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Preliminary communications

STABILITY AND STABILIZATION OF THE ANTHOCYANINS FROM *EUTERPE OLERACEA* MART

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The effect of light on the stability and stabilization of the anthocyanins isolated from freeze-dried aqueous extracts of fruits of *Euterpe oleracea* were studied, using crude and purified extracts at pH 2.2 and 3.0. Crude extract was 80.0 times more stable than the purified one at pH 2.2 and 24.3 at pH 3.0. Stabilization with tannic acid was attempted and resulted in 65% half-life increase of the anthocyanins of the crude and a considerable half-life increase (610%) of the purified one.

Keywords: anthocyanins, açaí, *Euterpe oleracea*, stability

The *E. oleracea*, whose fruits are known as "açaí", is a tropical palm tree that grows easily in the northern part of Brazil. It can reach a high of 25 meters and a diameter of 16 centimeters; its leaves are deeply cut.

From its small black fruits, a thick black extract is prepared with water and widely used in Brazil as an "energy and healthy drink". The complete composition of the extract is not known. The fruit mesocarp is rich in fat that was analyzed by LUBRANO and co-workers (1994). In the unsaponifiable fraction, delta-5-sterols and substantial amounts of β-sitosterol were found and among the tocopherols and trienols, α-tocopherol is predominant.

In a previous paper, BOBBIO and co-workers (2000) analyzed the skin pigment and reported that 19% of fruits weight correspond to the fruit peel. The amount of anthocyanin was found to be 263 mg/100 g of fresh peel. The anthocyanins found were cyanidin-3-arabinoside and cyanidin-3-arabinosylarabinoside (BOBBIO et al., 2000).

The amount of anthocyanins in the fruit of *E. oleracea* as well as the lack of reported toxicity makes this fruit an important source of natural red pigment for use in foods.

In this paper we wish to report the stability and stabilization of the pigments from *E. oleracea* and its stabilization with tannic acid.

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1. Materials and methods

All reagents were of analytical grade.

The spectra of the solutions were recorded with a UV-Vis Beckman DU 70, using a 1.0 cm pathlength cell.

The freeze-dried water extracts were commercially available in Campinas-SP. Three samples were analyzed.

1.1. Preparation of the crude extract

The anthocyanins were extracted overnight from freeze-dried commercial water extract of fruits from *E. oleracea* using a 0.1% solution of HCl in ethanol at 5 °C, in the dark and under nitrogen (BAILONI et al., 1998).

1.2. Preparation of the purified extract

The ethanolic crude extract obtained in 1.1. was purified by paper chromatography with 1% HCl and BAW (n-buthanol–acetic acid–water, 6:1:2) as solvent systems according to FRANCIS (1982), and the anthocyanins were eluted together with methanol.

1.3. Stability of the crude extract

The ethanolic extract obtained in 1.1. was concentrated under reduced pressure at 38±2 °C, and the residue was dissolved in citrate-phosphate buffer of ionic strength 0.2M (BARANAC et al., 1996). The solutions were prepared in sufficient amount of buffer to give an absorbance of approximately 1.5 at pH 2.2 and 3.0. The solutions were distributed into 10 ml screw cap tubes and irradiated between two 40 W lamps, day-light type with nominal intensity of 2,500 lm at 24±1 °C. At regular time intervals absorbances were measured at the $\lambda_{vis\ max}$ of the solutions, i.e. at 516 nm.

1.4. Stability of the purified extract

The solvent free anthocyanic fraction obtained in 1.2. was used to prepare solutions as in 1.3., and the stability of light was estimated as described for the crude extract. The absorbances were read at the $\lambda_{vis\ max}$ of each solution: 510 nm and 512 nm at pH 2.2 and 3.0, respectively.

1.5. Stabilization of the crude and purified extracts at pH 3.0 by tannic acid

Solutions of crude and purified extracts at pH 3.0 were prepared as described in 1.3. To each solution sufficient tannic acid was added in order to obtain a 3:1 proportion of tannic acid to anthocyanins (w:w) as described by BOBBIO and co-workers (1990; 1992).

The absorbances of each solution were measured at 516 nm, the $\lambda_{vis\ max}$ of the solutions.

2. Results and discussion

In all cases the rate of disappearance of the anthocyanins followed first order kinetics and the rate constants are in Table 1. These results were obtained from the average readings from 3 samples.

Table 1. First order rate constants for the degradation of anthocyanins from the *E. oleracea* under different conditions

	pH					
	2.2		3.0			
			Without tannic acid		With tannic acid	
Sample	Crude extract	Purified extract	Crude extract	Purified extract	Crude extract	Purified extract
k (h^{-1})	2.4×10^{-3}	1.4×10^{-1}	3.8×10^{-3}	9.3×10^{-2}	2.3×10^{-3}	1.3×10^{-2}
$t_{1/2}$ (h)	301.2	4.95	182.4	7.45	301.3	53.3

The buffer maintained the pH of the systems within 0.05 of a pH unit.

At pH 2.2 and 3.0 the purified pigments decompose very fast losing all colour after 15 h, while the crude extract has a residual colour of 52% and 49% at pH 2.2 and 3.0, respectively, after 188 h (Figs 1 and 2). The instability of the purified extract indicates a lack of protective effect generally attributed to the copigmentation with the non-anthocyanic flavonoids (NAF) (CHEN & HRAZDINA, 1981; BROUILLARD et al., 1991), which are conspicuously absent in the purified extract where only association of anthocyanin molecules could take place.

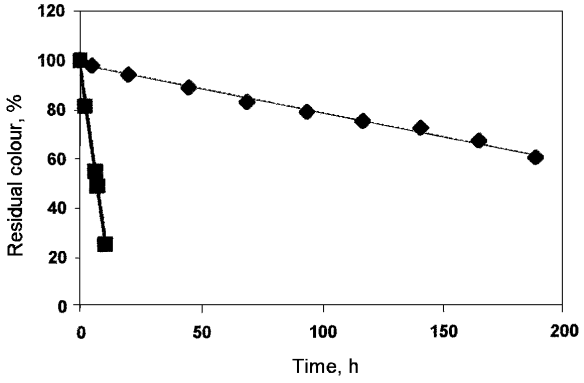

Fig. 1. Effect of purification on the degradation of anthocyanins from *E. oleracea* at pH 2.2:
♦: crude extract (y=–0.1983x+98.493, R^2=0.9925); ■: purified extract (y=–7.2766x+98.483, R^2=0.9969)

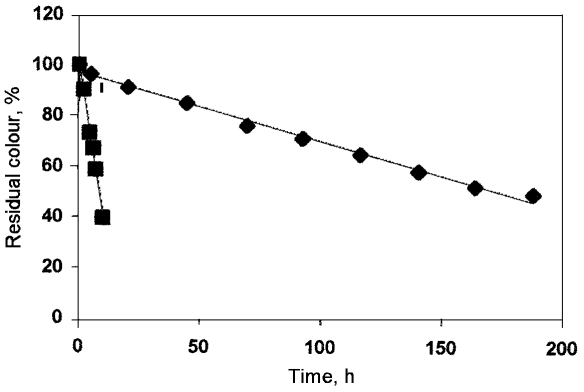

Fig. 2. Effect of purification on the degradation of anthocyanins from *E. oleracea* at pH 3.0: ♦: crude extract (y=–0.2715x+97.256, R^2=0.9915); ■: purified extract (y=–6.079x+101.24, R^2=0.9961)

The addition of tannic acid at pH 3.0, a pH close to that of the "açaí" juice, as a stabilizing compound (MACCARONE et al., 1987; BOBBIO et al., 1995), resulted in a increase of 62.5% in the residual colour of the crude extract and a considerable increase in the residual colour of 610% for the purified extract after 119 h (Figs 3 and 4).

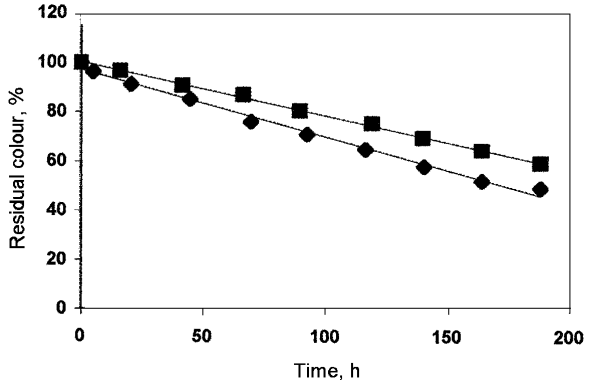

Fig. 3. Effect of purification on the degradation of anthocyanins from the crude extract of *E. oleracea* at pH 3.0: ♦: without tannic acid (y=–0.2717x+97.289, R^2=0.9915); ■: with tannic acid (y=–0.2158x+100.3, R^2=0.998)

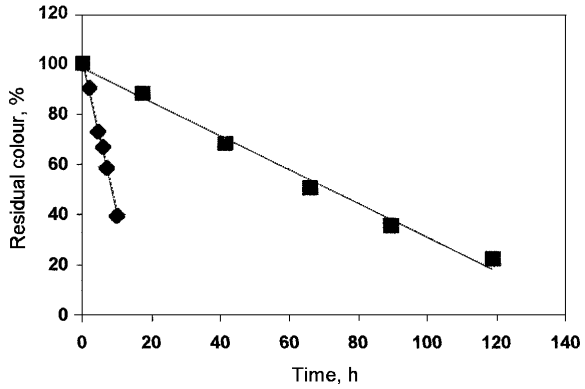

Fig. 4. Effect of purification on the degradation of anthocyanins from the purified extract of *E. oleracea* at pH 3.0: ♦: without tannic acid (y=–6.079x+101.24, R^2=0.9961); ■: with tannic acid (y=–0.6701x+98.099, R^2=0.9901)

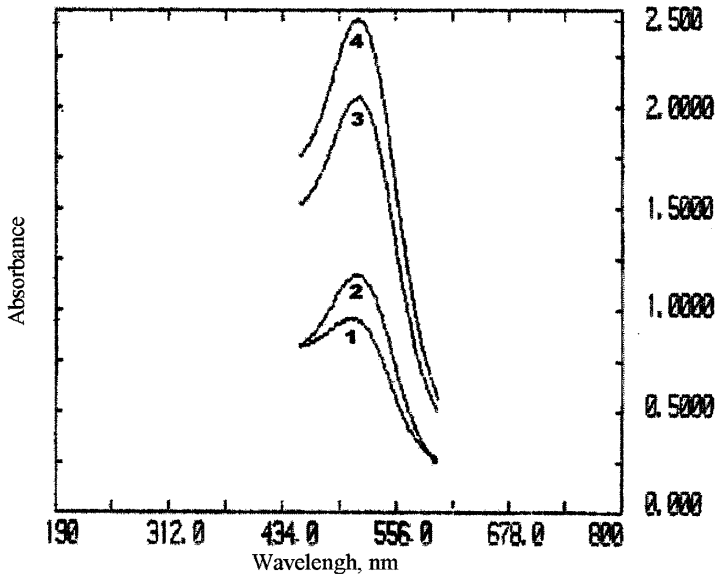

Fig. 5. Increase of the absorbances of the crude and purified extract of *E. oleracea* by addition of tannic acid: 1: purified extract; 2: crude extract; 3: crude extract plus tannic acid; 4: purified extract plus tannic acid

The proportionally lower effect of tannic acid on the stability of the crude extract when compared with the effect on the purified extract can be accounted by the fact that NAF present in the "açaí" offer better protection than the tannic acid, indicating also that there is a competition between NAF and tannic acid molecules for the anthocyanins which form a strong anthocyanin-NAF structure. According to MAZZA and BROUILLARD (1990), the values of $A\text{-}A_0/A_0$, where A is the absorbance of copigmented anthocyanins and A_0 the absorbance of anthocyanins without the addition of copigment, a reading at maximum visible wavelength can be used as a measure of copigmentation. Applying the same principle to the association of anthocyanin-tannic acid, the values obtained for the crude (0.43) and for the purified extracts (0.59) (Fig. 5) also indicate a stronger association of the tannic acid with the anthocyanins of the purified extract. The increase in the stability of the crude extract with tannic acid suggests a possible sandwich structure, as in the copigmentation anthocyanin-NAF where tannic acid might associate to the copigmentated structure, offering added protection at C_2.

3. Conclusion

At pH 2.2 and 3.0, purification has a destructive effect on the stability of the anthocyanins of the "açaí". At pH 3.0, copigmentation with tannic acid was an effective stabilizing agent for both extracts, and the purified extract showed an increase in the $t_{1/2}$ of 610%.

The strength of copigmentation is shown in Fig. 5.

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