PHYTOCHEMICAL, ANTIOXIDANT AND COLOR STABILITY OF AÇAI (*Euterpe oleracea* Mart.) AS AFFECTED BY PROCESSING AND STORAGE IN JUICE AND MODEL SYSTEMS

By

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Dedicated to those that have guided and inspired me.

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Açai (*Euterpe oleracea* Mart.), a palm fruit native to the Amazon region, has recently captured the attention of US consumers due to its novelty and potential health benefits associated with its high antioxidant capacity and polyphenolic composition. However, studies on the functional properties of this matrix and their stability during processing and storage are still lacking. These studies were undertaken to assess the polyphenolic and pigment stability of açai as affected by juice clarification, ascorbic acid fortification and storage and to determine matrix composition effects on the phytochemical and color stability of açai in both natural and model juice systems.

The results of clarification (non clarified, centrifuged and filtered açai pulp), ascorbic acid fortification (0 and 500 mg/L), and storage (4 and 20°C) on the phytochemical, antioxidant, and color stability of açai were evaluated through a storage period of 30 days. Matrix composition effects on açai stability were further assessed in a

study that compared natural juice systems with isolated fractions following ascorbic acid addition (0 and 500 mg/L) and storage (37ºC). Polyphenolic and anthocyanin fractions were isolated using C18 Sep-Pak mini-columns and redissolved in buffer (pH 3.5) or in the original aqueous matrix (C18 unbound). Samples were analyzed during a storage period of 12 days. Chemical analyses for both studies included total anthocyanin content, polymeric anthocyanin concentration, total soluble phenolics, antioxidant capacity and polyphenolics by HPLC.

Treatments stored at 4°C showed the highest stability for all variables measured by the end of the storage period. Clarification of açai pulp was responsible for initial losses in color (20%), total phenolics (25%), total anthocyanins (25%) and antioxidant capacity (20%). Ascorbic acid fortification markedly accelerated anthocyanin degradation in the clarified juice although no significant effects were observed in the non-clarified or centrifuged açai pulp. Losses in antioxidant activity were correlated to total anthocyanin ($r=0.88$) and phenolic ($r=0.73$) contents in both clarified and non-clarified treatments. In a second study, complex interactions among matrix components considerably influenced polyphenolic, antioxidant and color stability in açai juice and juice fractions. Color retention was favored by anthocyanin isolation while the retention of non-anthocyanin polyphenolics was enhanced by the natural juice matrix. Major losses occurred during the first 4 days of storage (37°C) in all treatments while in fortified fractions, the presence of non-anthocyanin polyphenolics exerted a protective effect against ascorbic acid oxidation. Maximum polyphenolic, antioxidant and pigment stability of açai products can be achieved if processing parameters are carefully controlled and low storage temperatures (4°C) are maintained throughout the distribution chain.

CHAPTER 1
INTRODUCTION

Consumption of fruits and vegetables has been associated with lowered incidence of various degenerative diseases including cancer and coronary heart disease (Van Poppel and others 1994; Burda and Oleszek 2001). The presence of phytochemicals such as flavonoids and other polyphenolics is thought to contribute to the protective effects and is generally associated with the antioxidant activity of these compounds (Chun and others 2005). Açai (*Euterpe oleracea* Mart.), one of the most abundant species in the Amazon estuary floodplains (Muñiz-Miret and others 1996), has captured the attention of US consumers in recent years due to its unique flavor, novelty and potential health benefits, possibly derived from its polyphenolic composition.

Polyphenolics are naturally occurring substances in plants, thought to have benefits on human health (Haysteen 1983; Bravo 1998; DiCarlo and others 1999; Burda and Oleszek 2001). Beneficial health effects of plant polyphenolics have been recognized to originate from its capability to inhibit oxidative degradation (Roginsky and Lissi 2005) hence, minimizing losses during processing is of particular interest. The stability of polyphenolic compounds present in açai has not been previously assessed and associated changes in antioxidant activity and functional properties are unknown. Among them, anthocyanins, members of the flavonoid group of phenolic compounds and the most abundant group of naturally occurring pigments in plants, have emerged as important alternatives to synthetic colorants (Kong and others 2003) while its high antioxidant activity and inhibitory effects on cancer cell growth has been indicated in several studies

(Renaud and De Lorgeril 1992; Wang and others 1997; Muth and others 2000; Tsuda and others 2000; Hou 2003). However, high raw material costs and poor stability during processing and storage considerably limit their industrial applications. Anthocyanin stability is considerably affected by structure, concentration, pH, light, temperature, the presence of copigments, metallic ions, oxygen, ascorbic acid, sugars, proteins and sulfur dioxide (Rodriguez-Saona and others 1999). Açai may constitute a novel source of these natural pigments and potential exists for its application as a functional ingredient in juice blends, functional and sport beverages, dairy products, desserts and as a dietary supplement. However, its stability during processing and storage has not been assessed and the conditions that favor detrimental changes in both color and functional properties have not been identified.

The edible pulp of açai fruits is commonly macerated with water to produce the thick puree of the same name, widely popular throughout the northern countries of South America (Strudwick and Sobel 1988). Further processing steps may include pasteurization, freezing, dilution, clarification, fortification and dehydration. Fortification of various fruit juices, nectars or purees with ascorbic acid is a common practice in the industry, not only to maintain quality while preventing browning reactions but also to enhance nutritional value (Freedman and Francis 1984). However, combining ascorbic acid with anthocyanins, naturally occurring in açai, has been shown to be mutually destructive, causing losses in color, functional properties and nutritional quality (Garcia-Viguera and Bridle 1999). Furthermore, phytochemical and matrix composition effects on açai polyphenolics and color stability in the presence of ascorbic acid have not been evaluated.

The present studies assessed clarification, fractionation, fortification and storage effects on antioxidant capacity, phytochemical content and pigment stability of açai. It was hypothesized that juice clarification would not affect polyphenolic stability whereas the presence of ascorbic acid would be detrimental for both polyphenolic and pigment stability. Additionally, isolated anthocyanin fractions would show decreased stability while the presence of naturally occurring polyphenolics would significantly improve color, antioxidant capacity and phytochemical stability. The specific objectives of these studies were

- To evaluate the antioxidant capacity, polyphenolic composition and pigment stability of açai as affected by juice clarification and fortification with L-ascorbic acid.
- To determine the effects of naturally occurring matrix components on the polyphenolic, antioxidant capacity and color stability of açai in the presence of ascorbic acid.
- To determine storage time and temperature effects on the color and pigment stability of açai.

CHAPTER 2
LITERATURE REVIEW

2.1 Açai Market and Distribution

Açai (*Euterpe oleracea* Mart) is a widely distributed palm in northern South America, attaining its greatest coverage and economical importance in the state of Pará, Brazil (Sergio and others 1999). The fruit of açai is a primary staple food for the region's inhabitants and according to Anderson (1988) its harvesting accounts for more than 60% of the forest products sold by a single family. Locally, fruits are used in several dishes like ice cream, pies, and jelly although a major product is a drink obtained from the cold maceration of its pulp. The beverage, also known as açai in the Amazonian region, is a dark purple juice of creamy texture, slightly oily appearance, and characteristic nutty flavor that contains a high anthocyanin and polyphenolic content (Muñiz-Miret and others 1996).

Due to its highly perishable nature, consumption and expanded commercialization of açai had long been restricted to a purely regional level in South America. According to Clay and Clement (1993) the market for açai in 1990, entirely domestic to South America, was nearly US$100 million. The main local market for the fruit is in Belém, Pará, where as many as 50,000 kg of unprocessed fruits are sold daily (Rogez 2000).

In the United States, interest in açai has intensified in recent years, due to increased import, its novel characteristics and potential health benefits associated with its high antioxidant capacity, derived from its rich anthocyanin content and polyphenolic composition. Consumer trends toward health and wellness have favored açai markets in

the form of juice drinks as well as a source of functional pigments and flavors for the food industry (Pszczola 2005). Currently, açai exists in US markets in a variety of forms including juices, nutraceutical beverages, and dietary supplements with excellent potential for its application as a functional ingredient in desserts, dairy, and organic products.

2.2 Açai Fruit

2.2.1 Occurrence

Açai is mainly found in the Amazon River estuary, covering an area of approximately 25,000 km^2. It is especially common on perennially flooded, black or clear water forest swamps. Population density is considered to be high at 2,500 to 7,500 plants/hectare but varies depending on soil conditions (Clay and Clement 1993).

2.2.2 Morphology

Açai is a slender, multistemmed, monoecious palm that can have more than 45 stems in different stages of growth, fruit development, and ripeness stage and trees can reach a height of over 25 meters. Fruit bunches per plant vary from 3 to 4 and produce from 3 to 6 kg of fruit per year (Clay and Clement 1993). Fruits are rounded, measuring from 1 to 1.4 centimeters in diameter, are purple, almost black when mature and appear in clusters. The seed comprises approximately 80% of the total volume and is covered by fibrous layers and a thin, slightly oily coating under which is a small edible layer (Rogez 2000).

2.2.3 Production

Fruiting occurs throughout the year, however, due to geographical variations in fruit ripening, heavy seasonal production occurs between July and December (Muñiz-Miret and others 1996). Harvesting the fruit bunches is an arduous and frequently

dangerous task, done by individuals accustomed to climbing the açai palms using a fiber ring to support their feet. A practiced harvester moves from one stem to another, without descending, until all ripe fruit bunches from a clump have been manually removed and collected in a basket. Fruits are then separated from the bunches and selected based on their firmness and color by the same harvesters, who can collect up to 180 kg in a day of work. Fruits are finally transported to the markets and commercialized in less than 24 h, to prevent significant nutritional and quality losses (Rogez 2000).

An estimated 180,000 tons of fruit are produced annually, limited only by demand since until recently, the high perishability of the fruits had restricted its consumption to a purely regional level (Rogez 2000).

2.2.4 Uses and Composition

Açai produces a wide variety of market and subsistence products while more than 22 different uses for all plant parts have been reported (Anderson 1988). However, the principal use is for the preparation of a thick, dark purple beverage obtained by cold maceration of its ripe fruits. In the process, water is incorporated to facilitate extraction and increase yields. The most important trade qualities are based on total solids and are “grosso” (14% total solids), “medio” (11%) and “fino” (8%). Açai is locally popular throughout all socioeconomic levels, and in Belém, Pará, an individual daily consumption of up to 2 L has been reported (Rogez 2000).

Nutrient content has been previously reported by Mota (1946), Campos (1951) and Altman (1996) (cited by Clay and Clement 1993), as follows: 1.25-4.34% (dry weight) protein, 7.6-11.0% fats, 1-25% sugar, 0.050% calcium, 0.033% phosphorous, 0.0009% iron, and traces of sulphur and vitamins A and B1. Additionally, it is characterized by a high caloric content, ranging from 88 to 265 calories per 100 g, depending upon the

dilution. Few studies have been conducted on açai juice (Bobbio and others 2002; Araujo and others 2004; Del Pozo-Insfran and others 2004) and data on its composition and functional properties are still lacking.

2.3 Polyphenolics

Vascular plants synthesize a diverse array of secondary metabolites, commonly referred to as phenolics. The term encompasses more than 8000 naturally occurring compounds, connected to a variety of physiological functions such as protein synthesis, nutrient uptake, enzyme activity, photosynthesis, resistance to microorganisms, pigmentation and organoleptic characteristics (reviewed by Robbins 2003). Several phenolic molecules have also been found to exert interesting biological activities and a vast body of evidence indicating healthful benefits in vitro and in vivo is accumulating (Visioli and others 2000).

2.3.1 Structure and Classification

Structurally, phenolic compounds are derivatives of benzene with one or more hydroxyl substituents that may include functional derivatives such as esters, methyl esters, glycosides or others (Visioli and others 2000). According to De Bruyne and others (1999), phenolic compounds are products of the plant aromatic pathway, which consists of three main sections: the shikimate segment that produces the aromatic amino acids phenylalanine, tyrosine and tryptophan, the phenylpropanoid pathway that produces the cinnamic acid derivatives, precursors of flavonoids and lignans and the flavonoid route that gives rise to a diversity of flavonoid compounds.

Simple phenols seldom occur naturally, so plant phenolics are divided into the following main groups: hydroxylated derivatives of benzoic acid or hydroxybenzoic acids, found in free state as well as combined as esters or glycosides (gallic acid);

phenolic derivatives of cinnamic acid or hydroxycinnamic acids (coumaric, caffeic, ferulic acid), which occur mainly esterified and the glycosidic phenylpropanoid esters (Skerget and others 2005). Polyphenols possessing two phenol subunits include the flavonoids, whereas compounds possessing three or more subunits are referred to as tannins (reviewed by Robbins 2003).

2.3.1.1 Phenolic acids

Phenolic acids are a distinct group of aromatic secondary plant metabolites that possess one carboxylic acid functional group. Two constitutive carbon frameworks can be distinguished: the hydroxycinnamic and hydroxybenzoic structures. Although the basic skeleton remains unchanged, the number and positions of the hydroxyl groups on the aromatic ring give rise to a variety of compounds. Only a minor fraction exists as free phenolic acids while the majority are linked through ester, ether or acetal bonds either to structural plant components (cellulose, proteins, lignin), to larger polyphenols (flavonoids) or to smaller organic molecules (glucose, organic acids); creating a variety of derivatives (reviewed by Robbins 2003).

Both benzoic and cinnamic acid derivatives have their biosynthetic origin from the aromatic amino acids tyrosine and L-phenylalanine, synthesized from chorismate, the final product of the shikimate pathway. Subsequent conversion of an aromatic amino acid to the various hydroxycinnamic acids involves a three-step sequence referred to as the general phenylpropanoid metabolism. Side chain degradation of hydroxycinnamic acid derivatives has been proposed to produce their corresponding benzoic acid derivatives, although these may also be originated by an intermediate in the shikimate pathway (reviewed by Herrmann 1995).

Phenolic acids have been connected with diverse plant functions, including nutrient uptake, protein synthesis, enzyme activity, photosynthesis, structural components and allelopathy (Wu and others 1999). Cinnamic and benzoic acid derivatives exist in virtually all plants and are physically dispersed throughout the plant in seeds, leaves, roots and stems (Robbins 2003). Due to their ubiquitous presence in plant based foods, humans consume phenolic acids on a daily basis. According to Clifford (1999), individual intake ranges from 25mg to 1g a day depending on the diet. Phenolic acids have also been associated with sensory properties, color and nutritional quality of foods (Tomas-Barberan and Espin 2001).

2.3.1.2 Flavonoids

Flavonoids are the most common and widely distributed group of plant phenolics (Le Marchand 2002). To date, over 5,000 different flavonoids have been identified and are classified into at least 10 chemical groups (Whiting 2001; Le Marchand 2002). Among them, flavonols, flavones, flavanones, catechins, anthocyanidins and isoflavones are particularly common (reviewed by Cook and Samman 1996).

Flavonols are the most abundant flavonoids in foods, with quercetin, kaempferol and myricetin being particularly common. Flavanones are mainly found in citrus fruits and flavones in celery. Catechins are predominantly found in green and black tea and in red wine. Anthocyanins are generally found in berries whereas isoflavones are almost exclusive of soy products (Le Marchand 2002).

Flavonoids are formed in plants from the aromatic amino acids phenylalanine and tyrosine and malonate. The basic flavonoid structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (C6-C3-C6), respectively labeled A, B and C (Figure 2-1). The level of oxidation and pattern of substitution of the C ring characterize

the various flavonoid classes, while individual compounds within a class differ in the substitutions of the A and B rings, which may include hydrogenation, hydroxylation, methylation, malonylation, sulphation and glycosylation (reviewed by Cook and Samman 1996). The pyran ring can also be opened in chalcone forms and recyclized into a furan ring or aurones (Skerget and others 2005).

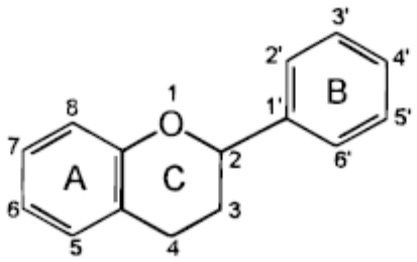

Figure 2-1. Basic flavonoid structure.

Flavonoids play various roles in plant ecology. Due to their attractive colors, flavones, flavonols and anthocyanidins may act as visual signals for pollinating insects. Catechins and flavonols, characterized by their astringency, might constitute a defense mechanism against predatory insects. Additionally, flavonoids function as catalysts in the light phase of photosynthesis while protecting plant cells from reactive oxygen species produced by the photosynthetic electron transport system. Furthermore, their favorable UV-absorbing properties protect plants against UV radiation and scavenge UV-generated radicals (reviewed by Pietta 2000). Flavonoid content of plants has been found to vary widely depending on variety, maturity stage and climatic conditions. Changes during storage and food processing, such as peeling and heating, have also been reported while oxidative reactions during processing can lead to brown polymers (Lairon and Amiot 1999).

Besides their physiological roles in plants, flavonoids are important components in the human diet. Dietary intake of flavonoids is considerably high, as compared to those of vitamin C (70mg/day) or vitamin E (7-10mg/day) and ranges between 50 and 800mg/day, depending on the diet (reviewed by Pietta 2000). According to Morton and others (2000), while many aspects of flavonoid and phenolic acid absorption and metabolism are still unknown, there is enough evidence to suggest that some of these compounds will be absorbed in sufficient concentration to have physiological effects.

2.3.1.3 Tannins

Plant tannins have been defined as water-soluble phenolic compounds having a molecular weight between 500 and 3000 daltons, show the usual phenol reactions and have the ability to precipitate alkaloids, gelatin and other proteins (Bate-Smith 1957, cited by Cos and others 2003) (Figure 2-2).

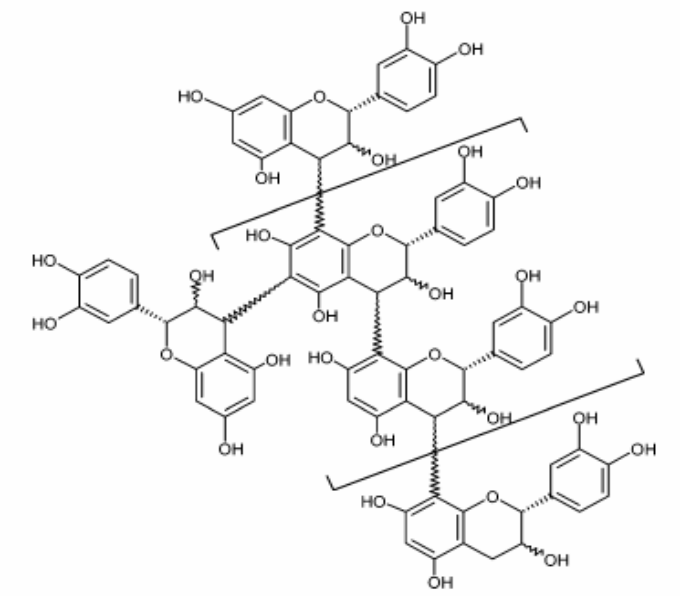

Figure 2-2. General structure of condensed tannins.

At present, tannins are classified on the basis of their structural characteristics in three major groups: the hydrolysable, the complex or partially hydrolysable and the

condensed or non hydrolysable tannins (Cos and others 2003). The first group encompasses polyesters of gallic acid and hexahydroxydiphenic acids (gallotannins and ellagitannins, respectively) whereas the latter groups, oligomers and polymers composed of flavan-3-ol nuclei (De Bruyne and others 1999). Complex tannins consist of a flavan-3-ol unit connected to a gallo- or ellagitannin through a glycosidic linkage. They are only partially hydrolyzable due to the carbon-carbon coupling on their flavan-3-ol unit with the glycosidic portion. Condensed tannins, commonly referred to as proanthocyanidins or polyflavanoids, are complex mixtures of oligomers and polymers composed of phenolic flavan-3-ols. In contrast to hydrolyzable tannins, condensed tannins do not have a polyol nucleus and are therefore not readily hydrolyzed. However, upon heating in acidic alcohols, red anthocyanidin pigments are obtained (Cos and others 2003). All these tannins are derived from the shikimate/chorismate pathway (De Bruyne and others 1999).

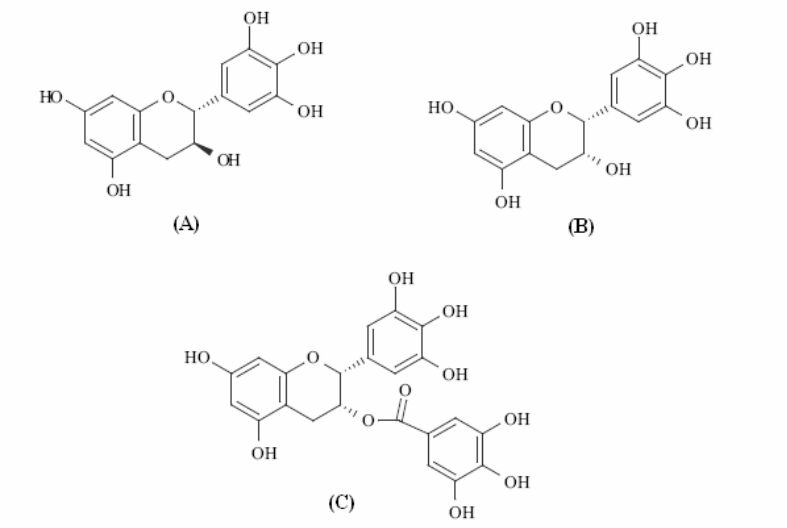

Figure 2-3. Chemical structures of some flavan-3-ols: (+)-Gallocatechin (A), (-)-Epigallocatechin (B) and (-)-Epigallocatechin 3-*O*-gallate (C).

Proanthocyanidins and flavan-3-ols derivatives are present in the fruits, leaves, bark and seeds of plants where its main function is to provide protection against

microbial pathogens, insects and larger herbivores (Dixon and others 2005). Besides their biological functions, proanthocyanidins have been suggested to account for a major fraction of the polyphenolics ingested in the Western diet, due to their ubiquitous existence (Santos-Buelga and Scalbert 2000). Although the bioavailability and metabolism of proanthocyanidins is still poorly understood, it seems clear that dimmers are the only proanthocyanidins that can be absorbed and found at low concentrations in plasma. Additionally, oligomeric and polymeric proanthocyanidins have been shown to be degraded by microbial flora in the gut into simple phenolic acids, which are readily absorbed (Prior and Gu 2005).

2.3.2 Polyphenolics as Antioxidants

Several in vitro studies have suggested that polyphenolics are endowed with interesting biological activities, linked to their antioxidant capacity and associated with a reduced incidence of oxidative damage diseases, including cancer and coronary heart disease (Visioli and others 2000). According to Cos and others (2003), the basic concept of free radical scavenging activity of an antioxidant is a redox transition involving the donation of a single electron or hydrogen atom to a free radical, transferring the radical character to the antioxidant, leading to a more stable compound.

Mechanisms of antioxidant action include: suppressing reactive oxygen species formation either by inhibition of enzymes or chelation of trace elements involved in free radical production; scavenging of reactive oxygen species and protection of antioxidant defenses (reviewed by Pietta 2000). Although there are several mechanisms, the predominant mode of antioxidant activity is believed to be radical scavenging via hydrogen atom donation. Additional radical quenching actions include electron donation and singlet oxygen quenching (Shahidi and Wanasundara 1992).

According to Rice-Evans and others (1996), for a polyphenol to be defined as antioxidant, two conditions must be satisfied: first, when present in low concentrations relative to the substrate to be oxidized, it delays, retards or prevents the autoxidation or free radical mediated oxidation; second, the resulting radical formed after scavenging must be stable. Chemical properties of polyphenolics, in terms of the availability of the phenolic hydrogens as hydrogen-donating radical scavengers, predict their antioxidant activity. Substituents in the aromatic ring affect the stabilization and therefore the radical-quenching ability of phenolic compounds (reviewed by Robbins 2003).

Polyphenolics have shown to inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase and protein kinase C as well as cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase and NADH oxidase, all involved in reactive oxygen species generation. Additionally, certain phenolics efficiently chelate trace metals that play an important role in oxygen metabolism and constitute potential enhancers in the formation of reactive oxygen species. Furtherly, due to their lower redox potentials, ranging from 0.23 to 0.75V, phenolics are thermodynamically able to reduce oxidizing free radicals with redox potentials in the range 1.0-2.13 V, such as superoxide, peroxyl, alkoxyl and hydroxyl radicals by hydrogen atom donation. In addition to their radical quenching ability, phenolics may stabilize free radicals by complexing with them, acquiring a stable structure (reviewed by Pietta 2000 and Cos and others 2003).

2.3.3 Polyphenolics in Açai

Characterization of phenolic compounds present in açai has only been previously reported twice in scientific literature. The predominant polyphenolics in açai pulp were characterized by Del Pozo-Insfran and others (2004) as ferulic acid, epicatechin, p-

hydroxy benzoic acid, gallic acid, protocatechuic acid, (+)-catechin, ellagic acid, vanillic acid and p-coumaric acid, in order of abundance and at concentrations that ranged from 17 to 212 mg/L. In addition, five compounds were tentatively identified as gallotannins while an ellagic acid glycoside was also detected. Some hydroxybenzoic and hydroxycinnamic acids have been also described by Coisson and others (2005). Vanillic acid was identified as the main phenolic acid in fresh açai juice while chlorogenic and p-hydroxybenzoic acids were also detected (Coisson and others 2005).

2.4 Anthocyanins

2.4.1 Structure and Occurrence

Anthocyanins (from the Greek *anthos*, flower and *kyanos*, blue) represent the most important class of naturally occurring pigments that impart color to fruits, vegetables, roots, tubers, bulbs, legumes, cereals, leaves and flowers (Bridle and Timberlake 1997). They belong to the flavonoid group of phenolic compounds and are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium cation or flavylium salts (Kong and others 2003). The flavylium cation constitutes the main part of the anthocyanin molecule and contains conjugated double bonds responsible for light absorption around 500 nm, causing pigments to appear red to the human eye. The aglycones, called anthocyanidins, are usually penta- (3,5,7,3´,4´) or hexa-substituted (3,5,7,3´,4´,5´). To date, 22 naturally occurring anthocyanidins have been identified, although only six of them (Figure 2-4) are common in higher plants: pelargonidin (Pg), peonidin (Pn), cyanidin (Cy), malvidin (Mv), petunidin (Pt) and delphinidin (Dp) (Francis 1989).

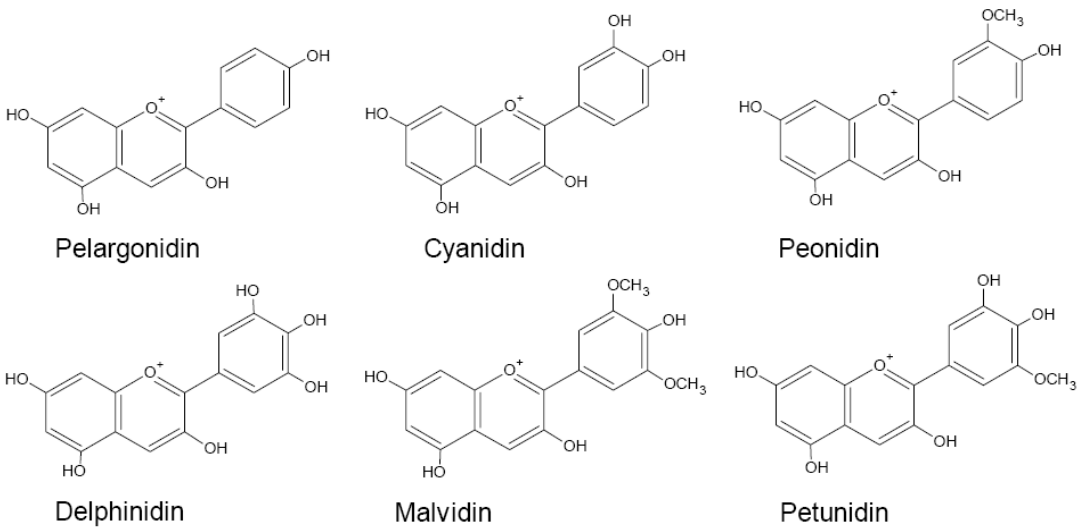

Figure 2-4. Chemical structures of the most abundant anthocyanidins.

Anthocyanidins are seldom found in nature as such, occurring mainly in their glycosylated forms, as anthocyanins. Due to their glycosylation, anthocyanins are more soluble and stable in aqueous solutions than their corresponding aglycones (Harborne 1964). Classification of anthocyanins is based on the number of glycosyl units that constitute them. Monoglycosides are comprised of one saccharidic moiety, primarily attached to the 3-hydroxyl group of the aglycone. In diglycosides, two monosaccharides are attached to the 3- and 5-hydroxyl groups and less commonly to the 3- and 7-hydroxyl groups although is also possible to find both monosaccharides attached to C-3. In triglycosides, attachment of monosaccharides occurs in such a way that two of them are in the C-3 and one in the C-5 or C-7 although a linear or branched attachment of three monosaccharides at C-3 is also possible (Brouillard 1983). Sugars most commonly found in anthocyanins are the monosaccharides glucose, rhamnose, galactose, arabinose and xylose (De Ancos and others 1999). According to Kong and others (2003), more than 400 anthocyanins have been reported, of which, cyanidin 3-glucoside is the most widespread.

Individual anthocyanins may be also differentiated by the nature and number of organic acids attached to the anthocyanin glycosyl units, usually aromatic phenolic acids or aliphatic dicarboxyl acids or a combination of both. These are generally linked to the 6- position of the monosaccharide, but anthocyanins with acyl substitution at the 2-, 3-, and 4- positions of the monosaccharide have been elucidated (Cabrita and others 2000). The most common acylating agents include derivatives of hydroxycinnamic acids (*p*-coumaric, ferulic, caffeic and sinapic), hydroxybenzoic acids (gallic) and a range of aliphatic acids (malonic, acetic, malic, succinic and oxalic acids). Aromatic and aliphatic acylation may occur in the same molecule and from zero to three or more acylating residues may also be present (reviewed by Clifford 2000).

According to Kong and others (2003), the most significant function of anthocyanins is their ability to impart color to the plants and plant products in which they occur. In addition, anthocyanins play a definite role in the attraction of animals for pollination and seed dispersal while they can also act as antioxidants, phytoalexins or antibacterial agents.

Besides their roles as secondary metabolites in the pigmentation of plants, anthocyanins have gained increasing interest as functional ingredients for coloring food and as potent agents against oxidative stress. Their success as natural alternatives for artificial dyes has been thought to depend on their economic feasibility, their chemical, biochemical and physical stability during processing and their appearance at food pH (Stintzing and others 2002). Nutritional interest in anthocyanins is based on the marked daily intake (180 to 215 mg/day in the United States), which is considerably higher than the estimated intake of other flavonoids (23mg/day), including quercetin, kaempferol,

myricetin, apigenin and luteolin (reviewed by Galvano and others 2004). Anthocyanins also posses known pharmacological properties and are used for therapeutic purposes, as in the treatment of illnesses involving tissue inflammation and capillary fragility (Kong and others 2003). In addition, anthocyanins have been shown to be effective scavengers of reactive oxygen species and to inhibit lipoprotein oxidation and platelet aggregation (Ghiselli and others 1998). Moreover, in a study evaluating the pharmacokinetics of anthocyanins in humans, Cao and others (2001) found that anthocyanins can be absorbed in their unchanged glycosylated forms.

2.4.2 Color stability

Anthocyanins are highly unstable molecules and its color stability is strongly affected by anthocyanin concentration and structure, pH, solvents, temperature, light, enzymes, oxygen, copigments, metallic ions, ascorbic acid, sugar and their degradation products (Rodriguez-Saona and others 1999; Stingzing and others 2002).

Studies regarding the storage stability of anthocyanins have been conducted in model beverage systems (Dyrby and others 2001), juices (Choi and others 2002; Zhang and others 2000) and jams (Garcia-Viguera and others 1998). Anthocyanin degradation rates followed first order kinetics and storage temperature was identified as the main factor responsible for anthocyanin loss (Marti and others 2001).

2.4.2.1 Structural and concentration effects

The substitution pattern, glycosyl and acyl groups attached and the site of their bonding have a significant effect on anthocyanin stability while in general, both acylation and glycosylation result in improved color stability (Baublis and others 1994; Turker and others 2004). According to Giusti and Wrolstad (2003), increased anthocyanin concentration also promotes higher color stability and produces a multifold increase in

color intensity. Self-association reactions among anthocyanins are thought to be responsible for improved stability observed at higher concentrations (Dao and others 1998).

2.4.2.2 pH

Anthocyanins are known to display a variety of color variations in the pH range from 1-14. The ionic nature of anthocyanins enables structural changes and resulting in different colors and hues at different pH values (Von Elbe and Schwartz 1996). In plant vacuoles, anthocyanins occur as the equilibrium of four molecular species (Clifford 2000), the basic colored flavylium cation and three secondary structures: the quinoidal base, the carbinol pseudobase or hemiacetal and the chalcone pseudobase (Figure 2-5).

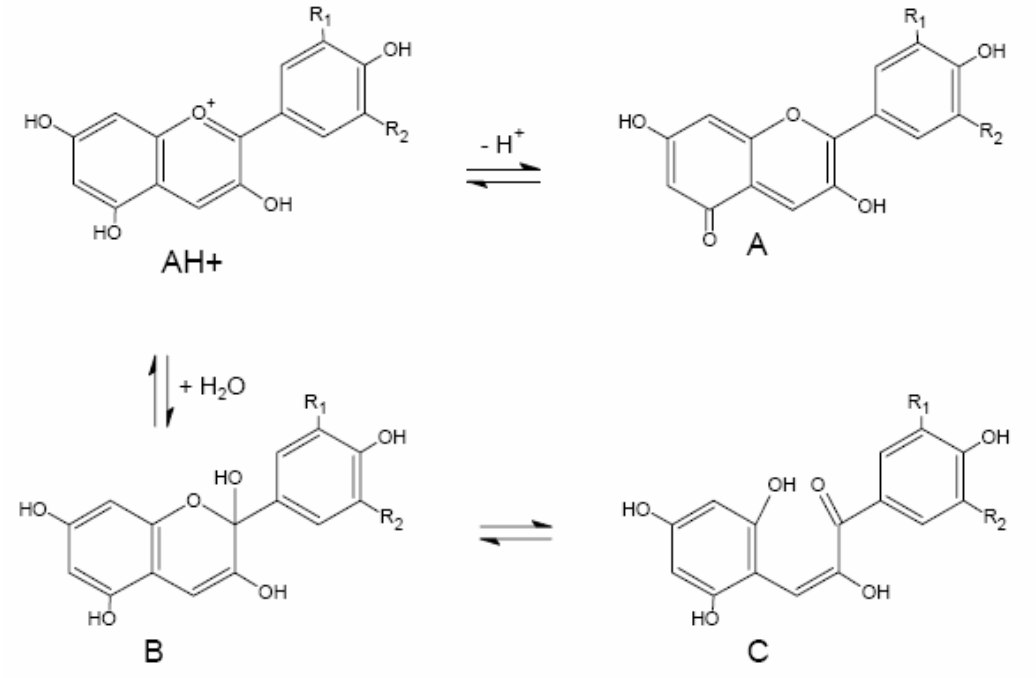

Figure 2-5. Four main equilibrium forms of anthocyanins in aqueous media: flavylium cation (AH+), quinonoidal base (A), carbinol- or anhydrobase (B) and chalcone (C).

The red flavylium cation is the only predominating equilibrium species in very acidic media (pH 0.5) and as the pH is increased hydration occurs, by nucleophilic attack

of water, to the colorless carbinol form, decreasing color intensity. When pH increases further, ring opening of the carbinol form yields the colorless chalcone (Brouillard 1983).

2.4.2.3 Temperature

Anthocyanin degradation rate during processing and storage markedly increases as the temperature rises. Increased temperature induces hydrolysis of glycosidic bonds in anthocyanin molecules, leading to accelerated pigment losses of the unstable aglycones (Maccarone and others 1985).

2.4.2.4 Oxygen

Oxygen amplifies the impact of other anthocyanin degradation factors. According to Jackman and others (1987), the deleterious effects of oxygen on anthocyanins can take place through direct oxidative mechanisms or through indirect oxidation, where the oxidized components of the media further react with anthocyanins, giving rise to colorless or brown products.

2.4.2.5 Light

Light has been shown to greatly accelerate anthocyanin degradation. Furtado and others (1993) found the end products of light induced degradation of anthocyanins to be the same as in thermal degradation, however, the kinetic pathway of the degradation differed, involving the excitation of the flavylium cation.

2.4.2.6 Enzymes

The most common anthocyanin degrading enzymes are glycosidases, which break covalent bonds between glycosyl residues and aglycones, resulting in the rapid degradation of the highly unstable anthocyanidin. Peroxidases and phenolases, naturally present in fruits, are also common anthocyanin degradation enzymes (Kader and others 1997). Enzymes oxidize phenolic compounds in the media to their corresponding

quinones, which then react with anthocyanins and lead to brown condensation products (Kader and others 2001).

2.4.2.7 Sugars

Sugars and their degradation products are known to decrease anthocyanin stability. According to Krifi and others (2001), anthocyanins react with both the degradation products of sugars, furfural and hydroxy-methylfurfural, and ascorbic acid to yield brown pigment polymers. In contrast, sugars have also been found to protect anthocyanins from degradation during frozen storage, preventing polymerization and browning, probably due to the inhibition of enzymatic or condensation reactions by sucrose (Wrolstad and others 1990).

2.4.2.8 Ascorbic acid

Ascorbic acid and its derivatives are widely used as antioxidants in fruit juices, acting primarily as singlet oxygen quenchers (Elliot 1999). Ascorbic acid and anthocyanins have long been known to be mutually destructive, especially in the presence of oxygen (Sondheimer and Kertesz 1953; Starr and Francis 1968). Ascorbic acid has been reported to enhance polymer pigment formation and bleach anthocyanin pigments (Poei-Langston and Wrolstad 1981). Hydrogen peroxide produced by copper-catalyzed breakdown of ascorbic acid was first believed to be responsible for pigment degradation (Timberlake 1960). The presence of anthocyanin breakdown products in decolorised systems showed, according to Iacobucci and Sweeny (1983, reviewed by Garcia-Viguera and Bridle 1999), that color bleaching of anthocyanins by ascorbic acid occurs by oxidative cleavage of the pyrilium ring. Direct condensation between anthocyanins and ascorbic acid has been also postulated as a mechanism for mutual destruction, reinforced by the work of Poei-Langston and Wrolstad (1981), who noted that anthocyanin color

decreased more rapidly under oxygen free conditions than under oxygenated conditions, favoring a predominant condensation mechanism to explain the observed color loss. However, the stability of acylated anthocyanins has been observed to increase in presence of ascorbic acid (Del Pozo-Insfran and others 2004). In addition, anthocyanins have been considered to be protected by ascorbic acid against enzymatic degradation (Talcott and others 2003).

2.4.2.9 Copigmentation

Colored forms of plant anthocyanins in nature are strongly stabilized by other natural components, so-called copigments, which allow them to be colorful despite the prevailing weakly acidic pH conditions (Brouillard 1983). Copigmentation results in higher absorbance values (hyperchromic shift) and a shift in the wavelength at which the maximum absorbance in observed (bathochromic shift), typically 5 to 20 nm higher, providing a blue-purple tone in an otherwise red solution (Boulton 2001). A wide range of different molecules has been found to act as copigments, including flavonoids and other polyphenols, alkaloids, amino acids and organic acids (Brouillard and others 1989). For a given pigment-cofactor pair, the observed color enhancement depends on the concentration of pigment, the molar ratio of cofactor to pigment, pH, the extent of non aqueous conditions and the anions in solution (Boulton 2001). In natural systems, such as juices, a competition between the various cofactors and pigments would be expected.

The most important copigmentation mechanisms are intermolecular and intramolecular interactions, although self-association and metal complexation may also occur. Intermolecular interactions can occur with both the flavylium cation and the quinonoidal base of the anthocyanins. Since both these colored equilibrium forms are almost planar, interactions between the flavylium cation or quinonoidal base and

copigment result in an overlapping arrangement of the two molecules, preventing the nucleophilic attack of water on the anthocyanin molecule (Chen and Hrazdina 1981).

2.4.3 Anthocyanins in Açai

Reports on the identification of anthocyanins in açai differ, and while Bobbio and others (2000) reported the presence of cyanidin-3-arabinoside and cyanidin-3-arabinosylarabinoside, Del Pozo-Insfran and others (2004) found cyanidin and pelargonidin glycosides while HPLC-MS analysis of açai pulp revealed the predominant presence of cyanidin-3-glucoside and cyanidin-3-rutinoside, in the studies by Gallori and others (2004) and Lichtenthaler and others (2005).

CHAPTER 3
PHYTOCHEMICAL, ANTIOXIDANT AND PIGMENT STABILITY OF AÇAI AS AFFECTED BY CLARIFICATION, ASCORBIC ACID FORTIFICATION AND STORAGE

3.1 Introduction

Açai, one of the most abundant plants in the Amazon estuary, has recently captured international attention due to potential health benefits associated with its high antioxidant capacity and polyphenolic composition. The emerging açai market is experiencing rapid growth and numerous internet businesses and retail merchants already offer a wide array of products to US consumers. Among them, Sambazon, that offers açai pulp, açai-based smoothies, supplement capsules, açai powders and shelf-stable concentrates. Similarly, Zola Açai offers an unfiltered form of açai in a single-serve presentation while Monavie markets dietary supplement juice blends and gels, using a partially clarified form of açai. Alternatively, Bossa Nova sells a highly clarified form of açai through retail, gourmet, and natural food stores. Even though a variety of açai-based functional beverages and dietary supplements are currently available in the US, studies on the functional properties of this matrix are still lacking. Processing effects on color, phytochemical stability and antioxidant capacity of açai products have not been previously assessed while only few studies on açai have been reported in scientific literature (Bobbio and others 2002; Araujo and others 2003; Del Pozo-Insfran and others 2004; Coisson and others 2005).

Açai pulp is commonly clarified to improve aesthetic properties and market acceptability while removing fats and insoluble solids. In addition, some juices may be fortified with ascorbic acid, not only to prevent browning but to improve nutritional

quality. However, the effects of ascorbic acid fortification are mostly unknown in açai-based food products. The objective of this study was to investigate the effects of clarification, ascorbic acid fortification, and storage temperature on the phytochemical content, antioxidant capacity and color attributes of açai pulp. Results from these investigations are aimed to assist the açai juice industry optimize processing and storage practices to achieve maximum aesthetic quality and retention of biologically active compounds.

3.2 Materials and Methods

3.2.1 Materials and Processing

Pasteurized, frozen açai pulp was obtained from Bossa Nova Beverage Group (Los Angeles, CA) and shipped overnight to the Department of Food Science and Human Nutrition at the University of Florida. Pulp was thawed and divided into three different portions. One portion remained unprocessed (pulp control) while the two remaining portions were subsequently centrifuged (2000*g*) at 4°C for 15 min to separate insoluble solids and lipids from the aqueous fraction (centrifuged juice). The third portion was further processed by passing it through a 1 cm bed of diatomaceous earth to remove insoluble solids and remaining lipids (clarified juice). Each portion was then divided into two subfractions, one fortified with L-ascorbic acid (500mg/L) and the other with an equal volume of citric acid buffer (pH 3.5) as a non fortified control. All processing regimes were adjusted to pH 3.5 for subsequent storage and sodium azide was added to retard microbial growth. Treatments were finally loaded into 15 mL glass test tubes, sealed and stored in the dark at 4 and 20°C for 30 days. Samples were collected for evaluation every 10 days and frozen (-20°C) until analysis.

3.2.2 Chemical Analysis

3.2.2.1 Spectrophotometric determination of total anthocyanins

Total anthocyanin content was determined spectrophotometrically by the pH differential method of Wrolstad (1976, cited by Wrolstad and others 2005). Juice treatments at each storage time were initially diluted 50-fold with buffer solutions at pH 1.0 and pH 4.5. Absorbance was read on a Beckman DU® 640 spectrophotometer (Beckman, Fullerton, CA) at a fixed wavelength of 520nm and total anthocyanin concentration calculated using mg/L equivalents of cyanidin-3-glucoside with an extinction coefficient of 29,600 (Jurd and Asen 1966).

3.2.2.2 Determination of polymeric anthocyanins

The percentage of monomeric and polymeric anthocyanins was determined based on color retention in the presence of sodium sulfite (Rodriguez-Saona 1999). Juice treatments were diluted 10X in pH 3.5 buffer and each sample subdivided into two fractions. A solution containing 5% sodium sulfite was added to one fraction while an equivalent volume of water (400 µL) was added to the remaining 4mL fraction. Absorbance at 520nm was recorded for all fractions on a Beckman DU® 640 spectrophotometer (Beckman, Fullerton, CA) and the concentration of polymeric anthocyanins calculated as the percentage of absorbance remaining after the addition of sodium sulfite.

3.2.2.3 Determination of total soluble phenolics

Total soluble phenolics were determined by the Folin-Ciocalteu assay (Singleton and Rossi 1965). Juice samples were diluted 10-fold and 100µL of each were loaded into test tubes, to which 1 mL of 0.25N Folin-Ciocalteu reagent (Sigma Chemical Co. St. Louis, MO) was added. After a 3 min reaction, during which the phosphomolybdic-

phosphotungstic acid was reduced by phenolic compounds and other reducing agents in the juice, 1 mL of 1N sodium carbonate was added to form a blue chromophore. A final dilution with 5 mL of distilled water was performed after 7 min. Samples were held for 30 min, after which absorbance was read using a UV-Vis microplate reader (Molecular Devices Spectra Max 190, Sunnyvale CA) at 726nm. Total soluble phenolics were quantified based on a linear regression against a gallic acid standard curve and data was expressed in mg/L gallic acid equivalents.

3.2.2.4 Quantification of antioxidant capacity

Antioxidant capacity was determined by the oxygen radical absorbance capacity (ORAC) method of Cao and others (1996), adapted to be performed with a 96-well Molecular Devices fmax® fluorescent microplate reader (485 nm excitation and 538 nm emission). The assay measures the ability of an antioxidant to inhibit the decay of fluorescein induced by the peroxyl radical generator 2,2-azobis (2-amidinopropane dihydrochloride) compared to Trolox, a synthetic, water-soluble vitamin E analog. For analysis, juice samples were diluted 200-fold in pH 7.0 phosphate buffer and 50µL of each were then transferred to a microplate along with a Trolox standard curve (0, 6.25, 12.5, 25, 50 µM Trolox) and a phosphate buffer blank. Readings were taken every 2 min over a 70 min period at 37°C. Antioxidant capacity was quantified by linear regression based on the Trolox standard curve and results were expressed in µmol of Trolox equivalents per gram (µmol TE/g).

3.2.2.5 Analysis of polyphenolics by HPLC

Polyphenolic compounds were analyzed by reverse phase HPLC using modified chromatographic conditions of Talcott and Lee (2002) with a Waters 2695 Alliance system (Waters Corp., Milford, MA) equipped with a Waters 996 photodiode array

(PDA) detector. Separations were performed on a 250 x 4.6 mm Acclaim 120-C18 column (Dionex, Sunnyvale, CA) with a C18 guard column. Mobile phases consisted of water (phase A) and a 60:40 methanol and water (phase B), both adjusted to pH 2.4 with o-phosphoric acid. The gradient solvent program ran phase B from 0 to 60% in 20 min; 60 to 100% in 20 min; 100% for 7 min; 100 to 0% in 3 min and final conditions were held for 2 min at a flow rate of 0.8 mL/min. Polyphenolics were identified by UV/VIS spectral interpretation, retention time and comparison to authentic standards (Sigma Chemical Co., St. Louis, MO). All treatments were filtered through a 0.45µM filter and directly injected (50µL) into the HPLC.

3.2.3 Statistical Analysis

The study was designed as a 3 x 2 x 4 full factorial that included three levels of açai pulp clarification, presence or absence of ascorbic acid and four storage times. Data for each treatment is the mean of three replicates. Analysis of variance, multiple linear regression, Pearson correlations and means separations by Tukey's HSD test ($P < 0.05$) were conducted using JMP software (SAS Institute, Cary, NC).

3.3 Results and Discussion

Clarification, ascorbic acid addition and storage temperature effects were compared to a control within each treatment. Results for all analysis, except for polymeric anthocyanins, were reported as a percentage of the initial concentration for each treatment.

3.3.1 Anthocyanin Color Retention

Anthocyanin color stability was assessed spectrophotometrically and decreased in a linear, temperature-dependent manner (Figures 3-1 and 3-2). Clarification, fortification with ascorbic acid and storage temperature significantly affected color stability

($p<0.0001$). Initial anthocyanin concentration in açai pulp (729.3 ± 3.4 mg/L) was only slightly affected by centrifugation (714.8 ± 3.2 mg/L), but experienced a 20% decrease after filtration (580.7 ± 3.6 mg/L). Color loss was probably due to irreversible binding or physical trapping of anthocyanins to the diatomaceous earth filter, since a deep purple color remained on filters following clarification. No significant differences ($p<0.05$) were found among degradation rates of non clarified treatments during storage at 4 or 20°C (Figures 3-1 and 3-2).

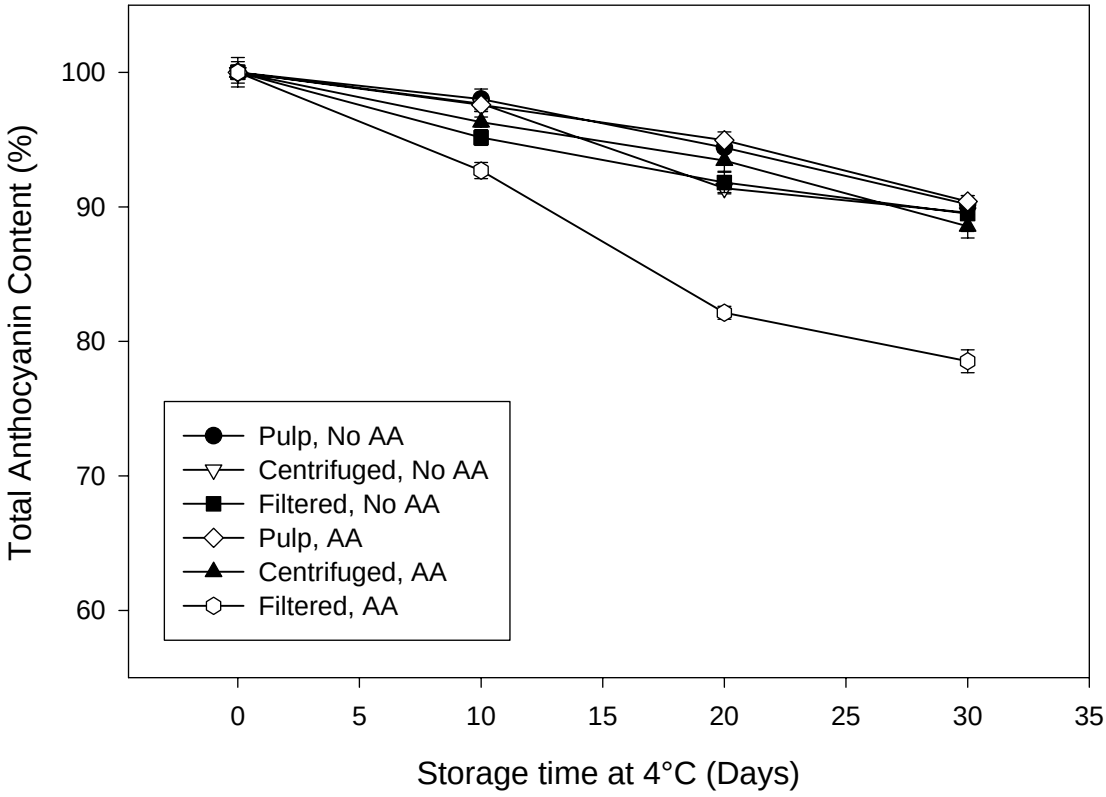

Figure 3-1. Total anthocyanin content of açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 4°C. Error bars represent the standard error of the mean, n=3.

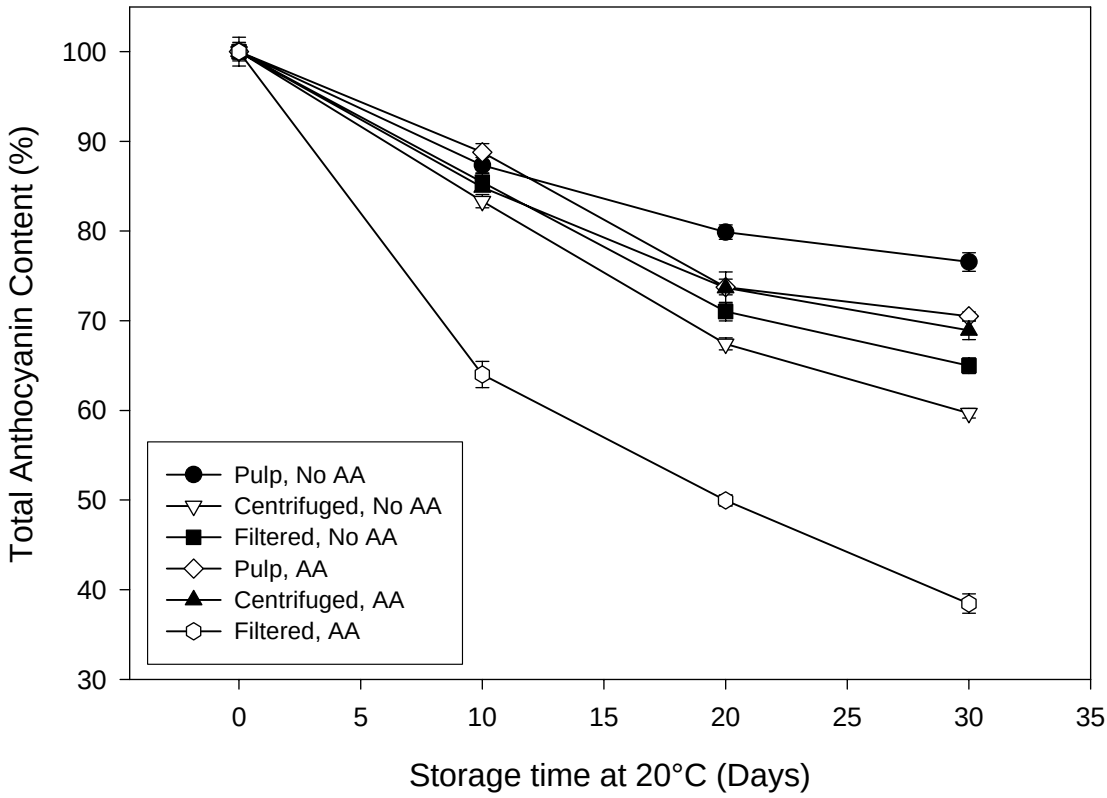

Figure 3-2. Total anthocyanin content of açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 20°C. Error bars represent the standard error of the mean, n=3.

The presence of ascorbic acid was particularly detrimental for clarified açai juice, which following fortification, experienced from 2.5 and 2.1 times higher losses compared to non fortified controls when stored at 4 and 20°C respectively. Both pulp and centrifuged juice remained unaffected by ascorbic acid addition at both storage temperatures. It has been suggested that disruption of plant tissues by juice extraction and clarification destroys the protective tertiary structure or intermolecular copigmentation that protects anthocyanins from nucleophilic attack by water (Goda and others 1997). This may explain the poor stability of filtered açai juice in the presence of ascorbic acid as compared to unfiltered treatments, if an additional processing step is considered.

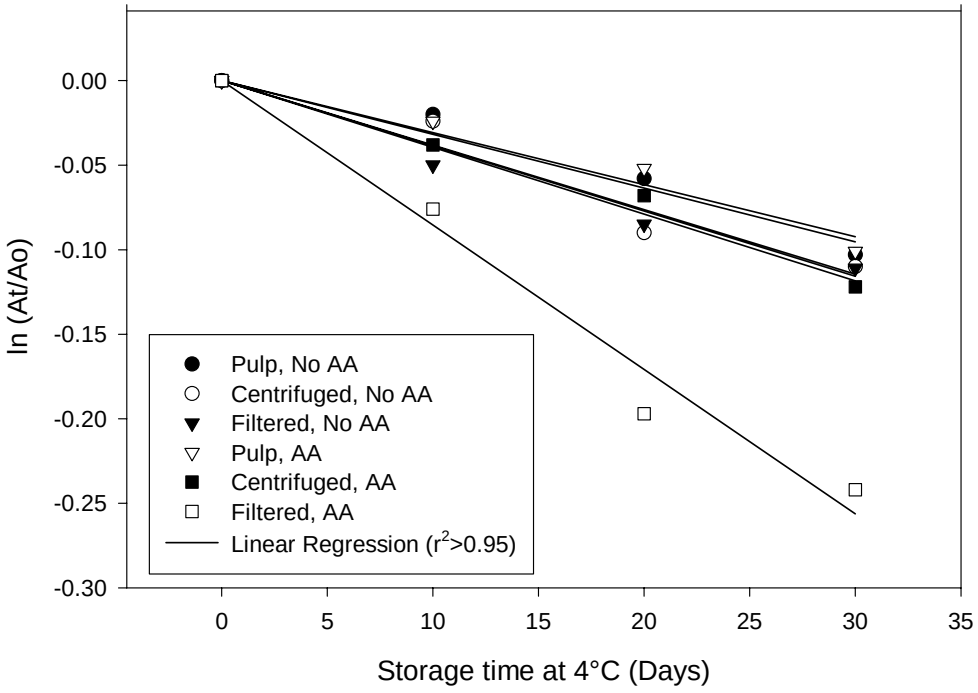

Figure 3-3. Linear regression of total anthocyanin content of açai as affected by clarification, fortification with ascorbic acid (AA) and storage at 4°C.

Although it has also been suggested (Brouillard and Dangles 1994) that the formation of anthocyanin-copigment products also occurs in solution, as in fruit juices, Clifford (2000) observed that it does not necessarily follow that the nature of the copigmentation that occurs in intact cells is identical to that which occurs in juices. In addition, loss of polyphenolic copigments during filtration, might have also affected the nature and extent of copigmentation, since the molar ratio of cofactor to pigment greatly affects copigmentation reactions (Boulton 2001; Malien-Aubert and others 2001). Moreover, decreased anthocyanin concentration following filtration might have negatively affected color stability in fortified treatments since quantitative differences in anthocyanin content have been found to protect against pigment losses induced by

ascorbic acid in strawberry puree and juice (Garzon and Wrolstad 2002) and in strawberry and blackcurrant model systems (Skrede and others 1992).

Storage temperature had a significant effect on color degradation rates and while 10 to 22% color was lost during storage at 4°C, losses between 24 and 61% were experienced by treatments stored at 20°C. Regression analyses were used to describe color degradation during storage, which followed first-order kinetics (Figure 3-3) and were in agreement with previous reports (Cemeroglu and others 1994; Iversen 1999). First-order reaction rates (k) and half-lives (t½) or time needed for 50% degradation of anthocyanins under the experimental conditions were calculated using the equations: $\ln(A/Ao)=-kt$ and $t½=\ln 0.5/k$; where *Ao* is the initial absorbance of appropriately diluted juice and *A* is the absorbance value after t days of storage at either 4 or 20°C (Table 3-1).

Table 3-1. Clarification and ascorbic acid (AA) fortification effects on kinetic parameters of color degradation in açai juice stored at 4 and 20°C.

		4°C		20°C	
		k[1]	t½ [2]	k	t½
No AA	Pulp	3.57	194.0a[3]	8.91	77.8a
	Centrifuged	3.97	174.6a	14.28	48.6b
	Filtered	3.35	206.6a	14.78	46.9ab
AA	Pulp	3.20	216.6a	12.34	56.2ab
	Centrifuged	3.95	175.3a	11.31	61.3ab
	Filtered	8.46	81.9b	31.15	22.3c

[1]Reaction rate constant (k x 10^{-3} $days^{-1}$). [2]Half life (days) of initial absorbance value for each treatment. [3]Values with similar letters within columns are not significantly different (LSD test, $p<0.05$).

Degradation kinetics showed the marked effect of temperature on anthocyanin color stability and all treatments experienced 3.5 times higher degradation rates when stored at 20°C than when kept refrigerated. In filtered juice, the presence of ascorbic acid was particularly detrimental at the highest temperature, losing 50% of the initial color in 22 days, as compared to a projected 82 days when the same treatment was stored at 4°C.

3.3.2 Polymeric Anthocyanins Concentration

Color deterioration of anthocyanin containing products during storage not only results from anthocyanin degradation but also from brown pigment formation (Skrede and others 1992). These color changes have been associated with the transformation of monomeric anthocyanins into polymeric forms (Baranac and others 1996; Francia-Aricha and others 1997; Johnston and Morris 1997). Polymerization of anthocyanins present in açai was significantly affected by clarification, storage temperature, and ascorbic acid addition; increasing linearly throughout the storage period (Figures 3-4 and 3-5).

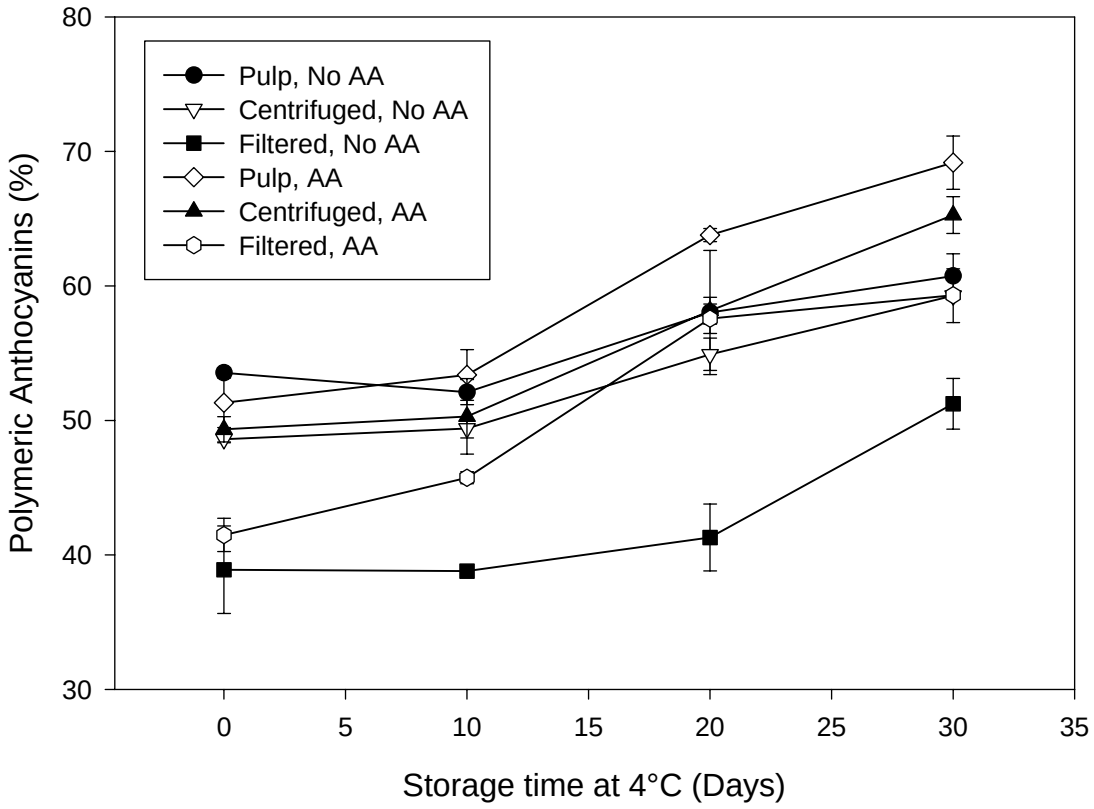

Figure 3-4. Polymeric anthocyanins (%) in açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 4°C. Error bars represent the standard error of the mean, n=3.

Initial differences on polymeric anthocyanin content between clarified (38.9-41.5%) and non clarified (48.6-54.1%) treatments were attributed to irreversible binding of polymeric compounds to the diatomaceous earth used for filtration. Moreover, higher degrees of polymerization were observed in non clarified treatments at the end of storage, probably due to higher initial concentrations of both polymeric and total anthocyanins. Differences in polymeric anthocyanin content may explain the higher stability of non clarified treatments, especially in presence of ascorbic acid. According to Dao and others (1998), self-association reactions among anthocyanins are thought to be responsible for the improved stability observed at higher concentrations.

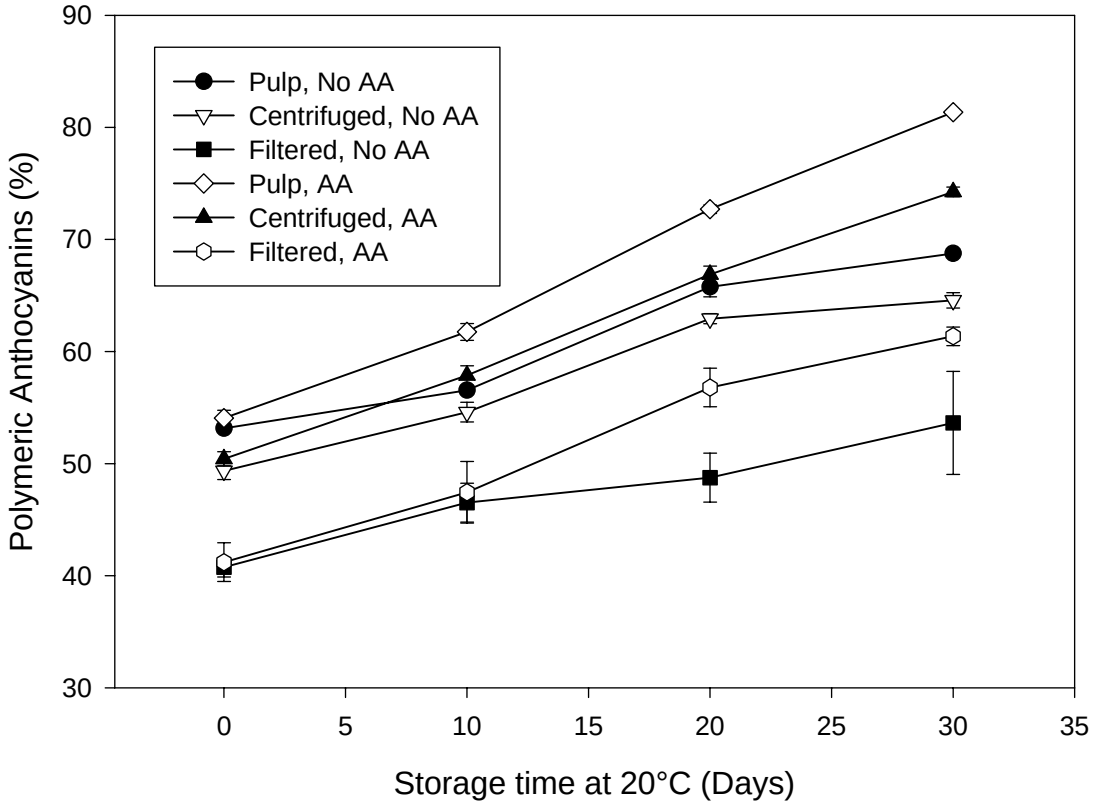

Figure 3-5. Polymeric anthocyanins (%) in açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 20°C. Error bars represent the standard error of the mean, n=3.

The presence of ascorbic acid markedly increased anthocyanin polymerization, and significant differences ($p<0.05$) were found between all fortified treatments and their corresponding non fortified counterparts when stored at both 4 and 20ºC. Final polymeric anthocyanin concentration in fortified treatments was higher than their non fortified counterparts by 6.0-8.5% when stored at 4ºC and by 7.7-12.6% following storage at 20ºC. Similar results have been reported in blackcurrant and strawberry syrups, where addition of ascorbic acid notably increased the proportion of polymeric color, while the highest degrees of polymerization were found in syrups with low anthocyanin concentration which had been fortified with ascorbic acid (Skrede and others 1992).

The rate at which polymerization occurred was also affected by storage temperature, even when similar results were obtained at both 4 and 20ºC. Final polymeric anthocyanin concentrations ranged from 51.2 to 69.2% when treatments were stored at 4ºC while 53.6 to 81.4% of anthocyanins were polymerized following storage at 20ºC. Polymerization rates notably increased when non clarified treatments were stored at either 4 or 20ºC but final polymeric anthocyanin concentrations in clarified juice treatments increased only slightly when the storage temperature was raised from 4 to 20ºC. Similar results were observed by Turker and others (2004) when studying the effects of storage temperature on the stability of black carrot anthocyanins in a fermented beverage and where only a minor increase in polymeric color percentages was observed in samples stored at 4 and 25ºC over a storage period of 90 days.

3.3.3 Total Soluble Phenolics

Total soluble phenolics in açai showed only minor losses during storage and were slightly influenced by temperature. Initial phenolic content of each treatment was determined by its degree of clarification, ranging from 197.2 ± 6.9 mg gallic acid

equivalents (GAE)/100mL in pulp and centrifuged juice to 143.7 ± 7.0 mg GAE/100mL in clarified juice; representing a 25% loss during filtration. Differences were attributed to binding of polyphenolic compounds to the filter, and were consistent with previous observations on initial anthocyanin contents.

Further effects of clarification or ascorbic acid addition were not detected while storage temperature only had a minor influence on polyphenolic degradation rates (Figures 3-6 and 3-7).

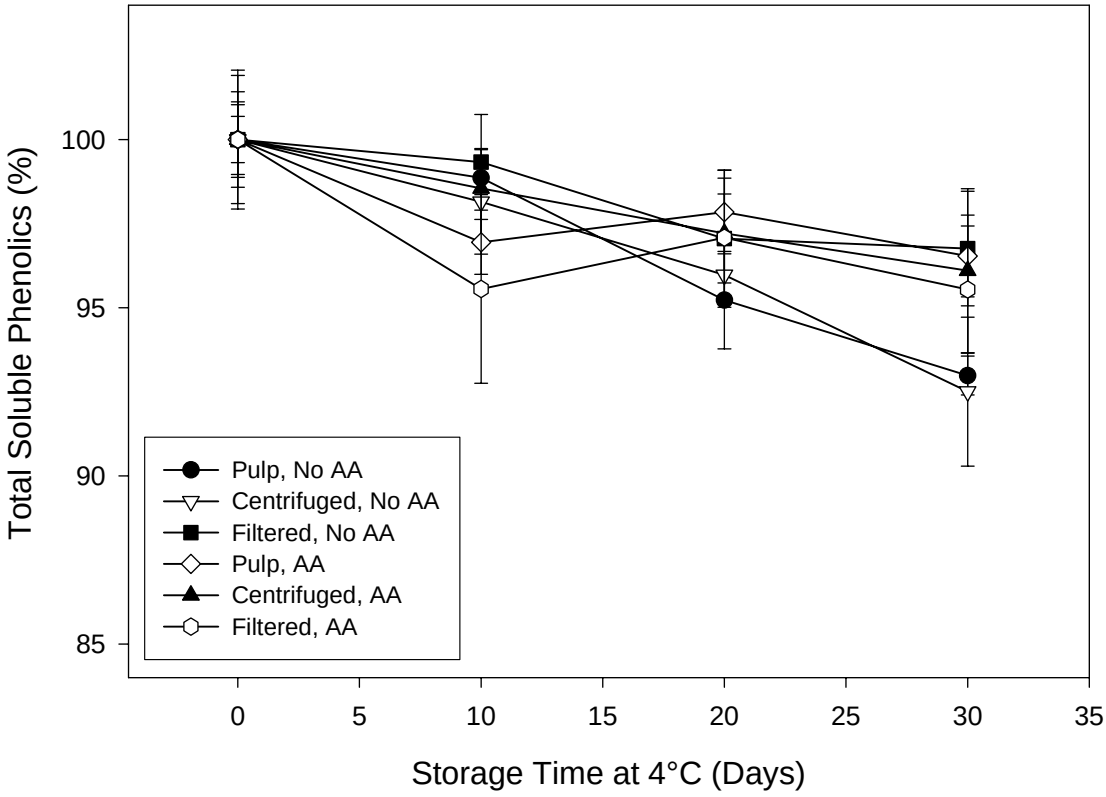

Figure 3-6. Total soluble phenolics (%) in açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 4°C. Error bars represent the standard error of the mean, n=3.

Maximum losses in total soluble phenolics at the end of storage ranged from 8 to 13% for all treatments maintained at 4 and 20ºC respectively. Minor losses in total

phenolic content, quantified by the Folin-Ciocalteu method, might be attributed to polymerization reactions, which reduce the number of free hydroxyl groups, measured by this assay (Klopotek and others 2005). This hypothesis is supported by the reports by Tsai and Huang (2004), where polymeric anthocyanin content was negatively correlated (r=0.84) to ferric reducing antioxidant power (FRAP). Polyphenolic oxidation via electron donation and singlet oxygen quenching might have also reduced total soluble phenolic contents (Shahidi and Wanasundara 1992).

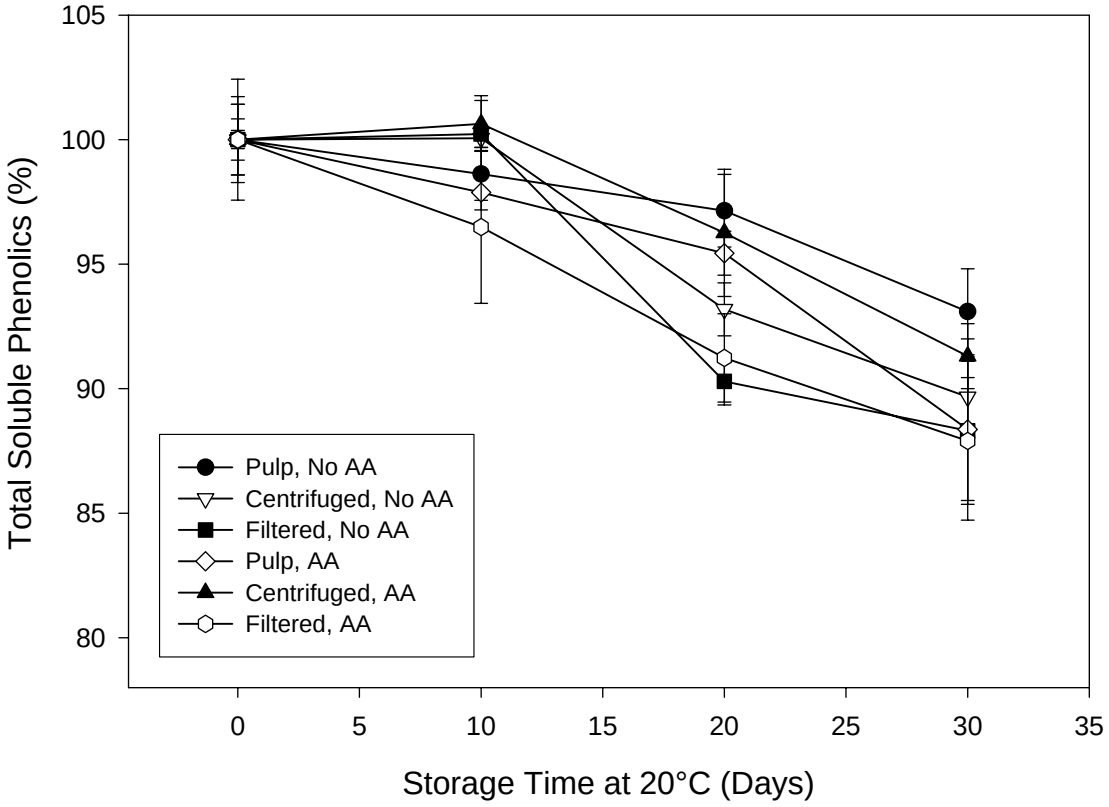

Figure 3-7. Total soluble phenolics (%) in açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 20°C. Error bars represent the standard error of the mean, n=3.

3.3.4 Polyphenolics by HPLC

Polyphenolics present in açai were analyzed by HPLC and changes in relative peak area during storage monitored over time at 360 and 520nm. Peaks with the highest relative areas at each wavelength were used for quantification of non-anthocyanin polyphenolics and total anthocyanins respectively.

3.3.4.1 Anthocyanins by HPLC

HPLC analysis of açai juice confirmed the predominant presence of cyanidin-3-glycosides, as previously reported by Bobbio and others (2002), Del Pozo-Insfran and others (2004), Gallori and others (2004) and Coisson and others (2005). Previous reports on the specific identity of anthocyanins in açai differ, and while Del Pozo-Insfran and others (2004) only detected the presence of cyanidin and pelargonidin after hydrolysis, Gallori and others (2004) and Lichtenthaler and others (2005) reported cyanidin-3-glucoside and cyanidin-3-rutinoside as the most abundant anthocyanins in açai following HPLC-mass spectrometry analysis. Moreover, hydrolysis and TLC analysis of açai juice have revealed the presence of cyanidin-3-arabinoside, cyanidin-3-glucoside and cyanidin-3-rutinoside in other studies (Bobbio and others 2000, Gallori and others 2004). Geographical, seasonal and fruit specific variations might also account for these differences. In this study two major anthocyanins (Figure 3-8), both with maximum absorbance at 520 nm, were detected and tentatively identified as cyanidin glycosides, based on spectral characterization described by Hong and Wrolstad (1990b). Maximum absorbance coincided with typical absorption maxima in the 520 to 526 nm range for cyanidin 3-glycosides in acidic methanol solutions while no differences in spectral characteristics were noted between peaks, in support of previous observations where the nature of the sugar substitution had no significant effects on anthocyanin spectra. The

absence of the characteristic spectral absorption maxima of hydroxylated aromatic acids in the 310 nm range led to the conclusion that anthocyanins were not acylated. Furthermore, the presence of different glycosidic substituents was identified based on their retention characteristics in reversed-phase HPLC. Previous studies characterizing black currant, blackberry, raspberry, plum and cherry anthocyanins have shown that both cyanidin-3-glucoside and cyanidin-3-rutinoside elute sequentially and share similar spectral characteristics (reviewed by Hong and Wrolstad 1990a). Based on these observations and the reports of Gallori and others (2004) and Lichtenthaler and others (2005), anthocyanins in açai were tentatively identified as cyanidin-3-glucoside and cyanidin-3-rutinoside respectively.

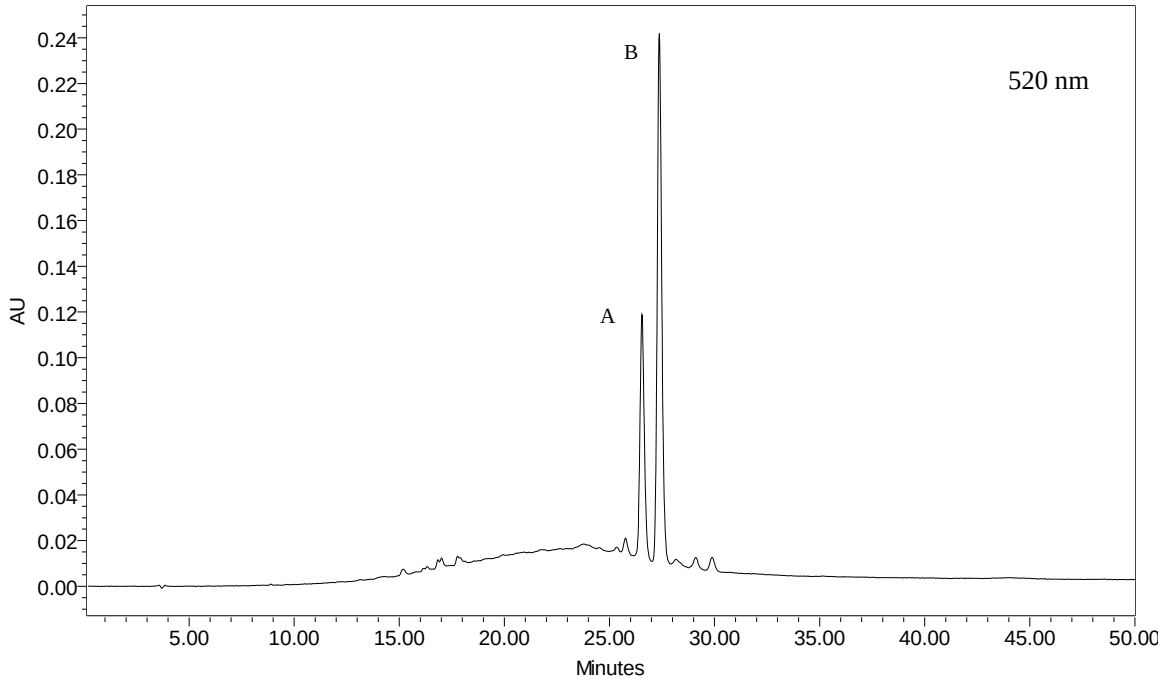

Figure 3-8. HPLC chromatogram of anthocyanins present in açai juice. Compounds were tentatively identified as cyanidin-3-glucoside (A) and cyanidin-3-rutinoside (B) based on their spectral characteristics.

Addition of the corresponding peak areas for cyanidin-3-glucoside and cyanidin-3-rutinoside yielded the total anthocyanin content for each treatment and was used for monitoring relative changes in total anthocyanin contents over the storage period.

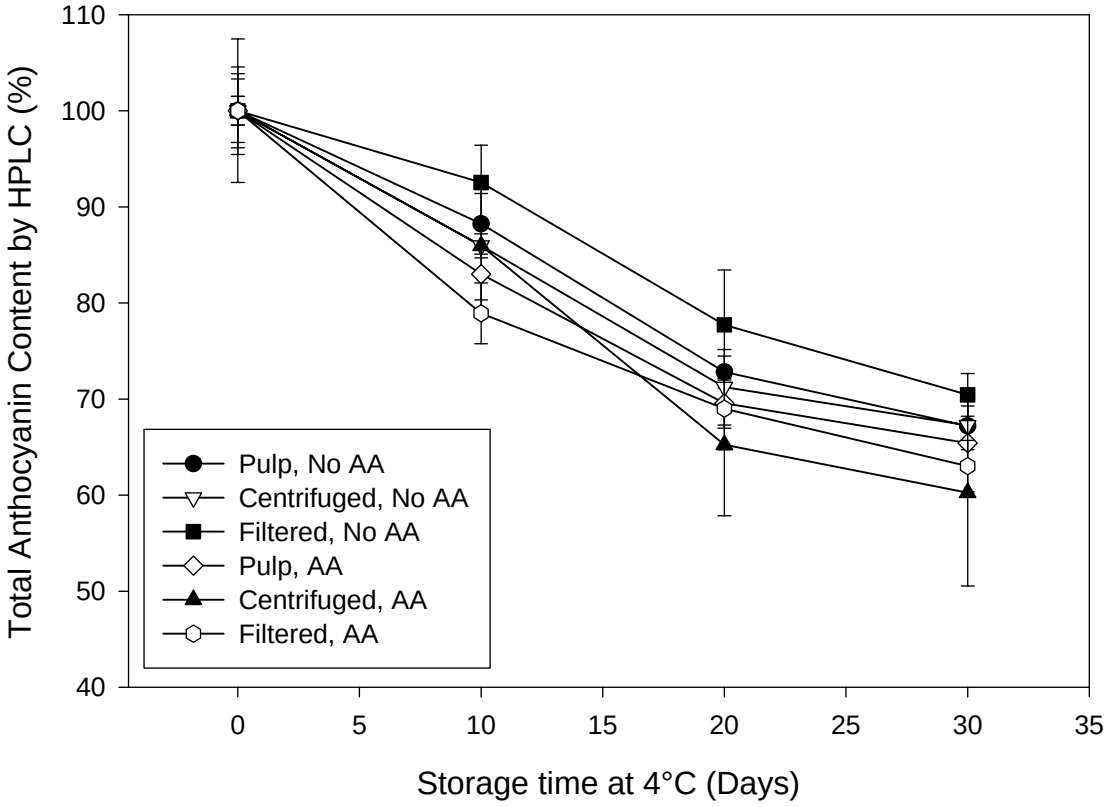

Figure 3-9. Total anthocyanin content (HPLC) of açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 4°C. Error bars represent the standard error of the mean, n=3.

Degradation of anthocyanins present in açai occurred in a linear, temperature-related manner (Figures 3-9 and 3-10); moderately correlated to color degradation (r=0.78). Initial anthocyanin contents varied with clarification level, and even when no differences were found between açai pulp and centrifuged juice, a 25% decrease in total anthocyanin content was observed after filtration. This behavior paralleled color losses observed in clarified juice during filtration (20% loss) and was probably due to

irreversible binding of anthocyanins to the filter, as previously discussed. Aside from initial differences among non clarified and clarified treatments, no further differences ($p<0.05$) in anthocyanin degradation rates were observed among treatments stored at 4ºC (Figure 3-9).

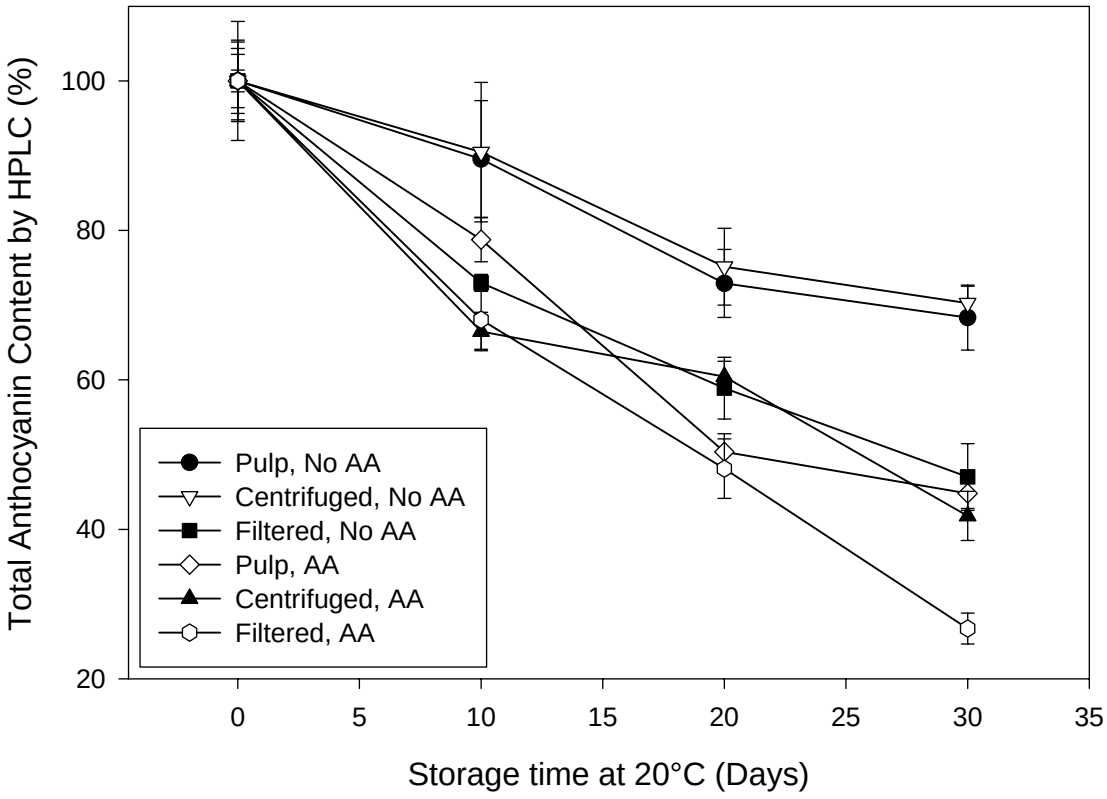

Figure 3-10. Total anthocyanin content (HPLC) of açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 20°C. Error bars represent the standard error of the mean, n=3.

In contrast, treatments stored at 20ºC (Figure 3-10) showed significant loses with clarification and ascorbic acid addition. Kinetics of anthocyanin degradation were described by a first-order reaction model (Figure 3-11), analogous to color degradation, and used to calculate anthocyanin degradation rate constants and half-lives for each treatment (Table 3-2).

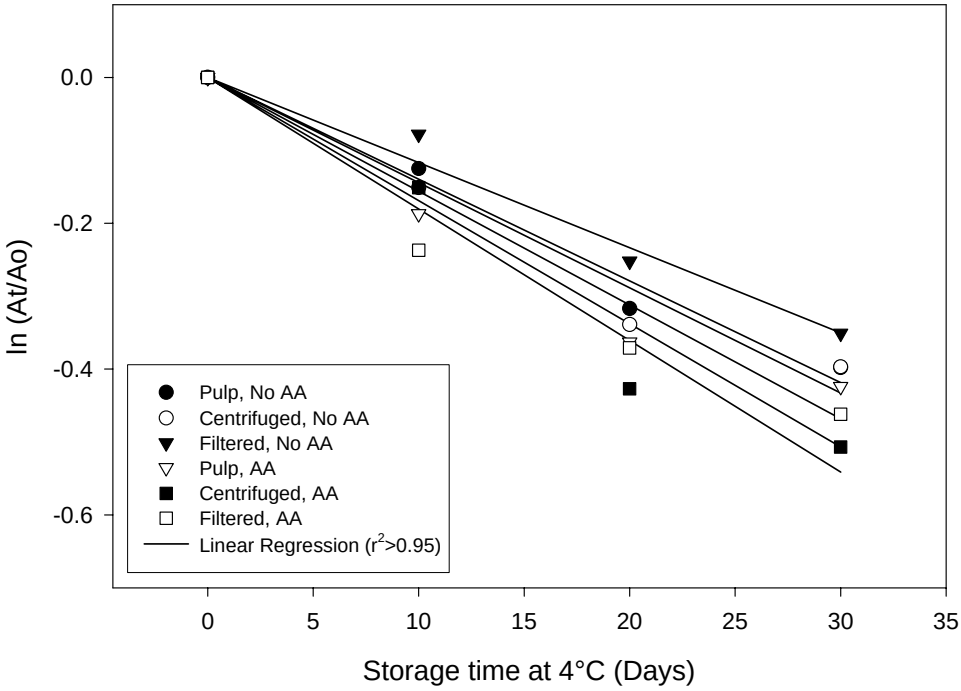

Figure 3-11. Linear regression of total anthocyanin content (HPLC) of açai as affected by clarification, fortification with ascorbic acid (AA) and storage at 4°C.

Significantly higher ($p<0.05$) anthocyanin degradation rates were observed in clarified juices when compared to non clarified açai pulp, and half-lives were reduced from 56-61 to 28 days in non clarified and clarified juices respectively. Decreased anthocyanin degradation in non clarified treatments was probably due to the stabilizing effect that higher anthocyanin and polyphenolic concentrations have on anthocyanins (Skrede and others 1992; Garzon and Wrolstad 2002).

In addition, treatments fortified with ascorbic acid showed significantly higher degradation rates ($p<0.05$) than their corresponding non-fortified treatments, shortening half-lives from 61, 56 and 28 to 30, 29 and 16 days in pulp, centrifuged and clarified açai juice respectively. Similar detrimental effects of ascorbic acid addition on anthocyanin stability have been reported in strawberry syrups, juices and concentrates (Skrede and

Table 3-2. Clarification and ascorbic acid (AA) fortification effects on kinetic parameters of anthocyanin degradation in açai juice stored at 4 and 20°C.

		4°C		20°C	
		k[1]	t½ [2]	k	t½
No AA	Pulp	11.7	59.3a[3]	11.4	61.0a
	Centrifuged	12.4	56.1ab	12.4	55.7a
	Filtered	12.2	56.5ab	24.8	28.0b
AA	Pulp	13.6	51.0ab	23.0	30.1b
	Centrifuged	13.8	50.3b	23.5	29.4b
	Filtered	12.7	54.5ab	43.1	16.1c

[1]Reaction rate constant (k x 10^{-3} $days^{-1}$). [2]Half life (days) of initial anthocyanin content for each treatment. [3]Values with similar letters within columns are not significantly different (LSD test, $p<0.05$).

others 1992; Garzon and Wrolstad 2002), blackcurrant juice (Iversen 1999), sour cherries and pomegranate juices (Ozkan 2002), blood oranges (Choi and others 2003) and anthocyanin model systems (Garcia-Viguera and Bridle 1999; Brenes and others 2005). Proposed mechanisms of degradation include the direct condensation between anthocyanins and ascorbic acid (Poei-Langston and Wrolstad 1981) and a free radical mechanism where cleavage of the pyrilium ring resulted from molecular oxygen oxidation reactions induced by ascorbic acid or its degradation products (Garcia-Viguera and Bridle 1999; Ozkan 2002).

3.3.4.2 Non anthocyanin polyphenolics by HPLC

HPLC analysis of non-anthocyanin polyphenolics in açai juice revealed the presence of gallic acid, catechin, (-)epicatechin, chlorogenic acid and various gallic acid derivatives and procyanidins, in agreement with similar characterizations performed by Del Pozo-Insfran and others (2004) and Coisson and others (2005). Major polyphenolic compounds in açai showed characteristic spectral absorption maxima at 272.8/348 nm (E,F), 272.8/339.2 nm (G), 258.6/353.5 nm (H) and 249.2/339.2 nm (I) respectively, although identification was not possible since standards were not available. Total

polyphenolic content was expressed as the sum of the total corresponding areas of peaks F-I and assessed over time for changes in relative concentration.

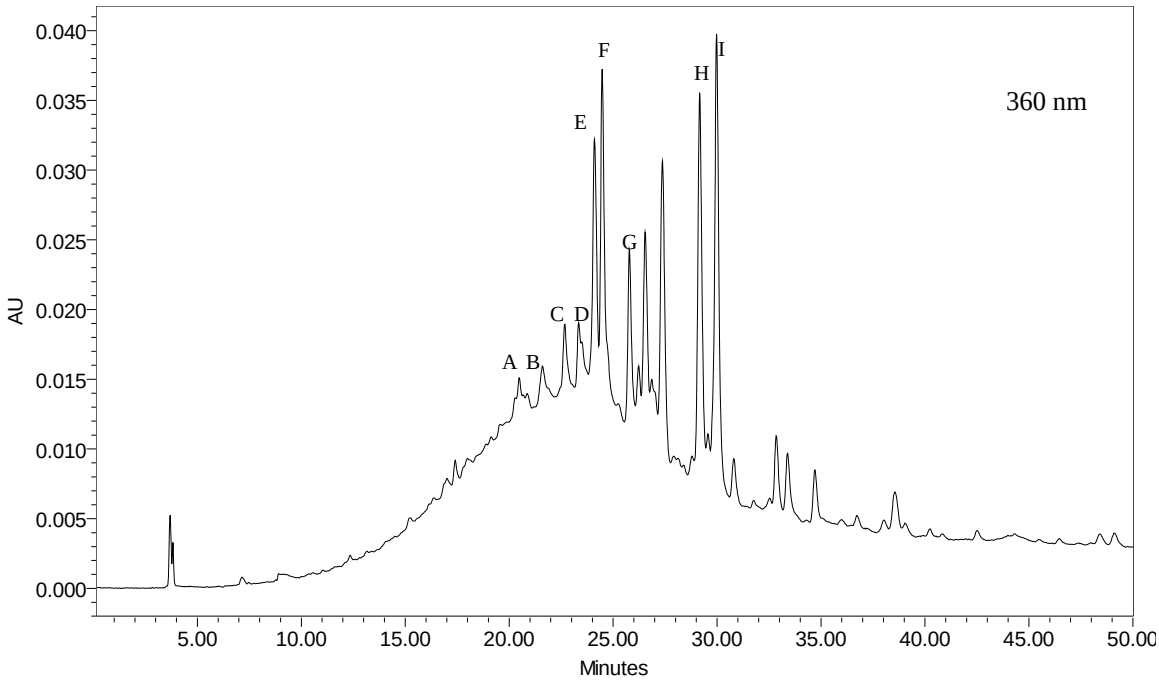

Figure 3-12. HPLC chromatogram of polyphenolics present in açai juice: catechin (A), procyanidin (B), chlorogenic acid (C), (-)epicatechin (D), unknown characteristic açai polyphenolics (E-I). Identification (360 nm) was done based on spectral characteristics and comparison to authentic standards.

While conducting HPLC analysis of treatments, a “hump” was observed in all chromatograms (Figure 3-12), probably originated from high polymerization degrees among anthocyanins and other polyphenolics. This hypothesis was supported by previous reports in wines, pigment extracts and concentrated fruit juices, which decline in the anthocyanin content during storage followed by the subsequent transformation of formerly sharp peaks into a “hump” with residual compounds “floating” over it (Francis 1989). Four types of transformations have been identified: direct condensation between anthocyanins and flavanol monomers or oligomers, direct condensation between anthocyanins and quinones, aldehyde bridging of anthocyanins with flavanol monomers

and dimers involving acetaldehyde and cycloaddition of acetaldehyde, vinylphenols or pyruvic acid (reviewed by Clifford 2000). Moreover, Yanagida and others (2003) have observed that reversed-phase HPLC is an effective separation method for polyphenolics, except for highly polymerized oligomers, which appear as broad unresolved peaks due to the enormous variety of isomers and oligomers with different degrees of polymerization. No significant changes in hump height occurred during storage, while a 12% in total area was observed by the end of the storage period, a potential limitation for polyphenolic quantification.

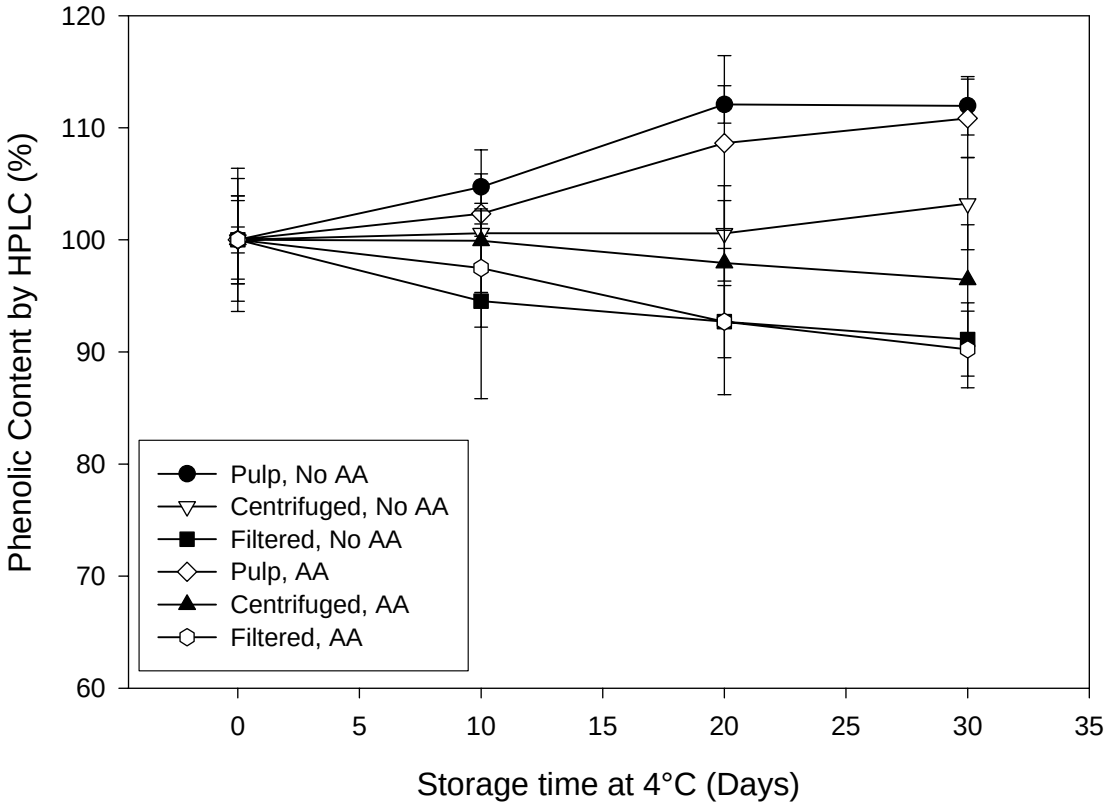

Figure 3-13. Phenolic content (HPLC) of açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 4°C. Error bars represent the standard error of the mean, n=3.

Total peak area of major non-anthocyanin polyphenolics in açai was initially higher for clarified and centrifuged juice than for non clarified pulp (25% lower), contrary to what was observed for total soluble phenolics. These results might indicate loss of minor polyphenolics during processing, which are accounted for in the total soluble phenolics content but were not included in the computation of total polyphenolic peak area. Further differences among treatments with varying degrees of clarification were observed during storage, following similar trends at both storage temperatures (Figures 3-13 and 3-14).

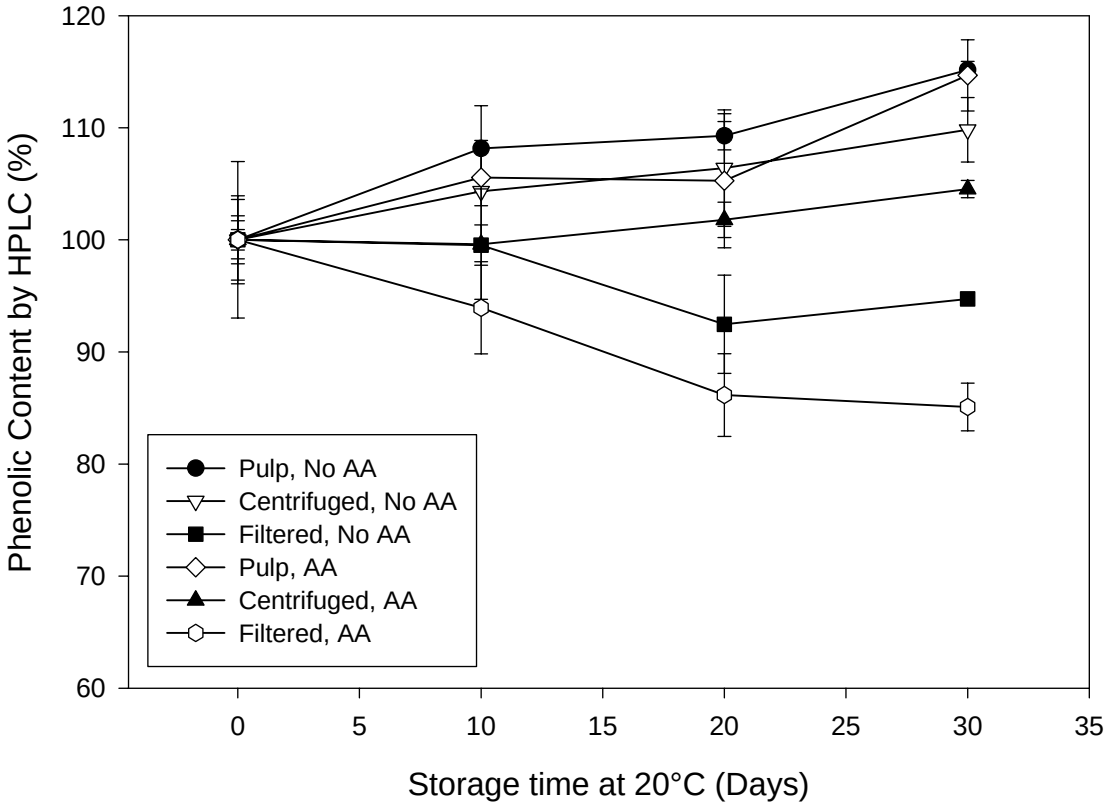

Figure 3-14. Phenolic content (HPLC) of açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 20°C. Error bars represent the standard error of the mean, n=3.

Overall, phenolic content increased by 10 to 15% in non clarified pulp and by 2 to 10% in centrifuged juice when stored at 4 and 20ºC respectively while phenolics in clarified juice decreased 15% at both storage temperatures. The high stability of these compounds in clarified juices and their relative increase in the non fortified pulp led to the hypothesis that selected major polyphenolics in açai constitute polymers with varying degrees of polymerization. Their appearance in the middle of the chromatographic "hump", thought to indicate the presence of highly polymerized compounds, seems to support this hypothesis.

Ascorbic acid addition had no effect on treatments stored at 4ºC but significant differences were detected among non fortified and fortified forms during storage at 20ºC (Figure 3-14). Total phenolic area in treatments fortified with ascorbic acid was 15% lower than their corresponding non-fortified counterparts at the end of storage; a pattern followed by all three clarification levels. These results may indicate a protective effect of non anthocyanin polyphenolics against ascorbic acid breakdown products. This protective effect, attributed to their actions as free radical acceptors and metal chelators (Ozkan 2002), has been linked to increased anthocyanin stability in black currant juice (Clegg and others 1968), sour cherry, pomegranate and strawberry juices (Ozkan and others 2002) and has also been observed in model systems (Brenes and others 2005).

3.3.5 Antioxidant Capacity

Antioxidant capacity of açai (54.4±1.2 µmol Trolox equivalents (TE)/mL) was found to be higher to values reported by Kalt and others (1999) and Ehlenfeldt and Prior (2001) for similar anthocyanin-rich fruits, such as highbush blueberry (4.6-31.1µmol TE/mL), raspberry (19.2-22.6µmol TE/mL) and strawberry (18.3-22.9µmol TE/mL). Antioxidant contents in açai varied within clarified (44.5±1.4µmol TE/mL) and non

clarified (54.4±1.7µmol TE/mL) treatments, a reduction that paralleled previously observed losses in anthocyanin and polyphenolic content after filtration. Antioxidant capacity decreased in a linear, temperature related manner, experiencing losses that ranged from 25 to 45% following storage at 4 and 20ºC respectively (Figures 3-15 and 3-16).

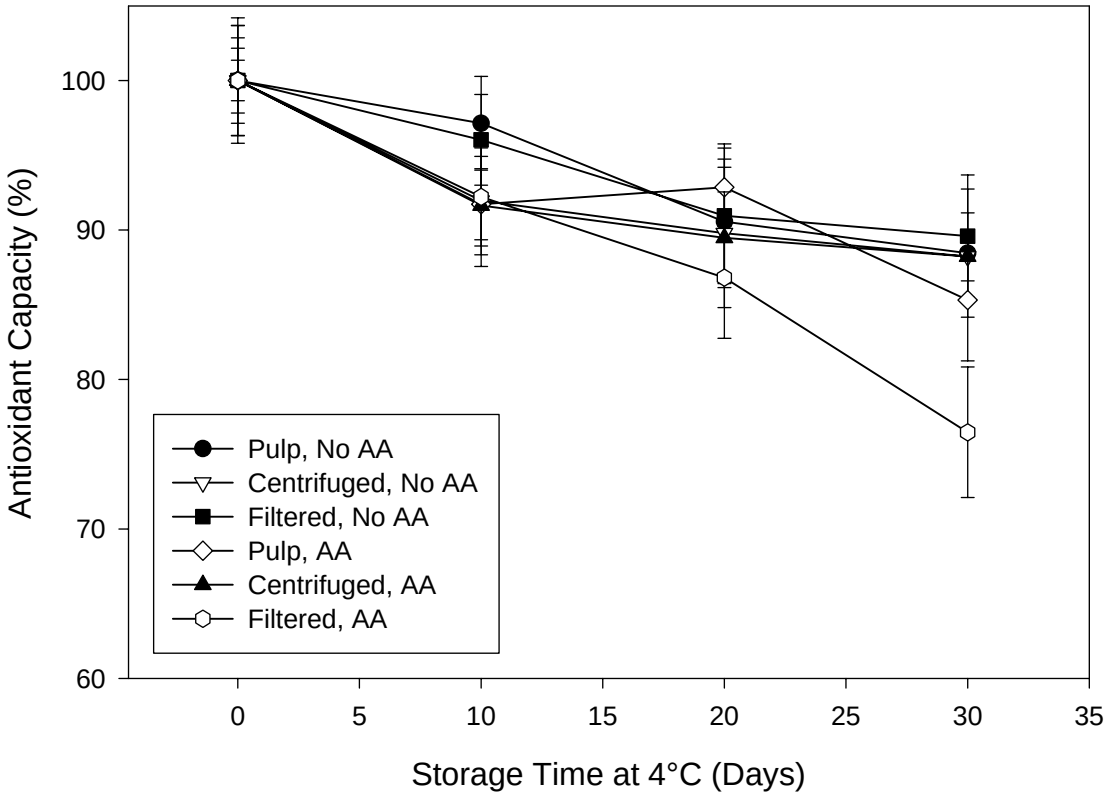

Figure 3-15. Antioxidant capacity (%) of açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 4°C. Error bars represent the standard error of the mean, n=3.

No differences were detected among treatments stored at 4ºC while those stored at 20ºC, only clarified açai juice with added ascorbic acid degraded at a significantly higher rate. Losses in antioxidant capacity were attributed to decreased anthocyanin and polyphenolic contents during storage and results were correlated to analogous trends observed for color

(r=0.88), total anthocyanins by HPLC (r=0.79) and total phenolics (r=0.73). Similar correlations between antioxidant capacity and total anthocyanin (r=0.90 and 0.77) or phenolic (r=0.83 and 0.92) contents were respectively obtained by Kalt and others (1999) in strawberries, raspberries and blueberries and Prior and others (1998) in highbush and lowbush blueberries.

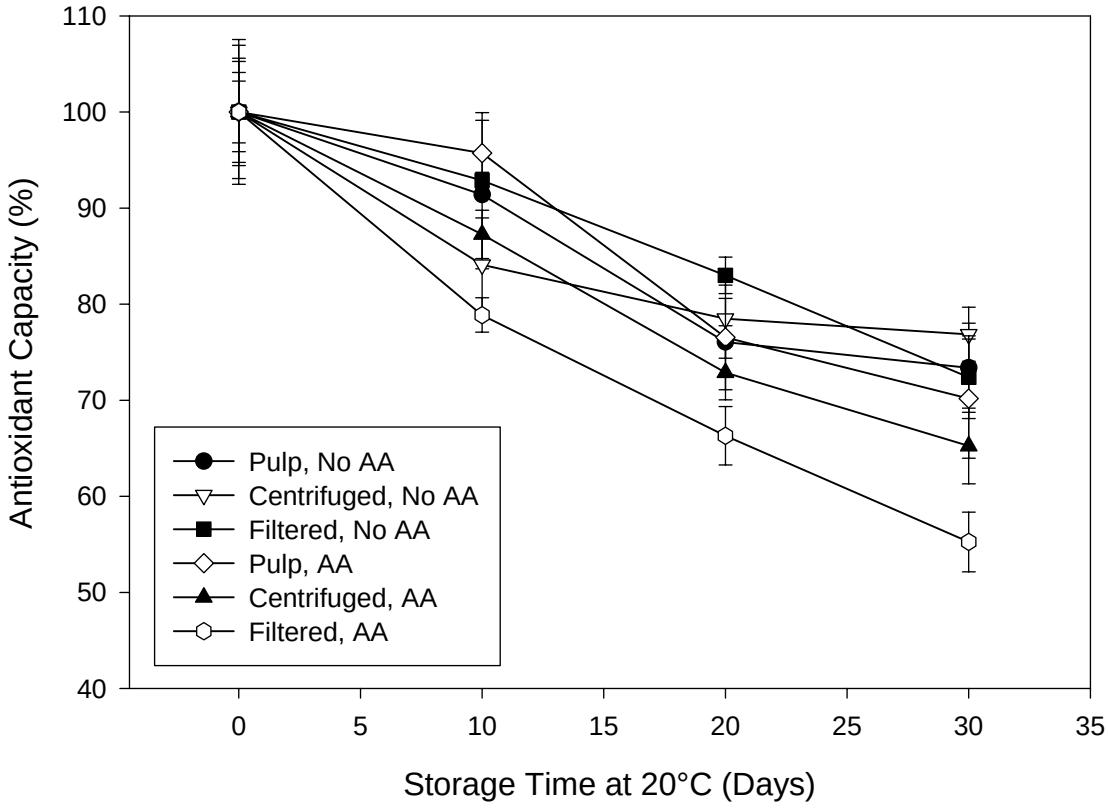

Figure 3-16. Antioxidant capacity (%) of açai as affected by clarification and fortification with ascorbic acid (AA) during storage at 20°C. Error bars represent the standard error of the mean, n=3.

3.4 Conclusions

Treatments stored at 4°C showed the greatest stability for all variables measured. Clarification of açai pulp negatively affected initial anthocyanin and polyphenolic contents, causing losses in color (20%), total phenolics (25%), total anthocyanins (25%)

and antioxidant capacity (20%). Clarified açai juice also exhibited poor stability in the presence of ascorbic acid, experiencing significantly higher losses in color, anthocyanin content and antioxidant capacity than all other treatments at the end of the storage period, especially when kept at 20ºC. Clarification of açai pulp without affecting its color and polyphenolic stability is then possible if low temperatures (<5°C) are maintained throughout the distribution chain. Fortification of clarified açai juice with ascorbic acid is not recommended since it markedly accelerates anthocyanin and color degradation.

CHAPTER 4
MATRIX COMPOSITION AND ASCORBIC ACID FORTIFICATION EFFECTS ON THE PHYTOCHEMICAL, ANTIOXIDANT AND PIGMENT STABILITY OF AÇAI

4.1 Introduction

Functional foods and beverages are finding global success, partially due to major consumer trends toward health maintenance (Milo 2005). Açai, catalogued as one of the most nutritious fruits from the Amazon (Pszczola 2005), is experiencing accelerated growth, not only due to its perceived novelty and exotic flavor but also to potential health benefits that have led to numerous successful new product launches in the United States. At present, numerous açai based ingredients used for coloring or flavoring beverages, fruit fillings, and frozen desserts are already available (Pszczola 2005) while its main use is as a key ingredient in various functional beverages and juice drinks.

Fruit juices are commonly fortified with ascorbic acid to prevent enzymatic browning reactions and to enhance nutritional properties (Freedman and Francis 1984). According to Ozkan and others (2004), poor sources of ascorbic acid, such as açai juice (Rogez 2000), are particularly good candidates for fortification. Several açai containing products are already being fortified with up to 225% (Jamba Juice açai smoothie) of the recommended daily intake for vitamin C (90 mg/day, National Academy of Sciences 2004) per 240mL serving.

Unfortunately, the presence of ascorbic acid in anthocyanin-rich systems has been shown to promote oxidation and negatively impact color, nutritional quality and functional properties in the final product (Shrikhande and Francis 1974). However,

results have been contradictory, and while ascorbic acid addition was shown to accelerate anthocyanin degradation in certain juices and model systems (Poei and Wrolstad 1981; Skrede and others 1992; Marti and others 2001; Brenes and others 2005), a protective effect of ascorbic acid on anthocyanin stability has been observed in others (Kaack and Austed 1998). Moreover, Kirca and others (2006) recently reported both positive and negative effects of ascorbic acid fortification on stability of black carrot anthocyanins in various fruit juices and nectars, indicating a major role of the matrices on anthocyanin stability. Factors such as extent of glycosylation, presence of acylated moieties and other compositional factors such as sugars, organic acids, proteins and polyphenolics likely influence the role of ascorbic acid on anthocyanin degradation.

While investigating the effects of ascorbic acid fortification on the anthocyanin stability of açai pulp, centrifuged, and filtered juice (Chapter 3), no significant differences were found in pulp or centrifuged juices. However, a pronounced detrimental effect was observed in filtered juice, probably indicating a protective effect of the natural açai matrix on anthocyanin stability. The results were tentatively attributed to initial differences in anthocyanin and polyphenolic composition; however, further studies are needed to confirm the possible protective role of naturally occurring polyphenolics as well as to identify potential detrimental components that may have led to accelerated anthocyanin degradation in the filtered juice. Therefore, the aim of this study was to investigate the effects of matrix composition and ascorbic acid addition on the phytochemical, antioxidant and color stability of açai juice and juice fractions under accelerated storage conditions.

4.2 Materials and Methods

4.2.1 Materials and Processing

Procedures for açai pulp clarification were followed as outlined in Chapter 3, and the clarified juice portion used for further preparation of fractions (Figure 4-1). Polyphenolics were isolated from the clarified açai juice stock (Fraction I) using C18 Sep-Pak Vac 20 cm^3 mini-columns (Waters Corporation, MA) and residual sugars and organic acids removed with water (unbound fraction). For preparation of fractions II and III, C18 bound polyphenolics were recovered by elution with acidified methanol (0.01% HCl). In a second procedure, initially bound phenolic acids and flavonoids were first removed from the column by elution with ethyl acetate, followed by elution with acidified methanol (0.01% HCl), which recovered the remaining anthocyanin bound fraction (Fractions IV and V).

Methanol was removed from the polyphenolic and anthocyanin fractions by vacuum evaporation at <40ºC. Each isolate was redissolved in an equal original volume of either the unbound fraction (Fractions III and V) or a citric acid buffer (Fractions II and IV), both adjusted to pH 3.5. Each treatment was subdivided into two fractions, one fortified with L-ascorbic acid (500 mg/L) and the other with an equal volume of citric acid buffer (pH 3.5) as a non-fortified control. Treatments were finally sealed in screw-cap glass vials and stored in the dark at 37°C for 12 days. Samples were collected every 48 hours and held frozen (-20°C) until analysis.

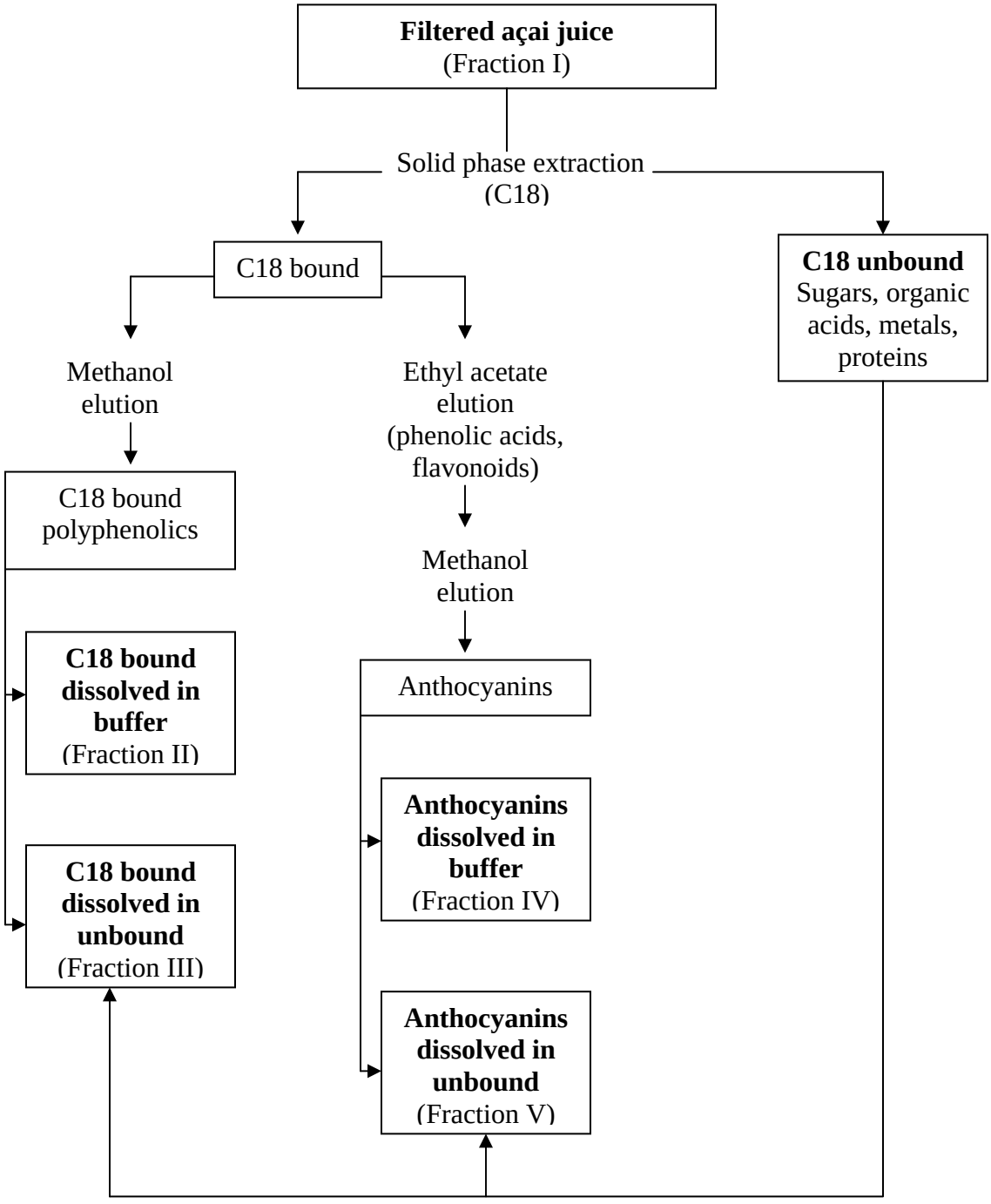

Figure 4-1. Açai juice fractionation process.

In addition, the effects of ascorbic acid addition on the anthocyanin stability of açai at various storage temperatures were assessed. Filtered açai juice and juice from which non-anthocyanin polyphenolics (NAP) were removed by liquid/liquid extraction with

ethyl acetate were fortified with ascorbic acid (0, 100 and 500 mg/L) and stored at 4, 20 and 37°C. Samples were taken every 8h from treatments stored at 37°C and every 48h from those stored at 4 or 20°C, for a total storage period of 4 to 12 days respectively.

4.2.2 Chemical Analysis

Chemical analysis (total anthocyanin content, polymeric anthocyanins, total soluble phenolics, antioxidant capacity and polyphenolics by HPLC) were conducted according to the procedures outlined in Chapter 3.

4.2.3 Statistical Analysis

The study was designed as a 5 x 2 x 7 full factorial that included five açai fractions, two ascorbic acid levels and seven storage times. For each treatment, the mean of three replicates is reported. Analysis of variance, multiple linear regression, Pearson correlations and means separation by Tukey's HSD test ($P < 0.05$) were conducted using JMP software (SAS Institute, Cary, NC).

4.3 Results and Discussion

4.3.1 Anthocyanin Color Retention

Anthocyanin color degradation followed first order kinetics (Figure 4-2), as previously reported by several studies (Cemeroglu and others 1994; Garzon and Wrolstad 2002; Kirca and others 2006). Initial anthocyanin recoveries varied, from 85.3% for anthocyanin fractions to 98.2% for C18 bound fractions.

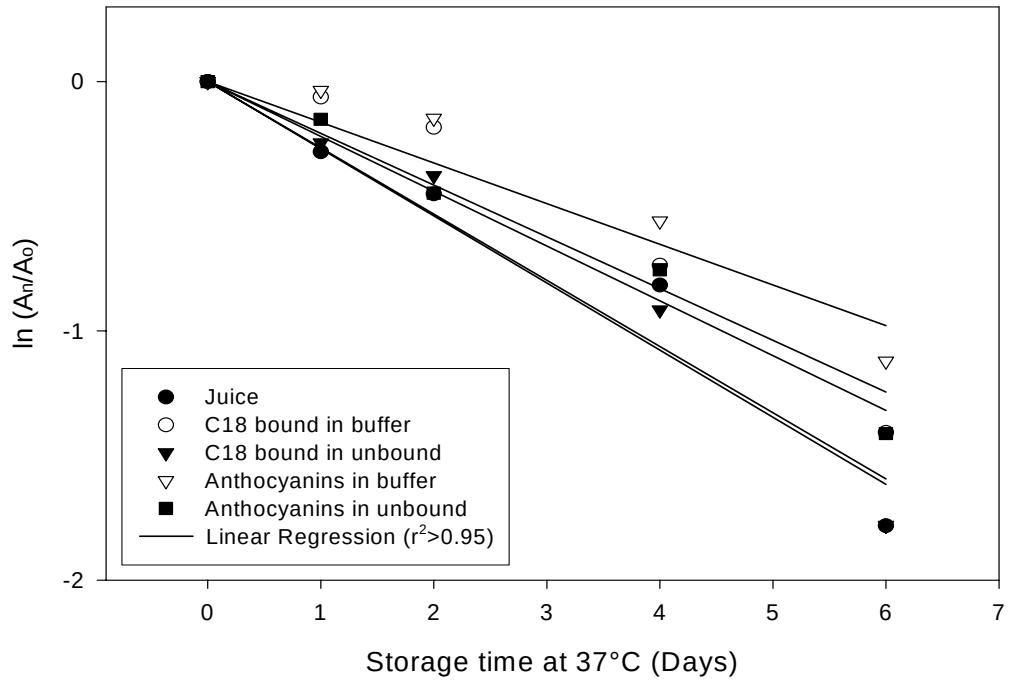

Figure 4-2. Linear regression of total anthocyanin content for non-fortified açai fractions as affected by storage at 37°C.

Non-fortified treatments experienced accelerated anthocyanin losses (67-78%) during the first 6 days of storage, after which only a small change in concentration was detected (Figure 4-3). At the end of the storage period, all treatments retained approximately 15% of their corresponding initial anthocyanin content. No differences were detected among degradation rates for juice and C18 or anthocyanin fractions redissolved in the C18 unbound fraction throughout the storage period. Fractions redissolved in buffer showed remarkably higher color stability during the first four days of storage, probably indicating a detrimental effect of unbound fraction components, such as proteins, sugars and its degradation products on anthocyanin stability. Similar results were observed by Kirca and others (2006), who studied the stability of black carrot anthocyanins in various juices and nectars and observed higher color stability in buffer

solutions when compared to the original juice systems. Anthocyanin half lives ranged from 13h in juice and nectar matrices to 25h in citrate-phosphate buffer solutions heated to the same temperature (90°C). Decreased stability in juices and nectars was attributed to the detrimental effects of sugars and ascorbic acid, commonly present in these systems, and suggested elsewhere by Dyrby and others (2001).

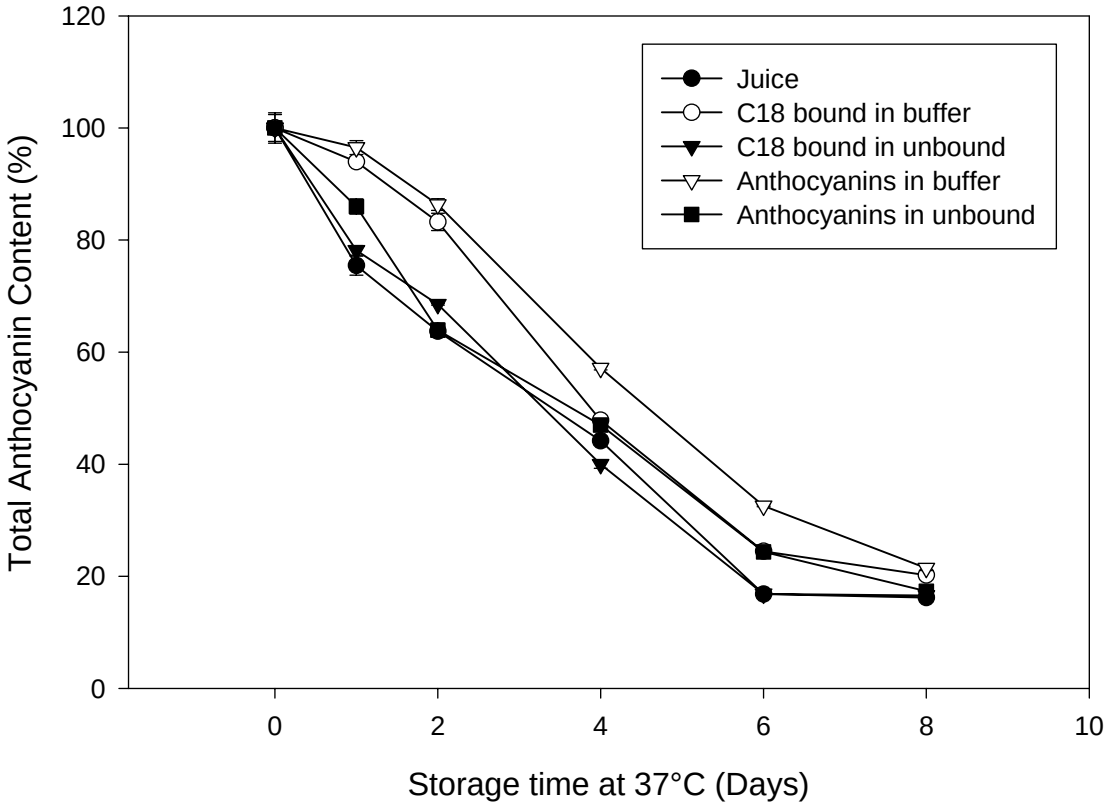

Figure 4-3. Total anthocyanin content of non-fortified açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

Moreover, Maccarone and others (1985) had previously found that only 25% of blood orange anthocyanins were lost after three months of storage (16-18ºC) when dissolved in a buffer solution (pH 3.2), while losses reached 65% in blood orange juice under the same conditions. Similarly, Rodriguez-Saona and others (1999) observed faster

anthocyanin degradation in juice model systems colored with vegetable juice concentrates than those with chemically extracted pigments, an effect that was attributed to the complex composition of the concentrates. Results from this study further support the influence of matrices on anthocyanin stability while also indicating detrimental effects of unbound matrix components, probably due to sugar degradation byproducts. Therefore, anthocyanin stability may be enhanced by processing and storage conditions that minimize the degradation of proteins, sugars and other juice components.

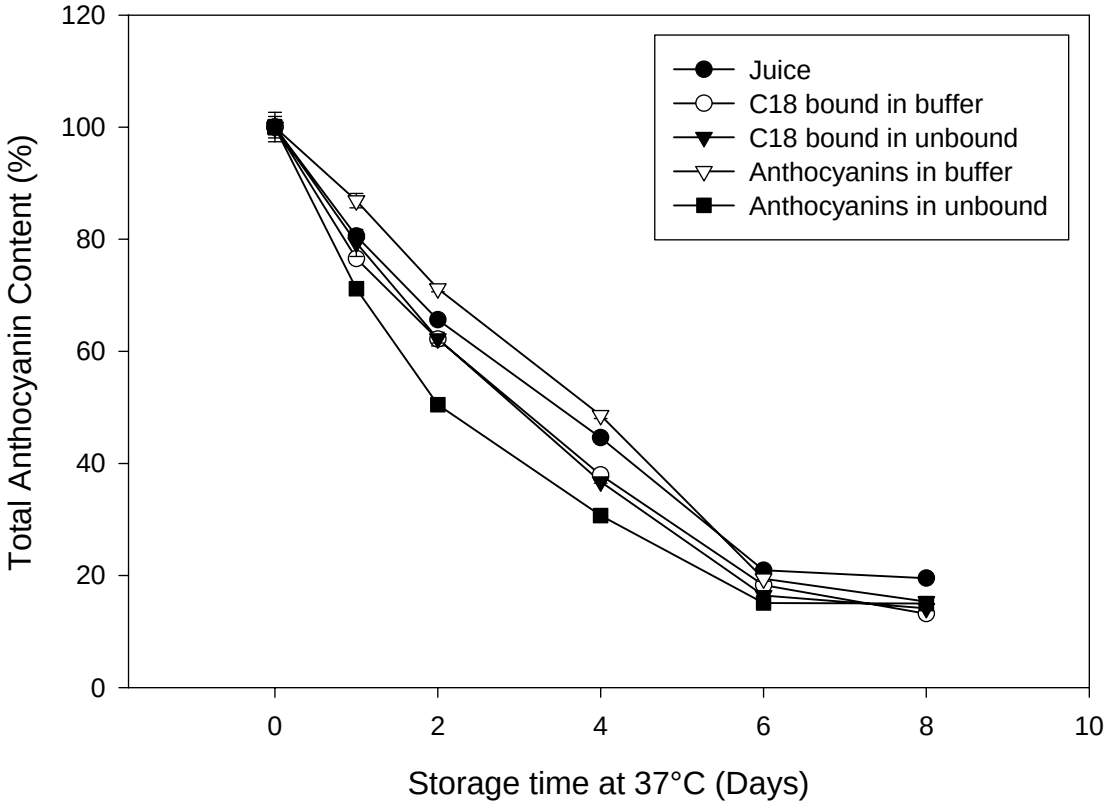

Figure 4-4. Total anthocyanin content of ascorbic acid fortified (500mg/L) açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

In presence of ascorbic acid, similar anthocyanin degradation patterns were observed (Figure 4-4). All treatments experienced accelerated losses during the first 6

days of storage while only minor changes occurred thereafter. The relatively higher stability of the anthocyanin fraction redissolved in buffer at initial stages of storage was maintained even in presence of ascorbic acid. A possible explanation may be in the formation of compounds with comparably high antioxidant activities during anthocyanin degradation, which has been previously shown to have a stabilizing effect on intact anthocyanins (Lichtenthaler 2004) and might be responsible for the relatively high stability of isolated anthocyanins in buffer solutions.

Isolated anthocyanin fractions redissolved in the unbound experienced the highest degradation rates among fortified treatments, indicating an overall negative effect of C18 unbound fraction components on anthocyanin stability. Among them, the combined presence of ascorbic acid, sugar degradation byproducts such as furfurals, and trace metals such as iron found at concentrations of 7.3mg/L in the unbound fraction could be particularly detrimental. While the presence of metals is known enhance the formation of reactive oxygen species (Pietta 2000), further oxidation might have also been promoted by furfurals (Es-Safi and others 2000) and other sugar or ascorbic acid degradation products, which have been correlated ($r>0.93$) to anthocyanin degradation (Iversen 1999; Ozkan 2002; Choi and others 2003; Brenes and others 2005).

Table 4-1. Kinetic parameters for color degradation in ascorbic acid (AA) fortified and non-fortified açai fractions stored at 37°C.

	No AA		AA	
	k[1]	t½ [2]	K	t½
Juice	0.279	2.48a[3]	0.252	2.75a
C18 bound/buffer	0.230	3.01a	0.270	2.56a
C18 bound/unbound	0.272	2.54a	0.297	2.34a
Anthocyanins/buffer	0.192	3.61a	0.295	2.35b
Anthocyanins/unbound	0.231	3.01a	0.307	2.26b

[1]Reaction rate constant (k $days^{-1}$). [2]Half life (days) of initial absorbance value for each treatment. [3]Values with similar letters within rows are not significantly different (LSD test, $p<0.05$).

Kinetic parameters of anthocyanin degradation (Table 4-1), including first order reaction rate constants (k) and half lives (t½) were calculated as previously outlined (Chapter 3). In the presence of ascorbic acid, color retention for juice seemed to be enhanced by fortification while overall color stability of both juice and C18 bound fractions was not significantly affected by ascorbic acid addition ($p<0.05$). However, color degradation rates of isolated anthocyanin fractions and C18 bound phenolics redissolved in buffer were markedly accelerated by the presence of ascorbic acid, especially on isolated anthocyanin fractions, where degradation rates increased by 25 to 35% when redissolved in the unbound or in buffer respectively. Differences between C18 bound and anthocyanin fractions were attributed to the presence of non-anthocyanin polyphenolics, which have been previously shown to protect both anthocyanin and ascorbic acid from degradation in several juice and model systems. Some examples are the studies by Ozkan and others (2004), who indicated a protective mechanism of blood orange flavonols on ascorbic acid retention, Rein and Heinonen (2004) also reported an stabilizing effect of phenolic acids on anthocyanins in raspberry, strawberry, lingonberry and cranberry juices, and Brenes and others (2005) found a protective effect of polyphenolic copigments on both ascorbic acid and anthocyanin retention in a grape juice model system. Protective effects of polyphenolic compounds have been repeatedly attributed to their role as antioxidants and metal chelators (Pietta 2000). According to Ozkan and others (2004), the formation of hydrogen peroxide during aerobic oxidation of ascorbic acid is responsible for ascorbic acid degradation and the formation of hydroperoxyl radicals that in turn feed the oxidation reactions. Polyphenolics may react with hydrogen peroxide (Ozkan 2002), thereby preventing ascorbic acid degradation.

Moreover, polyphenolics may act as metal-chelating agents, inhibiting the metal-induced Fenton reaction, which is an important source of reactive oxygen species (Cook and Samman 1996).

Interestingly, ascorbic acid addition was previously found to be detrimental for anthocyanin stability in açai juice stored at 4 and 20°C (Chapter 3). As a result, the potential effects of temperature and associated changes in anthocyanin degradation rates were further assessed. Regression analysis was used to describe anthocyanin degradation (Table 4-2), which followed first order kinetics ($p<0.05$). Degradation rates were significantly ($p<0.01$) influenced by storage temperature and while treatments experienced 3.5 times higher anthocyanin losses at 20°C than when stored at 4°C, treatments degraded almost 10 times faster when storage temperatures were raised from 20 to 37°C.

Table 4-2. Kinetic parameters for color degradation in açai juice as affected by the presence of non-anthocyanin polyphenolics (NAP), ascorbic acid addition (0, 100 and 500 mg/L) and storage (4, 20 and 37°C).

AA (mg/L)		4°C		20°C		37°C	
		k[1]	t½ [2]	k	t½	k	t½
0	Juice	3.83	181a[3]	0.073	37.8a	0.320	2.17b
	Juice without NAP	8.79	81.3b	0.121	22.9c	0.312	2.22b
100	Juice	3.93	176a	0.081	34.4b	0.199	3.48a
	Juice without NAP	13.1	52.8c	0.111	24.9c	0.322	2.15b
500	Juice	8.79	78.8b	0.129	21.5c	0.350	1.98bc
	Juice without NAP	14.5	47.7c	0.189	14.7d	0.425	1.63c

[1]Reaction rate constant (k x 10^{-3} $days^{-1}$ at 4ºC and k $days^{-1}$ at 20 and 37ºC). [2]Half life (days) of initial absorbance value for each treatment. [3]Values with similar letters within columns are not significantly different (LSD test, $p<0.05$).

Effects of ascorbic acid addition on anthocyanin stability of açai juices also varied with temperature and while its presence (500 mg/L) was detrimental at both 4 and 20°C slight differences were found between fortified and non-fortified treatments stored at

37°C and even positive effects were observed when a lower ascorbic acid concentration (100mg/L) was added. One possibility is that accelerated degradation rates in non-fortified treatments during storage at 37°C might have hindered detrimental effects of ascorbic acid addition on anthocyanin stability. Moreover, elevated temperatures alone have been reported to shift the anthocyanin equilibrium towards the chalcone form (Clifford 2000), which could be responsible for the accelerated degradation of non-fortified treatments when kept at 37°C. According to the author, while the nature of the transformation products is not known, there is clear evidence for the involvement of sugars and ascorbic acid, along with their degradation products, metal ions and hydrogen peroxide derived from ascorbic acid oxidation. However, effects of ascorbic acid addition were not always detrimental. Apart from the initial reports of Kaack and Austed (1998) on the protective effect of ascorbic acid on cranberry anthocyanins, further studies in other anthocyanin rich systems have found similar effects. Ozkan (2002) indicated a protective effect of ascorbic acid (60mg/L) on the anthocyanin stability of sour cherry and pomegranate juices and attributed this effect to the insufficient formation of ascorbic acid degradation products and the removal of hydrogen peroxide by reduced ascorbic acid; thereby protecting the anthocyanins from degradation. However, anthocyanin losses were accelerated when 80mg/L of ascorbic acid were added and attributed to an excess of ascorbic acid degradation products in relation to its antioxidant capacity. Furthermore, Kirca and Cemeroglu (2003) and Choi and others (2003) reported no significant effects of ascorbic acid fortification on the degradation of anthocyanins in blood orange juice while addition of ascorbic acid (300mg/L) to colored apple and grape juices and peach,

apricot and pineapple nectars did not affect the anthocyanin degradation rate (Kirca and others 2006).

Results from this study further suggest that ascorbic acid effects on anthocyanin color stability are not only concentration and temperature dependent but also matrix specific.

4.3.2 Polymeric Anthocyanins Concentration

Anthocyanins undergo a series of detrimental reactions during storage (Wrolstad and others 2005), among them, the transformation of monomeric forms into more stable oligomeric or polymeric pigments that give rise to important color changes towards brownish-red hues (Monagas and others 2006). Formation of polymeric anthocyanin compounds in açai juice and juice fractions was assessed spectrophotometrically and found to increase linearly during storage (Figure 4-5). Initially, no significant differences in polymeric anthocyanin concentrations were detected among treatments, suggesting an almost complete recovery of polymeric compounds during the isolation process. Significantly more polymerization occurred in the natural juice system when compared to either C18 bound or anthocyanin fractions, among which, no differences were detected. Higher degrees of anthocyanin polymerization and subsequent losses of monomeric forms were consistent with previous observations, where higher anthocyanin color degradation also occurred in the juice system when compared to isolated fractions. Dyrby and others (2001) observed 100% higher retention of monomeric anthocyanin forms in a model system than in a non-carbonated soft drink medium, both of which contained anthocyanins extracted from red cabbage, blackcurrant, elderberry or grape skin and were subjected to identical storage conditions. Accelerated anthocyanin degradation was attributed to detrimental effects of sugar and ascorbic acid in the soft drink systems.

Similar findings have been reported in studies by Maccarone and others (1985), Rodriguez-Saona and others (1999) and Kirca and others (2006), and previously discussed. However, more complex interactions seem to be responsible for higher polymerization rates in açai juice when compared to its fractions, since a higher retention of monomeric anthocyanin forms was also observed in the C18 bound fraction redissolved in the unbound, which presumably contains all original juice components.

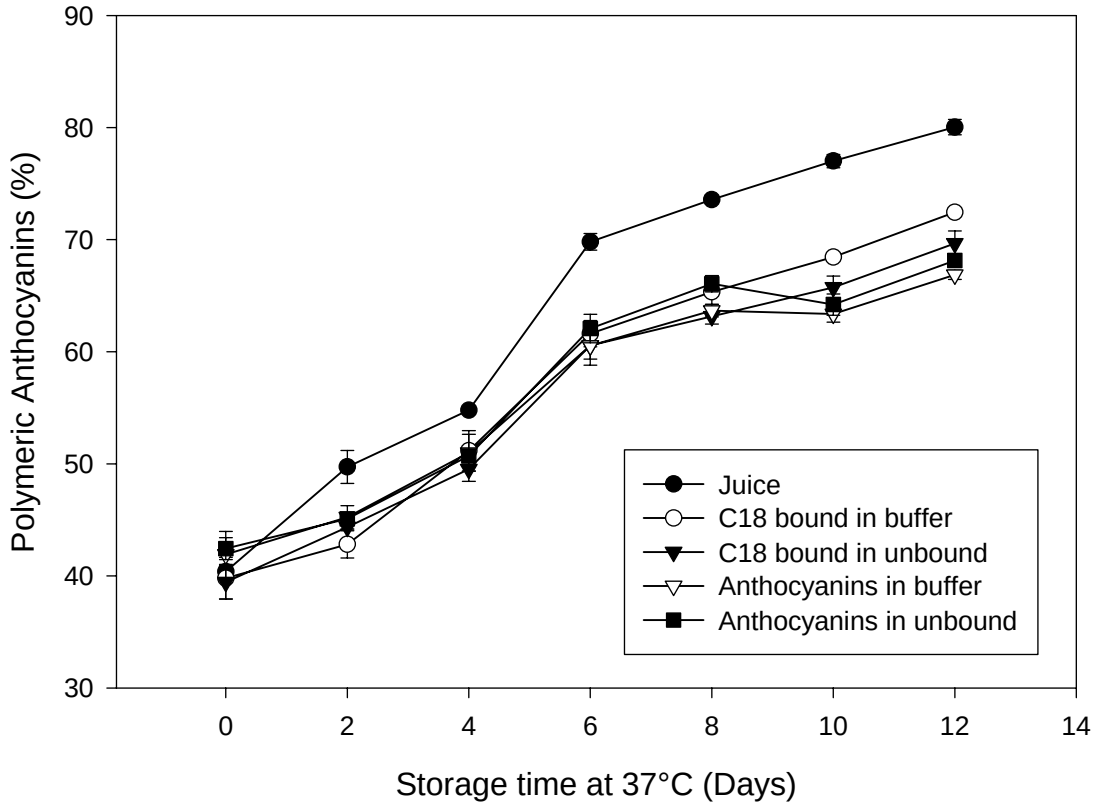

Figure 4-5. Polymeric anthocyanins (%) in non-fortified açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

The presence of ascorbic acid (Figure 4-6) markedly accelerated anthocyanin polymerization ($p<0.05$) in all model systems during the first four days of storage while it delayed polymerization in the natural juice system throughout the storage period. No

significant differences were found among fortified anthocyanin and C18 bound isolates, whose final polymeric anthocyanin contents were 10% higher when compared to its non-fortified counterparts.

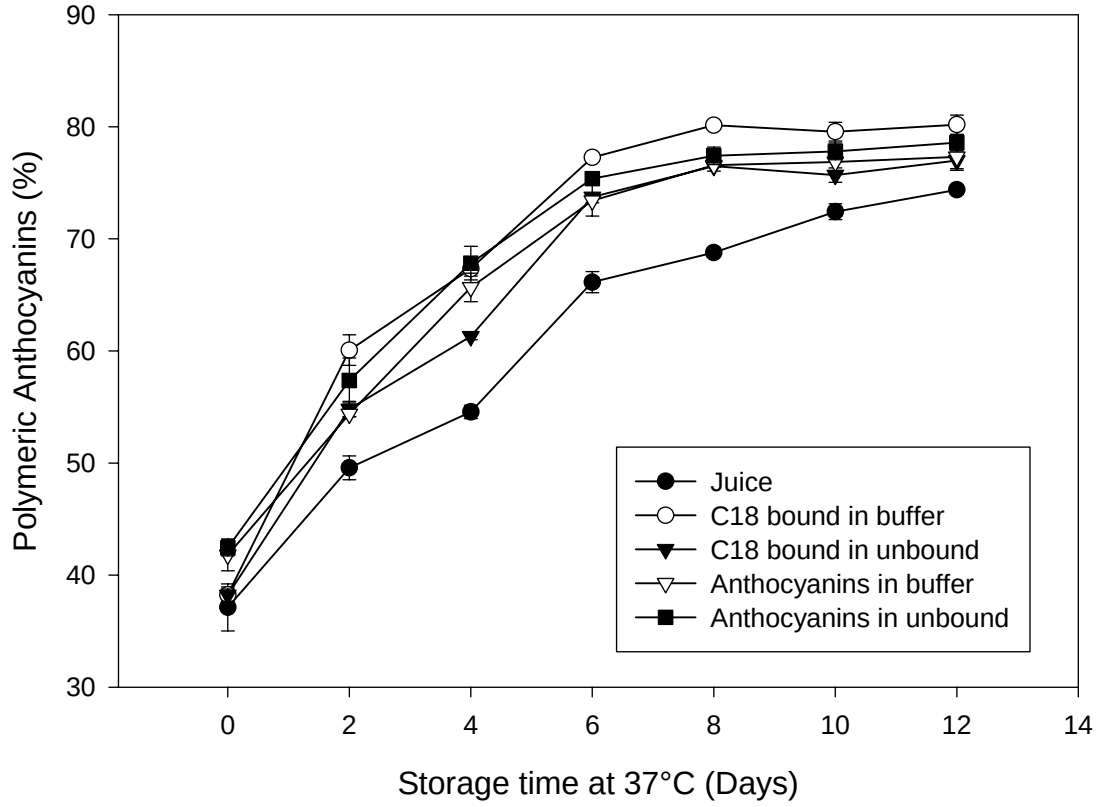

Figure 4-6. Polymeric anthocyanins (%) in ascorbic acid fortified (500mg/L) açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

Higher polymerization rates in anthocyanin rich systems following ascorbic acid fortification were also reported by Skrede and others (1992) in strawberry syrups fortified with ascorbic acid where more than 90% of the color originated from polymerized pigments at the end of storage. Moreover, the interaction between ascorbic acid degradation products (such as furfural and other aldehydic compounds) with phenolic compounds plays a major role in the formation of brown pigments during storage of fruit-

derived foods (Es-Safi and others 2000). While acetaldehyde was found to contribute to anthocyanin polymerization with flavanols, furfural and hydroxymethylfurfural, these driven reactions have resulted in colorless and yellowish compounds, which also contribute to browning reactions that affect color stability (Es-Safi and others 2002).

Polymerization rates of açai juice were not significantly affected by fortification, which may partially explain the previously observed positive effects of ascorbic acid addition on anthocyanin color stability in the juice system. Since reactions between anthocyanins and sugar or ascorbic acid degradation intermediates are thought to be the main factors responsible for the formation of polymeric compounds (Krifi and others 2000), protective effects of juice matrix components against ascorbic acid degradation might have decreased anthocyanin polymerization rates. However, these protective effects might not be explained by the sole presence of non-anthocyanin polyphenolics, since anthocyanins in the C18 bound fractions redissolved in the unbound also experienced higher polymerization rates than the original juice system in presence of ascorbic acid, even when similar components were theoretically present in both systems. Thus, more complex interactions among matrix components seem to be involved.

4.3.3 Total Soluble Phenolics

Soluble phenolics were quantified based on total reducing capacity of açai juice and juice fractions, according to the procedures of Singleton and Rossi (1965). Phenolic contents markedly decreased (33-42%) during the first 8 days of storage while only minor losses were subsequently observed (Figure 4-7). Initial phenolic contents varied among juice and juice fractions, depending not only on recovery rates (85-99%) during the isolation process but also on the presence of the unbound fraction components, which seemed to be responsible for 6-9% of the total reducing capacity of the systems.

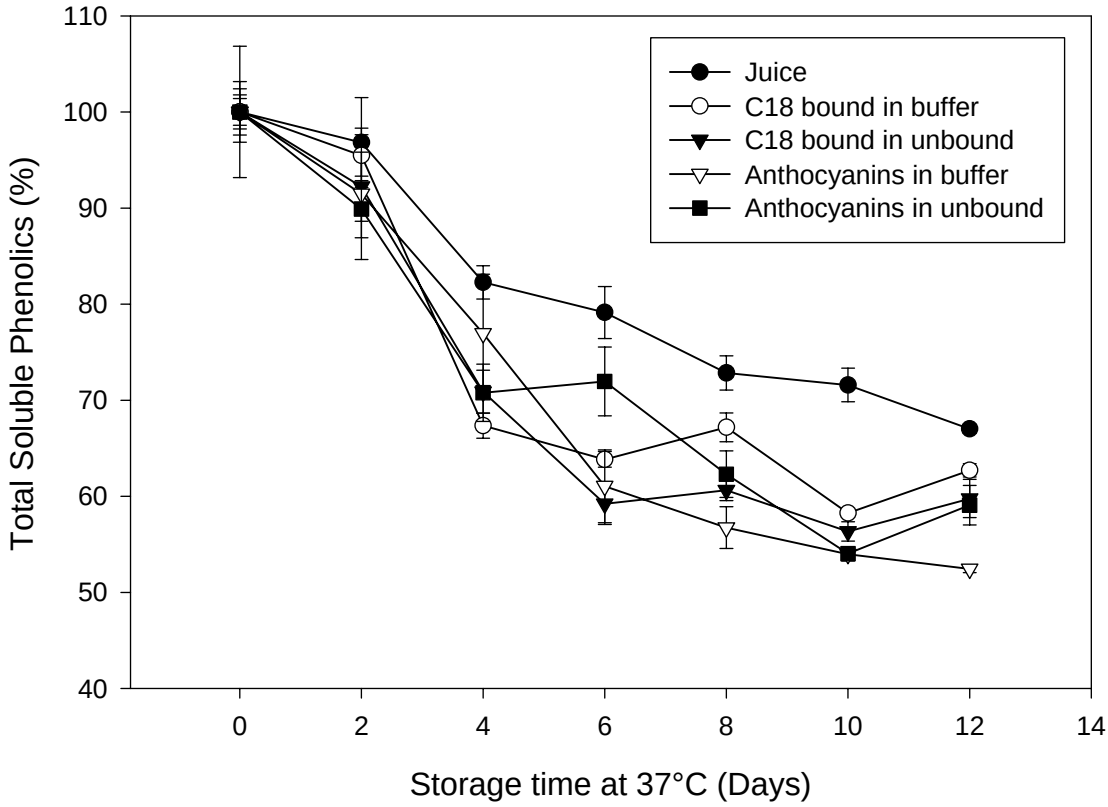

Figure 4-7. Total soluble phenolics (%) in non-fortified açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

Moreover, juice fractions also exhibited significantly higher degradation rates during storage, when compared to the original juice matrix. Losses in soluble phenolics were correlated to anthocyanin color degradation (r=0.88) and to changes on individual anthocyanin contents (r=0.81) during the first 8 days of storage. However, losses in total soluble phenolics could not be entirely attributed to anthocyanin degradation since different patterns were observed. While anthocyanins in the juice system decreased by more than 80% during the first six days of storage, only 20% of the total soluble phenolic content was lost during the same period. Similarly, all treatments lost more than 80% of their initial anthocyanin content by the end of storage while maximum losses in total

phenolics during the same period averaged 40%. Moreover, a previously indicated poor response of anthocyanins to the Folin-Ciocalteu assay (Singleton 1974) suggests that changes in soluble phenolics are mainly due to degradation of non-anthocyanin polyphenolics. Similar results were also obtained by Lichtenthaler (2004) when assessing anthocyanin and soluble phenolic contents measured by the Folin-Cioucalteu assay in several açai pulps from various sources. Even when anthocyanin contents were highly variable (13-463mg/L, a 36-fold variation), only minor differences in total phenolic contents (1900-4600mg/L, a 2.4-fold variation) were found among samples. In addition, anthocyanins were estimated to contribute to less than 10% of the total phenolic content, even when a moderate correlation ($r=0.73$) between phenolic and anthocyanin contents was also found.

As a result, changes in soluble phenolic contents during storage of açai juice and its fractions may only be partially attributed to anthocyanin degradation where the main changes were from the non-anthocyanin polyphenolics (NAP). However, the mathematical correlation between anthocyanin and soluble phenolic contents may denote similarities in their degradation patterns. Furthermore, the relatively high stability of these unidentified phenolic components in the juice system may indicate complex interactions involving other matrix components, of which anthocyanins are potentially included. Significant differences were additionally found among degradation patterns of fortified açai juice and juice fractions (Figure 4-8). While soluble phenolics in açai juice remained unaffected by ascorbic acid addition ($p<0.05$), fortified juice fractions experienced remarkably higher losses during the first 4 days of storage when compared to their non-fortified forms. However, no differences were found in the total soluble

phenolic contents of fortified and non-fortified juice or juice fractions at the end of storage.

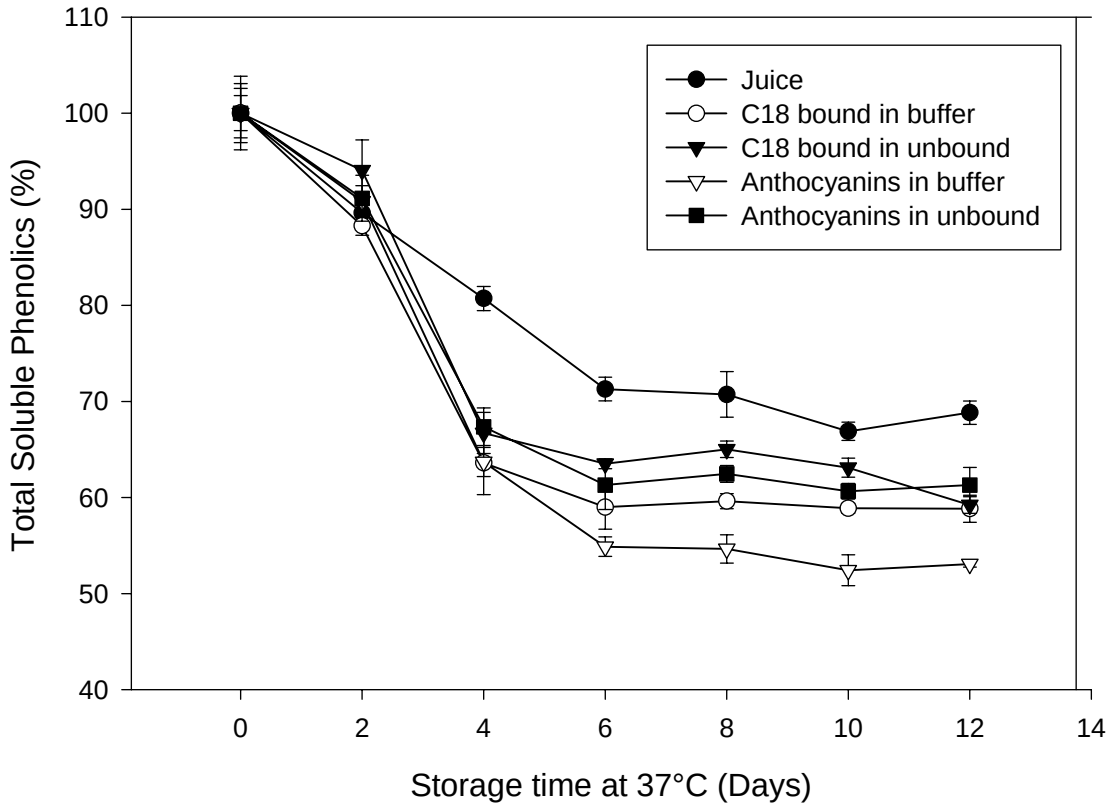

Figure 4-8. Total soluble phenolics (%) in ascorbic acid fortified (500mg/L) açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

In juice fractions, accelerated degradation of soluble phenolics in the presence of ascorbic acid may be attributed to ascorbic acid degradation products. In fact, Brenes and others (2005) indicated that ascorbic acid degradation products, particularly those formed after the oxidation of L-ascorbic acid to dehydroascorbic are probably responsible for anthocyanin degradation while the presence of non-anthocyanin polyphenolic copigments delayed ascorbic acid oxidation and the subsequent formation of its degradation products. Consequently, losses in total soluble phenolics might have favored the protection of

ascorbic acid by converting ascorbic acid radicals back to its original form, acting in these redox reactions (Cos and others 2003). Moreover, additional oxidation of ascorbic acid into dehydroascorbate and its free radical intermediate, semidehydroascorbate, might have catalyzed the formation of reactive oxygen species (Deutsch 1998), which promoted further oxidation of polyphenolic compounds. These hypotheses are consistent with the lower stability observed for the anthocyanin fraction redissolved in buffer, where reduced concentrations of non-anthocyanin polyphenolics were more prone to oxidation reactions induced by ascorbic acid degradation products, especially during the last stages of storage. However, the relatively high stability of polyphenolics present in the original juice system is not only explained by differences in non-anthocyanin polyphenolics, since isolates of presumably identical composition experienced significantly higher degradation rates. Again, unknown synergistic effects among matrix components seemed to enhance not only anthocyanin but overall polyphenolic stability when ascorbic acid was present in the juice system.

4.3.4 Polyphenolics by HPLC

Total anthocyanin and non-anthocyanin polyphenolics present in açai juice and juice fractions were analyzed by HPLC and quantified by the addition of major peak areas at 520 and 360nm respectively, as previously outlined (Chapter 3).

4.3.4.1 Anthocyanins by HPLC

Total anthocyanin contents in açai juice and fractions decreased following first order kinetics ($p<0.05$, Figure 4-9), in agreement with the reports of Rodriguez-Saona and others (1999), Garzon and Wrolstad (2002) and Ozkan and others (2004).

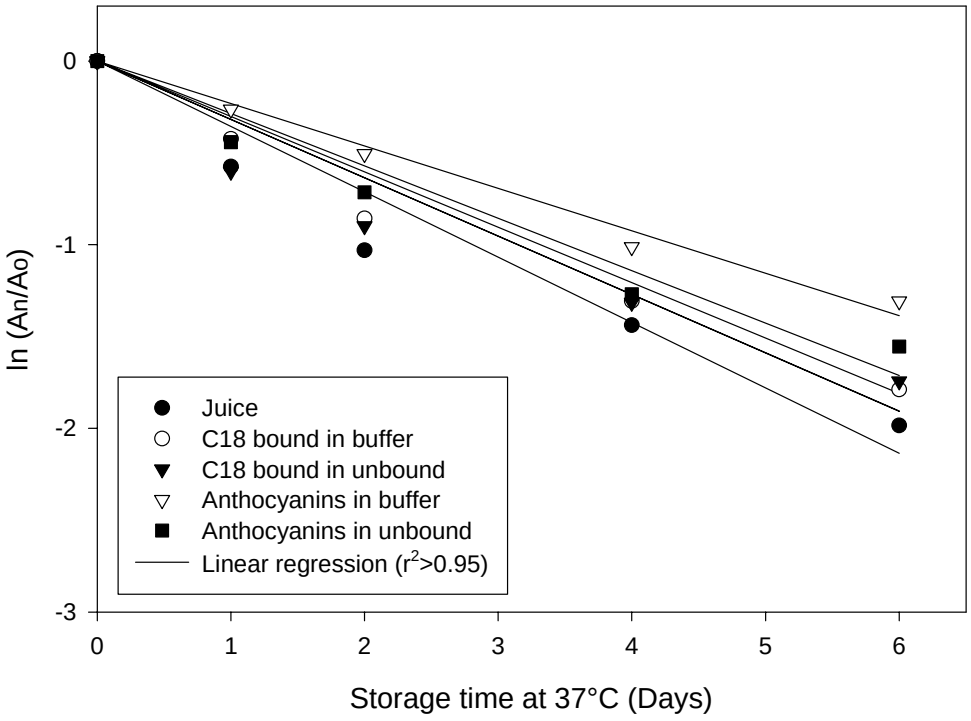

Figure 4-9. Linear regression analysis of total anthocyanin content (HPLC) for non-fortified açai fractions as affected by storage at 37°C.

Initial differences in anthocyanin content were determined by recovery rates, which ranged from 94 to 99% in both C18 bound and isolated anthocyanin fractions. Degradation patterns of HPLC determined anthocyanins were highly correlated (r=0.94) to those observed when anthocyanins were spectrophotometrically assessed. Accelerated anthocyanin losses occurred during the first six days of storage but only minor changes were subsequently recorded (Figure 4-10). No significant differences were found among juice, C18 bound fractions and treatments redissolved in the unbound while isolated anthocyanins redissolved in buffer showed significantly higher stability during the first 6 days of storage, following which, all treatments degraded to a similar extent. As previously discussed, similar observations have been made by several authors

(Maccarone and others 1985; Rodriguez-Saona and others 1999; Kirca and others 2006) in other anthocyanin rich systems.

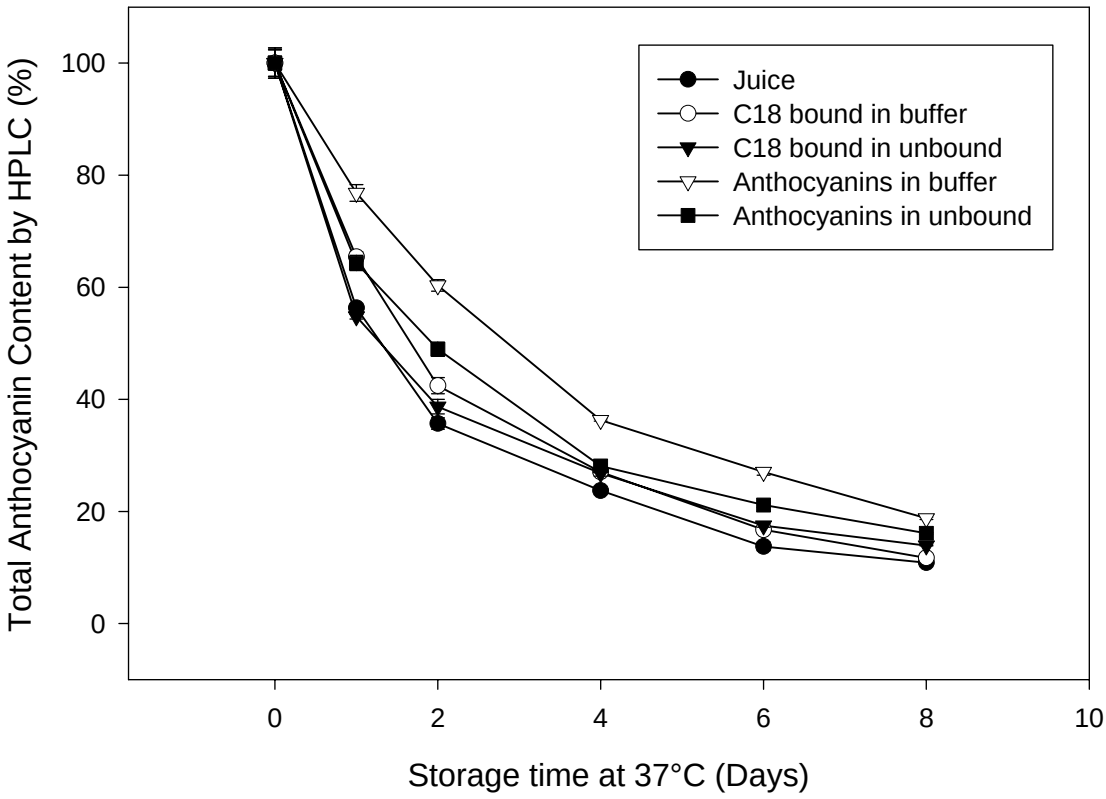

Figure 4-10. Total anthocyanin content (HPLC) of non-fortified açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

Moreover, similar results were obtained by Lichtenthaler (2004) when assessing anthocyanin stability of açai pulps during storage at 37°C and where the very much lower stability of anthocyanins in the pulps (14 to 21 days for complete degradation) compared to pure standard compounds (141 days for elimination of both cyanidin-3-glucoside and cyanidin-3-rutinoside) was attributed to the presence of degrading enzymes or prooxidants, as trace metals iron and copper, found in concentrations up to 26 and 2mg/100g dry matter respectively. Similar deductions can be made in the present study,

where the presence of metals (7.3mg/L iron) and sugar degradation products formed as a result of juice pasteurization and storage significantly accelerated anthocyanin degradation.

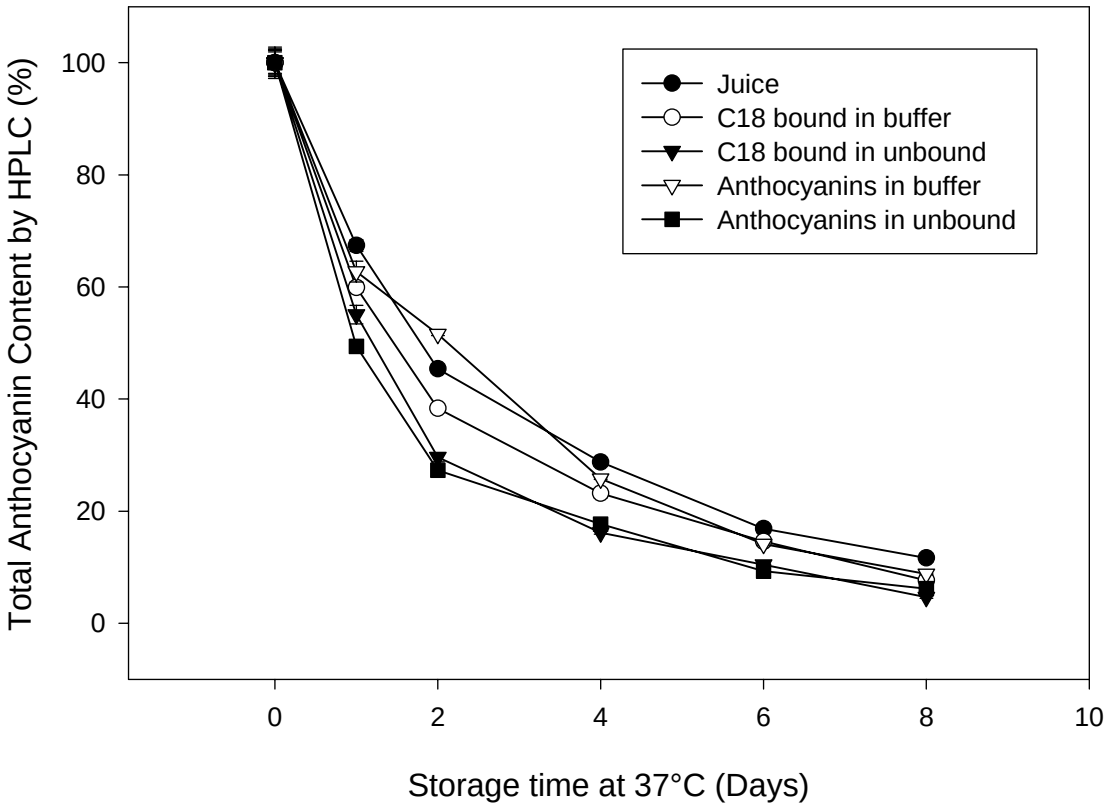

Figure 4-11. Total anthocyanin content (HPLC) of ascorbic acid fortified (500mg/L) açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

In presence of ascorbic acid, similar anthocyanin degradation patterns were observed, however, effects of ascorbic acid addition differed markedly among treatments. In general, açai juice and its fractions redissolved in buffer showed notably higher stability through the first stages of storage than their counterparts redissolved in the unbound. As previously discussed, these effects might be attributed to the presence of sugar degradation products and trace metals in the unbound fraction, which probably

accelerated anthocyanin degradation. However, no detrimental effects were observed in the juice system, indicating a protective effect of the original matrix on anthocyanin stability.

Kinetic parameters of anthocyanin degradation (Table 4-3) revealed no significant effects of ascorbic acid addition on either juice or the C18 bound fraction redissolved in buffer while detrimental effects were observed when the C18 bound fraction was redissolved in the unbound. Clearly, components present in the unbound fraction have a prooxidant effect in presence of ascorbic acid while complex interactions among matrix components seem to have a protective effect, which not only depends on the presence of non-anthocyanin polyphenolics. However, loss of other polyphenolic components during the isolation process might have also been detrimental for the system. Previous reports by Rommel and Wrolstad (1993), showed very low recovery rates (~10%) for benzoic standards, including gallic and protocatechuic acids isolated from C18 Sep Pak cartridges while recovery of flavanol aglycones, flavan-3-ols and ellagic acid approached 100% under the same conditions. Therefore, minor phenolic components present in juice might have been lost during the isolation process, further affecting the ability of the matrix to inhibit anthocyanin degradation reactions.

Table 4-3. Kinetic parameters for total anthocyanin (HPLC) degradation in ascorbic acid (AA) fortified and non-fortified açai fractions stored at 37°C.

	No AA		AA	
	k[1]	t½ [2]	k	t½
Juice	0.311	2.23b[3]	0.288	2.40a
C18 bound/buffer	0.290	2.39b	0.310	2.24ab
C18 bound/unbound	0.269	2.58b	0.363	1.91b
Anthocyanins/buffer	0.222	3.13a	0.320	2.17ab
Anthocyanins/unbound	0.255	2.71ab	0.364	1.90b

[1]Reaction rate constant (k days^{-1}). [2]Half life (days) of initial anthocyanin content for each treatment. [3]Values with similar letters within columns are not significantly different (LSD test, $p<0.05$).

Detrimental effects of ascorbic acid in isolated anthocyanin systems might be attributed not only to the formation of unstable degradation products (Ozkan 2002) but also to the absence of other protective polyphenolics. As observed by Iversen (1999), kinetics of degradation for both anthocyanins and ascorbic acid are strongly dependent on the nature of the total system, such as the presence of other antioxidants. In this study, effects of ascorbic acid addition seemed to be determined not only by the presence of non-anthocyanin polyphenolics but also by complex interactions among matrix components that have an overall protective effect on açai anthocyanins.

4.3.4.2 Non-anthocyanin polyphenolics by HPLC

Major non-anthocyanin polyphenolic compounds present in açai were analyzed by HPLC and their relative changes during storage monitored. Initial phenolic contents varied with recovery, which ranged from 88 to 99% in isolated anthocyanins and C18 bound fractions respectively. As observed, major polyphenolic compounds and anthocyanins in açai shared a similar affinity to the C18 cartridge. Moreover, the same degradation pattern was experienced by both C18 bound and anthocyanin fractions.

Similarly, Lichtenthaler (2004), following HPLC analysis of polyphenolics in açai pulps, detected the presence of non-anthocyanin polyphenolic compounds eluting during the same time span as anthocyanins (25-35 min during a 45min run), and to which, more than 40% of the total antioxidant capacity of the pulps was attributed. Following HPLC-MS analysis of the fractions, a large number of small signals was detected, indicating that several, yet unidentified compounds are included in this fraction.

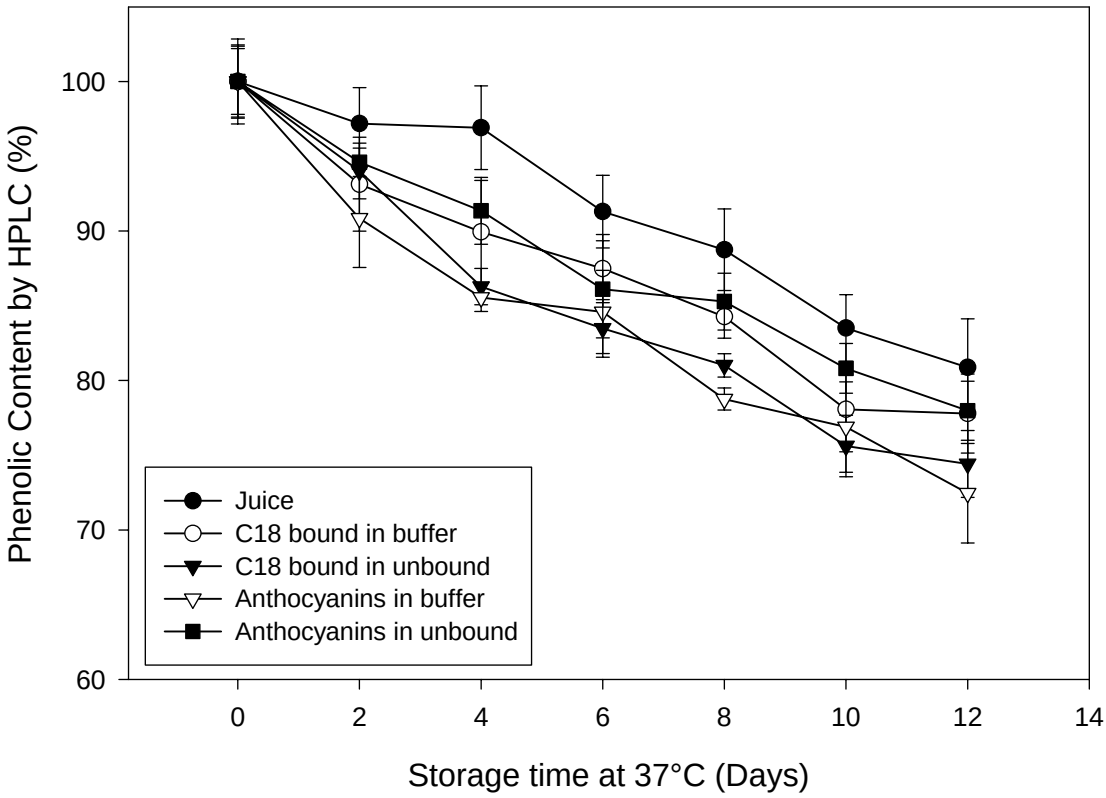

Figure 4-12. Phenolic content (HPLC) of non-fortified açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

Degradation of non-anthocyanin polyphenolics in non-fortified açai juice and fractions followed a linear pattern with maximum losses between 18 and 30% at the end of the storage period (Figure 4-12). Significant differences were found between juice and fractionated treatments, which generally degraded at faster rates. However, the higher polyphenolic stability of açai juice can not be explained solely by its composition since fractions on which the same components were presumably present degraded at a significantly higher rate, indicating additional protective interactions among matrix components. These observations were consistent with those previously made for total

soluble phenolics (Figure 4-7). In fact, a moderate correlation (r=0.71) was found between HPLC determined polyphenolics and soluble phenolic contents.

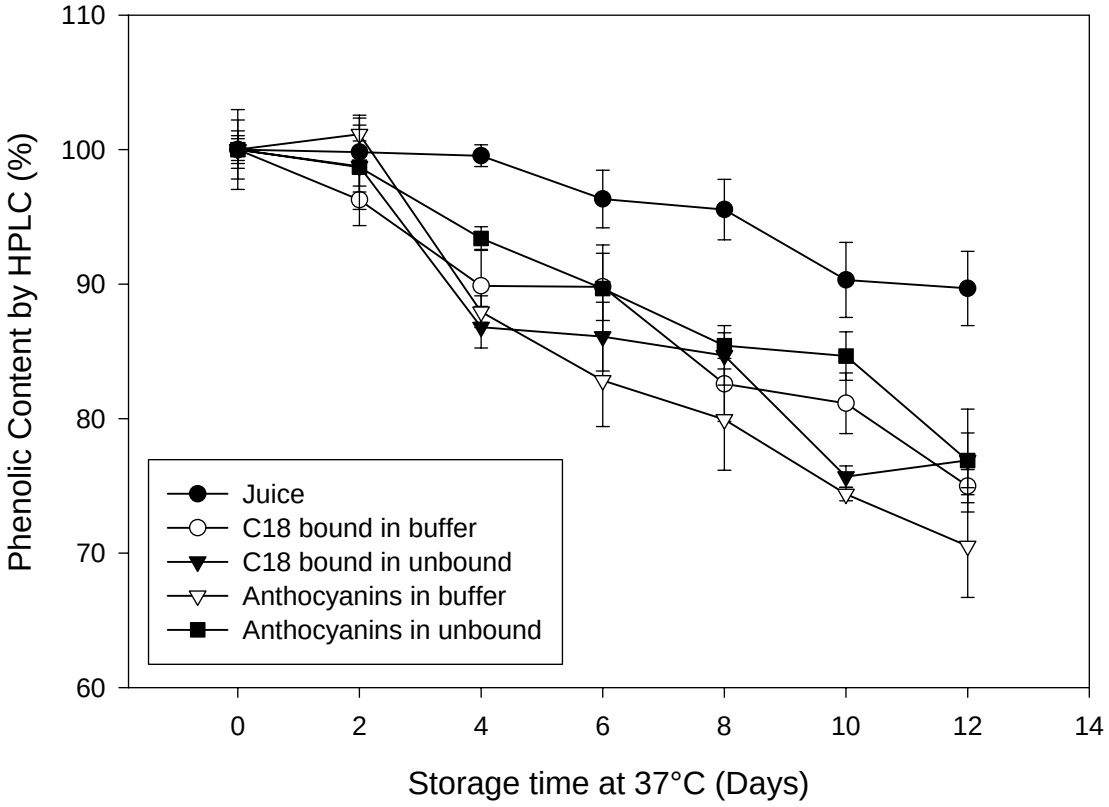

Figure 4-13. Phenolic content (HPLC) of ascorbic acid fortified (500mg/L) açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

Similar degradation patterns were observed in fortified and non-fortified juice fractions during storage while polyphenolics present in açai juice showed significantly higher stability in the presence of ascorbic acid. While results may suggest the presence of highly stable polyphenolic components, additional protective properties might also be derived from the presence of other phenolic constituents and interactions among matrix components. Moreover, the improved stability of fortified açai juice might indicate the preferential oxidation of other phenolic components when in presence of ascorbic acid.

However, more complex matrix interactions seem to exert a protective effect on the major polyphenolic components of açai juice in the presence of ascorbic acid since treatments with similar composition, as the C18 bound fraction redissolved in the unbound did not show improved stability following fortification. While no previous reports exist on the phytochemical stability of açai, further studies are needed to attain a better understanding of the chemistry behind the high polyphenolic stability of its juice.

4.3.5 Antioxidant Capacity

Parallel to anthocyanin and polyphenolic degradation, antioxidant activities of açai juice and juice fractions decreased significantly over time, retaining only 20 to 40% of their original antioxidant capacity by the end of the storage period. No differences were initially detected between juice and juice fractions or between fortified and non-fortified forms, suggesting that major contributors to the antioxidant activity of the juice were efficiently recovered in all fractions. Moreover, antioxidant capacity losses were highly correlated to anthocyanin content by HPLC ($r=0.88$), total anthocyanins ($r=0.85$), total soluble phenolics ($r=0.83$) and polyphenolics by HPLC ($r=0.64$), indicating a strong contribution of these components to the total antioxidant activity. However, compounds not yet identified and which share similar affinity characteristics to the C18 column as anthocyanins, are thought to be responsible for the major part of the antioxidant capacity in açai (Lichtenthaler and others 2005).

Non-fortified treatments experienced accelerated losses in antioxidant capacity during the first 4 days of storage (Figure 4-14), consistent with previously observed trends for total anthocyanins, individual anthocyanins by HPLC and total soluble phenolics. Major polyphenolic constituents were relatively stable during this period, suggesting that oxidation of additional non-anthocyanin phenolic components, including

anthocyanins, was probably responsible for initial losses in antioxidant activity. However, further degradation of major polyphenolic components in açai might have led to additional declines in antioxidant activity during the last 8 days of storage, since both experienced similar losses (10 to 15%) during the same period while essentially no changes in anthocyanin or soluble phenolic contents were detected. Moreover, a higher correlation (r=0.76) was obtained between the two variables when only data from the last 8 days of storage was included in the analysis.

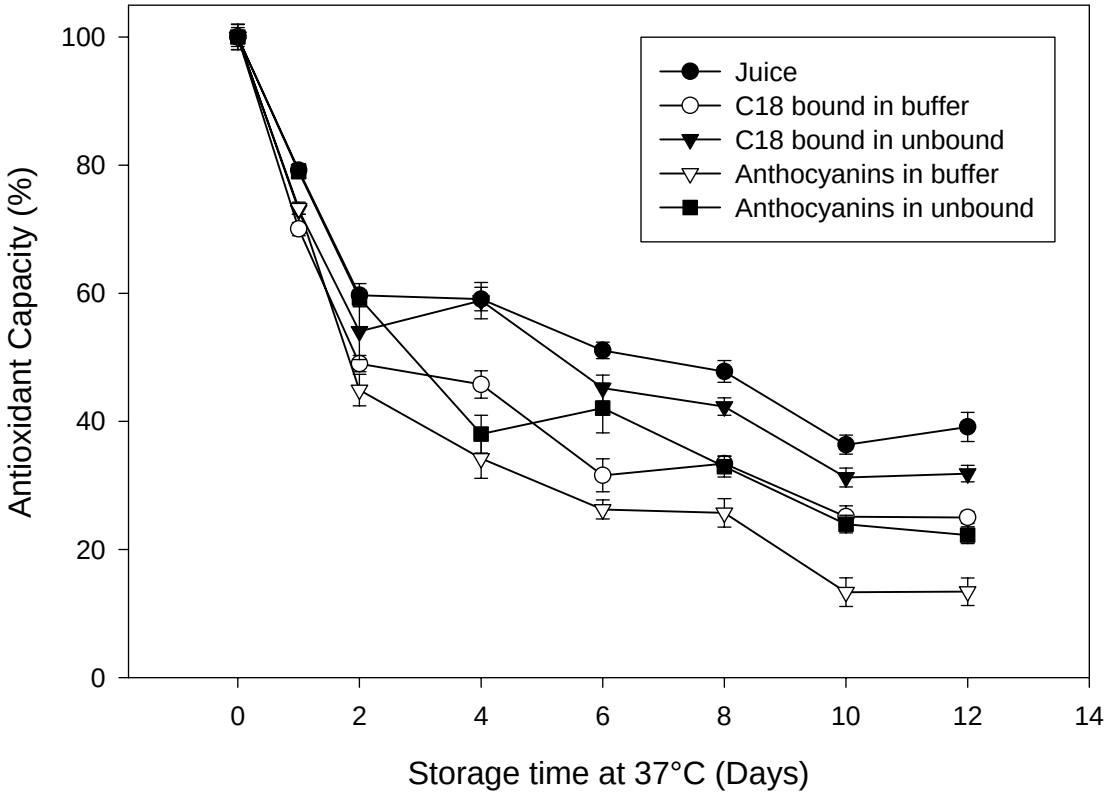

Figure 4-14. Antioxidant capacity (%) of non-fortified açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

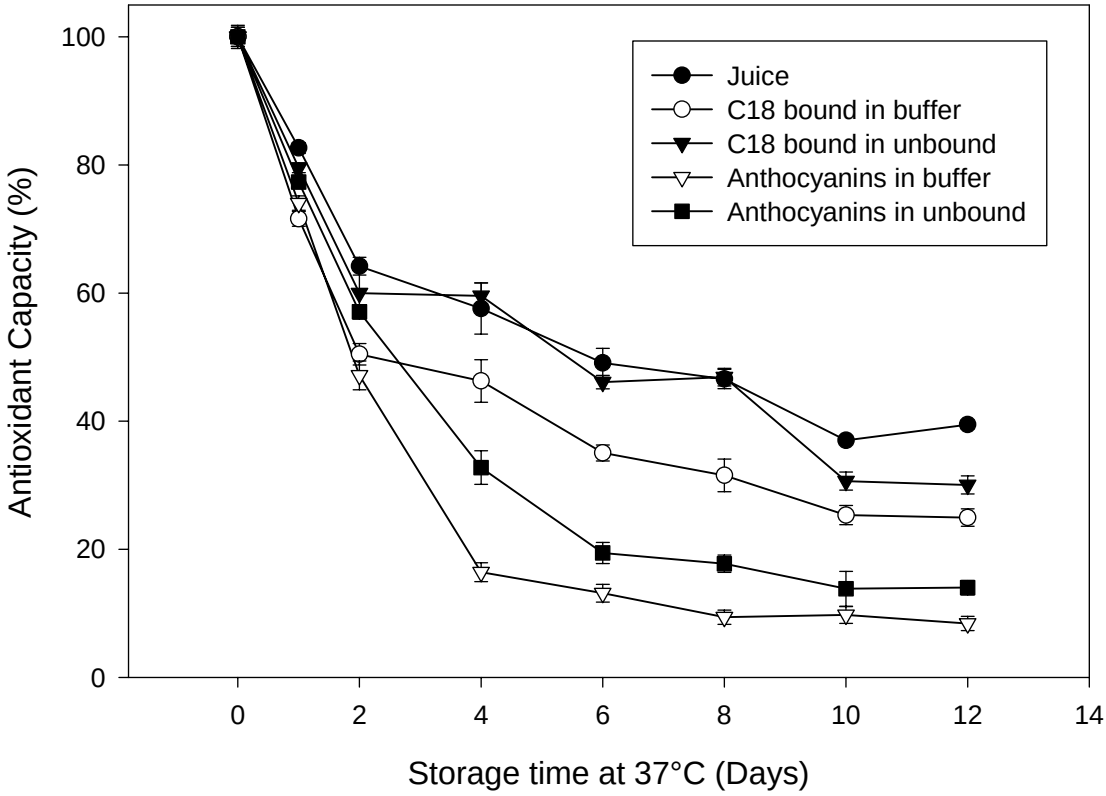

Figure 4-15. Antioxidant capacity (%) of ascorbic acid fortified (500mg/L) açai fractions as affected by storage at 37°C. Error bars represent the standard error of the mean, n=3.

Significant differences were detected among antioxidant degradation patterns for juice and juice fractions and overall, açai juice and fractions with the closest composition retained significantly higher antioxidant capacity levels, suggesting a positive effect of all juice components on antioxidant stability. The presence of non-anthocyanin polyphenolics induced a higher retention of antioxidant activity in the C18 bound fractions when compared to anthocyanin fractions while fractions redissolved in the unbound also exhibited higher antioxidant capacities compared to those redissolved in buffer throughout the storage period. Beneficial effects of non-anthocyanin polyphenolics are easily explained by its known roles as antioxidants and metal chelators (Robbins

2003), thus preventing further oxidation by unstable radicals, as previously discussed. However, positive effects of unbound components are less understood. Synergistic interactions among matrix components could explain a higher stability in terms of antioxidant capacity, since the presence of unbound components did not have any effect on the initial antioxidant capacity of the juice or juice fractions. Moreover, following HPLC analysis of the unbound fraction, no additional phenolic compounds were detected.

Fortified juice and juice fractions showed similar degradation patterns as their non-fortified counterparts, in terms of a higher stability in the original juice system and closely related fractions (Figure 4-15). Anthocyanin fractions experienced significantly more degradation in presence of ascorbic acid while no effects were observed in the juice or C18 bound fractions. Moreover, accentuated differences between C18 bound and anthocyanin fractions in presence of ascorbic acid further confirm the protective role of non-anthocyanin polyphenolics in preventing additional oxidation reactions. Finally, unknown interactions among juice components, including those present in the unbound fraction, had an overall positive effect on polyphenolic and antioxidant stability of juice and juice fractions, even in presence of ascorbic acid. Further research is needed to achieve a better understanding of these complex interactions.

4.4 Conclusions

Polyphenolic, antioxidant and color stability of açai is seemingly a function of complex interactions among matrix components, considerably influenced by processing, storage, temperature and composition. While color retention is favored by anthocyanin isolation, the retention of non-anthocyanin polyphenolics is enhanced by the natural juice matrix. In the presence of ascorbic acid, unknown synergistic effects among matrix

components seemed to favor not only anthocyanin, but overall polyphenolic and antioxidant stability in the juice system. Moreover, the presence of non-anthocyanin polyphenolics exerted a protective effect against ascorbic acid oxidation in fortified systems. Additional investigations are needed to attain a better understanding of the chemistry behind positive interactions among matrix components and their role on the polyphenolic, antioxidant and pigment stability of açai.

CHAPTER 5
SUMMARY AND CONCLUSIONS

Açai, a palm fruit native to the Amazon region, has recently captured the attention of US consumers due to potential health benefits associated with its high antioxidant capacity and polyphenolic composition. However, studies on the functional properties of this matrix are lacking while their stability during processing and storage has not been determined. These studies assessed the effects of clarification, ascorbic acid fortification, storage temperature and matrix composition on the polyphenolic, antioxidant, and anthocyanin pigment stability of açai. Clarification of açai pulp was responsible for initial losses (20-25%) in total anthocyanin, phenolic and antioxidant contents but no further effects were observed in non-fortified treatments. Ascorbic acid addition notably accelerated anthocyanin degradation in the clarified juice although no significant effects were observed in non-clarified treatments. Moreover, treatments stored at 4°C showed the highest stability for all variables measured. Clarification and fortification of açai pulp is then feasible if high product rotation rates and low temperatures (4°C) are maintained throughout the distribution chain. Complex interactions among matrix components considerably influenced polyphenolic, antioxidant and color stability in açai juice and juice fractions and while color retention was enhanced by anthocyanin isolation, a higher retention of non-anthocyanin polyphenolics was observed in the natural juice matrix. Furthermore, the presence of non-anthocyanin polyphenolics exerted a protective effect against ascorbic acid oxidation. All treatments experienced major color, antioxidant and polyphenolic losses during the first stages of storage (4 days at 37°C). Therefore,

maximum retention of polyphenolic and antioxidant activity of açai-containing products can be achieved if adequate industrial handling practices are maintained, especially at early stages of processing and storage. Additional research is needed to achieve a better understanding of the role of matrix interactions on the phytochemical and antioxidant stability of açai.

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BIOGRAPHICAL SKETCH

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