced peaches and syrup packaged in retortable pouches. Samples were stored at 51°C and 40°C for six weeks and 12 weeks, respectively. During these storages, vitamin C concentrations, color (CIELAB system) and browning index were measured at different periods of time. Rate constants and complete factorial designs were analyzed to determine any effect due to the Pycnogenol concentrations or Vitamin C type on the food systems. At each temperature, all treatments had similar rate constants. Even though the complete factorial design had some significant differences, they were not so clear to conclude that one treatment was better than the rest.

α-Glucosylrutin was added in systems made of diced peaches and syrup packaged in retortable pouches to prevent ascorbic acid browning. The use of $CaCl_2$ and different fruit sources (fresh, individually quick frozen and canned peaches) were

also evaluated. Samples were stored at 51°C and 40°C for 42 and 270 days, respectively. Vitamin C concentrations and color (CIELAB) were measured during storage. Rate constants were calculated to compare the different treatments. A complete factorial design was used to determine effects due to the flavonoid or $CaCl_2$. At 51°C, the treatments (control, $CaCl_2$, α-GR and $CaCl_2$-α-GR) did not slow the rate of AA or color deterioration. At 40°C, the treatments that had α-GR and were made using individually quick frozen or canned peaches had a lower rate of browning and loss of ascorbic acid than the rest of treatments. There is evidence which suggests that $CaCl_2$ could promote ascorbic acid browning under the evaluated conditions.

In summary, Ascorbyl-2-phosphate showed better stability than ascorbic acid at 40°C in the puree systems. Both Ascorbyl-2-phosphate and Pycnogenol did not show an improvement on the stability of the diced peaches packaged in the retortable pouches. α-Glucosylrutin improved the quality of diced peaches in pouches made of individually quick frozen and canned peaches during storage at 40°C.