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Chemical stability of açai fruit (*Euterpe oleracea* Mart.) anthocyanins as influenced by naturally occurring and externally added polyphenolic cofactors in model systems

Lisbeth A. Pacheco-Palencia, Stephen T. Talcott *

Department of Nutrition and Food Science, Texas A&M University, College Station, TX 77843, USA

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ABSTRACT

The influence of different classes of naturally occurring and externally added polyphenolic cofactors on the phytochemical and colour stability of anthocyanins in açai fruit (*Euterpe oleracea*) was investigated. Model systems were based on anthocyanin isolates from açai fruit, rich in cyanidin-3-rutinoside (311 ± 27 mg/l) and cyanidin-3-glucoside (208 ± 18 mg/l), and isolated groups of naturally occurring polyphenolic cofactors in açai fruit (phenolic acids, procyanidins, and flavone-*C*-glycosides, each adjusted to ~50 mg/l). Anthocyanin degradation kinetics were assessed as a function of pH (3.0, 3.5, and 4.0) and storage temperature (5, 20 and 30 °C). During storage, anthocyanins experienced pH and temperature-dependent losses, and the half life cyanidin-3-rutinoside ($t_{1/2}$ = 2.67–210 days) was consistently longer than cyanidin-3-glucoside ($t_{1/2}$ = 1.13–144 days). The presence of flavone-*C*-glycosides induced significant hyperchromic shifts and enhanced anthocyanin stability at all pH and temperature combinations, while no significant effects were attributed to the presence of phenolic acids or procyanidins. Additional models using externally added cofactors from rooibos tea, also rich in flavone-*C*-glycosides, resulted in up to 45.5% higher anthocyanin colour and up to 40.7% increased anthocyanin stability compared to uncopigmented anthocyanin isolates and had similar copigmentation effects to a commercial rosemary-based colour enhancer. Results suggest flavone-*C*-glycosides offer potential for their use as colour enhancers and stabilizing agents in products rich in cyanidin glycosides, particularly açai fruit-containing foods, juice blends, and beverages.

1. Introduction

Anthocyanins have been categorized as the most important group of water-soluble pigments in plants, and are responsible for most blue, red, and related colours in flowers and fruits (Clifford, 2000). Anthocyanin colour is an important sensory characteristic and often a major quality parameter for a variety of fruit products. Açai (*Euterpe oleracea* Mart.), a palm fruit native to the Brazilian Amazon, has been the focus of increased international attention as a functional ingredient in fruit juices, beverage blends, dietary supplements, and dairy products (Del Pozo, Brenes, & Talcott, 2004; Schauss et al., 2006) and is a potential rich source of anthocyanins (Gallori, Bilia, Bergonzi, Barbosa, & Vincieri, 2004; Lichtenthaler et al., 2005; Pacheco-Palencia, Hawken, & Talcott, 2007a, 2007b). Two predominant anthocyanins, cyanidin-3-rutinoside and cyanidin-3-glucoside, are responsible for most of its characteristic dark purple colour, and are often a major source of colour in açai-containing juices and beverages (Lichtenthaler et al., 2005; Pacheco-Palencia et al., 2007a; Schauss et al., 2006). However, anthocyanins are highly reactive and generally experience extensive degradation during long-term storage, leading to dark, dull, brown hues (Jurd, 1972).

Anthocyanin colour changes are known to be influenced by several factors, including pH, temperature, light, and the presence of enzymes, sugars, metals, and phenolic cofactors (Markakis, 1982). Among these, the presence of non-anthocyanin polyphenolics may significantly affect anthocyanin colour, as they participate in copigmentation reactions in enhanced colour and increased stability during storage (Singleton, 1972). Intermolecular copigmentation reactions are common in nature and occur when colourless phenolic cofactors are attracted to anthocyanins via weak hydrophobic forces (Mazza & Brouillard, 1990). Anthocyanin copigmentation reactions are detected by both a hyperchromic shift, where absorbance at the λ_{max} of the absorption spectrum increases, and by a bathochromic shift, where a change toward higher wavelengths (nm) at the λ_{max} of the absorption spectrum occurs (Baranac, Petranovic, & Dimitric-Markovic, 1996; Malien-Aubert, Dangles, & Amiot, 2001).

* Corresponding author. Tel.: +1 979 862 4056; fax: +1 979 458 3704.
E-mail address: stalcott@tamu.edu (S.T. Talcott).

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Previous studies have evaluated the use of externally added polyphenolic cofactors, particularly phenolic acids, to enhance and stabilize anthocyanin colour in berry juices (Eiro & Heinonen, 2002). External addition of commercial polyphenolic cofactors from rosemary (*Rosmarinus officinalis*) has also shown to result in enhanced anthocyanin colour and increased stability in grape juices (Brenes, Del Pozo-Insfran, & Talcott, 2005; Talcott, Brenes, Pires, & Del Pozo-Insfran, 2003). Thus, the natural occurrence and predominant presence of certain groups of polyphenolic cofactors in açai fruit, including phenolic acids, flavone-*C*-glycosides, and procyanidins (Gallori et al., 2004; Pacheco-Palencia et al., 2007b; Schauss et al., 2006), may play a significant role in the structural and pigment stability of anthocyanins in açai fruit juices. The role of flavone-*C*-glycosides is of particular interest, as their presence in other anthocyanin-rich fruits has not been previously reported. Moreover, the potential use of flavone-*C*-glycoside-rich extracts as anthocyanin colour enhancers and stabilizing agents has not been evaluated. Extracts rich in flavone-*C*-glycosides may be obtained from various botanical sources, among which, rooibos (*Aspalathus linearis)* tea, a leguminous shrub native to South Africa, is among the highest, most widely available, and easily extractable sources (Krafczyk & Glomb, 2008).

This study was conducted to evaluate the influence of different groups of naturally occurring polyphenolic cofactors on the stability of anthocyanins in açai models stored under different pH (3.0, 3.5, and 4.0) and temperature (5, 20, and 30 °C) conditions and assess the influence of externally added polyphenolic cofactors from rooibos tea, rich in flavone-*C*-glycosides, and from commercial rosemary extracts on the stability of açai anthocyanin models. Relations between anthocyanin degradation and sulfite bleaching resistance, reducing capacity, and antioxidant activity were additionally determined.

2. Materials and methods

2.1. Polyphenolic isolations

Phenolic acids and procyanidins were extracted from a previously characterized, polyphenolic-enriched açai oil (Pacheco-Palencia, Mertens-Talcott, & Talcott, 2008), which contained concentrated amounts of phenolic acids and procyanidins originally present in açai fruit pulp. Açai oil was extracted from a semi-solid filter cake obtained from the Bossa Nova Beverage Group (Los Angeles, CA), commercially used for açai pulp clarification, using a hydro-alcoholic solution, as described by Pacheco-Palencia et al. (2008). Açai oil was extensively extracted with an equal volume of a 1:1 (v/v) hexane:methanol solution until complete dissolution. A known volume of 0.1 M aqueous citric acid buffer at pH 3.0 was added to the mixture, to form a bi-layer, from which the hydrophilic phase was retained. Residual methanol in the hydrophilic phase was evaporated under reduced pressure (<40 °C) and the resulting aqueous solution recovered, adjusted to pH 7.0, and loaded onto C18 Sep-Pak Vac 20 cm^3 mini-columns (Waters Corporation, Milford, MA). Phenolic acids were eluted in the unbound fraction, while procyanidins were eluted with acidified methanol (0.01% HCl). The aqueous, phenolic acid-containing fraction was then acidified to pH 3.0, loaded onto a second Sep-Pak column, and eluted with acidified methanol (0.01% HCl). Methanol from each extraction was then evaporated under vacuum (<40 °C) and each fraction was re-dissolved in a known volume of the citric acid buffer.

In a second extraction, anthocyanins and naturally occurring flavone-*C*-glycosides in açai fruit were extracted from clarified açai pulp (Bossa Nova Beverage Group, Los Angeles, CA). Clarified açai pulp was likewise loaded onto Sep-Pak mini-columns (Waters Corporation, Milford, MA), and sequentially eluted with acidified water (0.01% HCl) to remove metals, sugars, and other water soluble components followed by ethyl acetate to remove phenolic acids. Flavone-*C*-glycosides and anthocyanins were finally eluted with acidified methanol. Following solvent evaporation and re-dissolution in the citric acid buffer, the methanolic fraction was loaded onto manually packed, lipophilic Sephadex LH-20 (Sigma–Aldrich, St. Louis, MO) 40 cm^3 mini-columns, previously equilibrated with water. Columns were washed with 30% aqueous methanol (v/v) to elute anthocyanins, followed by 60% aqueous methanol (v/v) to elute flavone-*C*-glycosides, and with 100% methanol to elute procyanidins. These procyanidins were combined with the procyanidin fraction previously obtained from açai oil. All solvents were finally evaporated under reduced pressure (<40 °C) and each resulting isolate was redissolved in the citric acid buffer (pH 3.0).

2.2. Anthocyanin models

Açai fruit models were created based on the predominant anthocyanins present in the fruit (cyanidin-3-rutinoside and cyanidin-3-glucoside), adjusted to a total concentration of approximately 500 mg/l in all models. Four different anthocyanin-based models were prepared by combining açai anthocyanins with a major group of naturally occurring polyphenolics present in the fruit. Models were created by combining phenolic acids, procyanidins, flavone-*C*-glycosides, and a non-copigmented anthocyanin control. Cofactor concentrations on each model were adjusted according to the original ratio of total phenolic cofactors to anthocyanins (1:10) naturally present in 100% clarified açai pulp. Models were adjusted to three different pH levels (3.0, 3.5, and 4.0), based on the pH range of commercial açai products, and equal amounts of each were loaded into screw-cap glass test tubes in triplicate. Treatments were stored in the dark at 5, 20, and 30 °C for up to 60 days. Sodium azide (50 mg/l) was added to prevent microbial growth during storage and individual tubes were removed from storage at predetermined time intervals and held at −20 °C until analysis.

Additional models with açai anthocyanins (~500 mg/l) were evaluated that contained externally added polyphenolic cofactors from a rooibos tea extract, rich in flavone-*C*-glycosides, as compared to a commercial rosemary extract (ColourEnhance, 3.5% rosmarinic acid, Naturex, South Hackensack, NJ) and a non-copigmented control. Rooibos tea extracts were obtained from rooibos tea, prepared by brewing 50 g of loose rooibos tea (Keekanne GmbH, Dusseldorf, Germany) in 50 ml boiling water for 30 min. Both rooibos tea and commercial rosemary extracts were subsequently diluted in citric acid buffer (0.1 M, pH 3.0) and further purified using Sep-Pak columns. Sugars, organic acids, and other water soluble components were removed with water, and polyphenolic components were recovered with ethyl acetate. Following solvent removal, compounds were redissolved in a known volume of citric acid buffer (0.1 M, pH 3.0), adjusted to equal soluble phenolic contents (10,000 mg gallic acid equivalents/l) and added to anthocyanin isolates to contain final concentrations of 0.2% v/v, based on previous reports using similar rosemary extracts (Brenes et al., 2005; Talcott et al., 2003). All models were finally adjusted to pH 3.0, 3.5, or 4.0, loaded into screw-cap glass tubes in triplicate, and stored at 30 °C for up to 30 days. Sodium azide (50 mg/l) was added to all treatments to retard microbial growth and pH was measured every other day to confirm its consistency during storage. Individual tubes were removed from storage every 3 days and held at −20 °C until analysis.

2.3. Chemical analyses

Polyphenolic compounds were analyzed by reversed phase HPLC with a Waters 2695 Alliance system (Waters Corporation, Milford, MA), using previously described chromatographic condi-