A rapid validated UHPLC-PDA method for anthocyanins quantification from *Euterpe oleracea* fruits (Açaí)

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INTRODUCTION

Euterpe oleracea is a palm tree widely distributed in the floodplains of the Amazonian delta (Brazil). The fruits called açaí (Figure 1) are an interesting source of anthocyanins [1] and they have gained polularity in North America and in the European countries in the food industry and in the health sector due to their extremely high antioxidant capacity and potential anti-inflammatory activities [2]. Accurate characterizations of these compounds are very important for the post-harvest and food industry. The aim of this work is to develop and validate an UHPLC-PDA method for major anthocyanins quantification in this fruit after fast extraction procedures and samples preparation.

Fig 1: *Euterpe oleracea* fruits (Açaí)

EXPERIMENTAL

☞Fruits were harvested in Abaetetuba (Brazil). Extractions and sample preparation were performed as shown in Figure 2.

☞U**HPLC conditions:** C18 HSS column, 1.8 µm, 100-2.1 mm, Waters, mobile phase gradient of solvent A: 5% formic acid in water and B: CH3CN. Flow rate of 400µL/min. Total run time of only 17 min.

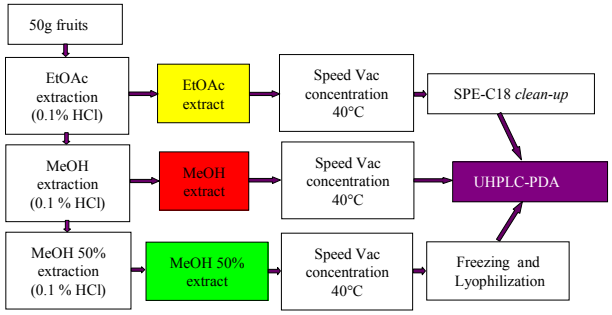

Fig 2: Extraction protocol and sample preparation

RESULTS

☞ **Identification of anthocyanins (Figure 3), validation of the UHPLC-PDA method (Table 1) and application of the method to samples of *Euterpe oleracea* fruits (Figure 4).**

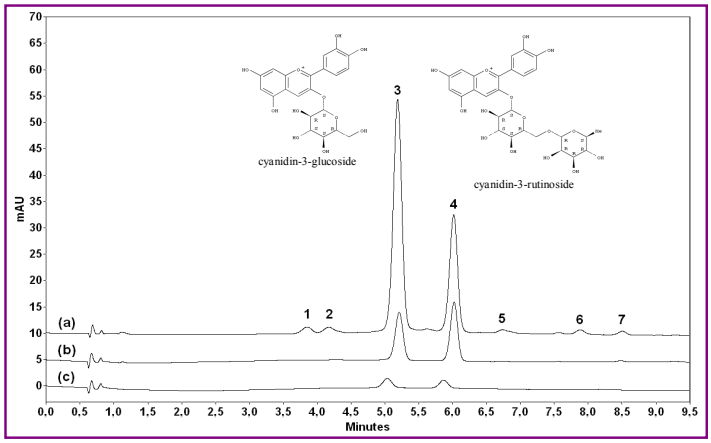

Fig 3: UHPLC-PDA chromatograms recorded at 520 nm from (a) MeOH, (b) MeOH50% and (c) EtOAc extracts. 1: cyanidin-di-O-glycoside, 2: cyanidin-di-O-glycoside (isomer of 1), 3: cyanidin-3-glucoside, 4: cyanidin-3-rutinoside, 5: pelargonidin-3-glucoside, 6: peonidin-3-glucoside, 7: peonidin-3-rutinoside

Table 1: Validation results for the quantification method of cyanidin-3-glucoside and cyanidin-3-rutinoside obtained in MeOH extract of *Euterpe oleracea* fruits

Validation criteria	**cy-3-glu**		**cy-3-rut**	
Response function	Linear regression Calibration in the matrix (5 points) range: 1.0-49.0 µg/mL		Weighted (1/X) linear regression Calibration in the matrix (5 points) range: 1.0-48.6 µg/mL	
Trueness	Concentration (µg/mL)	Relative bias (%)	Concentration (µg/mL)	Relative bias (%)
	1.0	6.76	1.0	3.60
	2.0	2.54	1.9	0.51
	4.9	0.67	4.9	0.38
	24.5	-0.07	24.3	-0.24
	49.0	-0.83	48.6	-1.29
Precision	Repeatability (RSD%)	Intermediate precision (RSD%)	Repeatability (RSD%)	Intermediate precision (RSD%)
	4.63	5.30	3.10	3.80
	2.49	2.49	1.86	2.85
	1.90	1.90	1.41	1.41
	2.99	2.99	2.47	2.47
	1.46	1.46	1.35	1.35
Accuracy	Relative 95% β-Expectation lower and upper tolerance limits (%)		Relative 95% β-Expectation lower and upper tolerance limits (%)	
	-7.6, 21.1		-7.2, 14.4	
	-3.8, 8.9		-8.8, 9.8	
	-4.1, 5.5		-3.2, 4.0	
	-7.6, 7.5		-6.5, 6.0	
	-4.7, 3.0		-4.9, 2.3	
Linearity				
Slope	0.9910		Slope	0.9873
Intercept	0.0910		Intercept	0.0829
r^2	0.9994		r^2	0.9995
LOD	0.32 µg/mL		0.12 µg/mL	
LOQ	1.07 µg/mL		0.97 µg/mL	

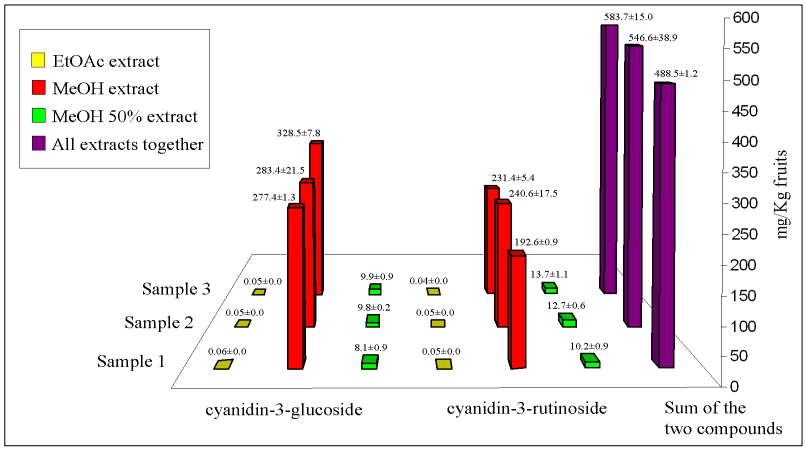

Fig 4: Anthocyanins quantification results from EtOAc, MeOH and MeOH50% extracts obtained from *Euterpe oleracea* fruits. mg/kg fruits ± standard deviation

CONCLUSION

We developed a fast protocol for anthocyanins extraction and sample preparation from *Euterpe oleracea* fruits. Elimination of lipophilic compounds can be done by an EtOAc extraction, while anthocyanins are extracted from the residue by MeOH. Calibrations in the matrix were used for cyanidin-3-glucoside and cyanidin-3-rutinoside quantifications and showed better accuracy profiles in comparison to calibrations without matrix. To our knowledge this is the first work that describes a validated UHPLC-PDA method for the quantification of the major anthocyanins from *Euterpe oleracea* fruits. Furthermore, this method was selective and gave good estimators of linearity, accuracy, trueness and precision from 1 to 48 µg/mL of cyanidin-3-glucoside and cyanidin-3-rutinoside. In addition minor diglycosilated anthocyanins were identified in the fruit for the first time.

REFERENCES

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[2] M. Heinrich *et al.*, Phytochem. Lett. 4(2011)10

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