A rapid UHPLC-DAD-ESI-MS^n method for anthocyanins quantification from *Euterpe oleracea* fruits harvested at different times

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Fig 1: Açai fruits in some different maturity stages

INTRODUCTION

Euterpe oleracea is a palm tree widely distributed in the floodplains of the Amazonian delta (Brazil). The fruits called açai (Fig 1) are an interesting source of different anthocyanins [1]. Lately they have gained popularity 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]. Açai fruits are normally sold from different maturation stages. The evaluation of the anthocyanin profile during different maturation stages is thus important for the post-harvest industry. Thus, the aim of this study was to characterize the anthocyanin profiles of açai fruits at different stages of maturity from specific region of harvest.

EXPERIMENTAL

- The fruits were harvested during the peak harvesting season, between July and October 2009, in the floodplains of the eastern Amazonian region (State of Pará, Brazil) from Abaetetuba region.
- The extracts were obtained as shown in Figure 2. The MeOH and MeOH 50% extracts were analysed at 0.25mg/mL.
- **UHPLC 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: CH_3CN. Total run time of 21 min instead of 55 min in the previously developed HPLC method.
- **Mass spectrometry conditions:** LTQ-Orbitrap XL (Thermofisher scientific) from the MASSMET plateform was used in ESI positive mode with the following conditions: a capillary temperature and voltage of respectively 275°C and 30V. The source voltage was set to 5 kV and the RF lens 1 voltage to 105V. N_2 was used at an arbitrary sheath and auxiliary flow rate of 20 and 10.

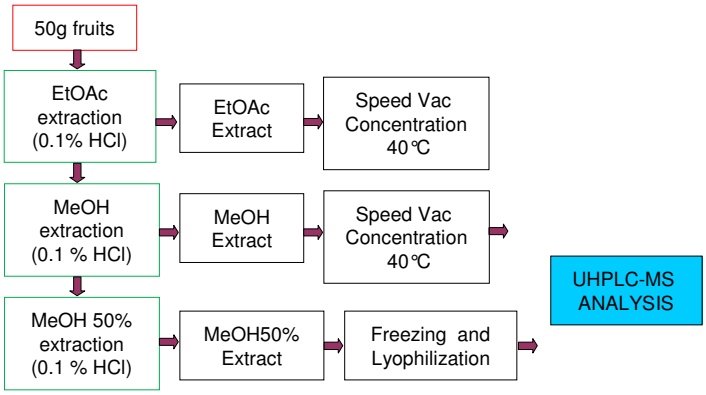


Fig 2: Extraction protocol

RESULTS AND DISCUSSION

- **UHPLC-DAD-MS identification of anthocyanins (fig 3 and table 1) identified on the basis of their high resolution mass spectra (with Δ<3ppm) and by comparison with the retention times of standards in MeOH and MeOH 50% extracts of fruits at different maturation stages.**

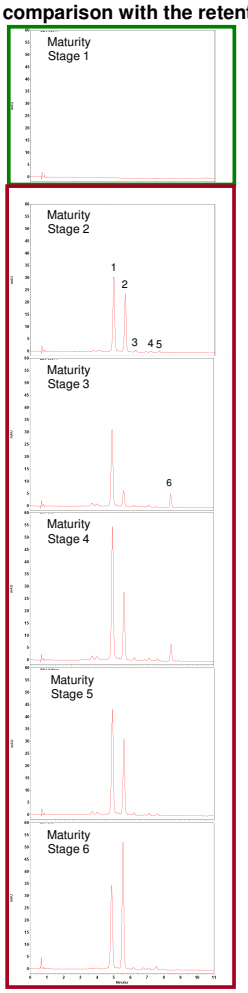
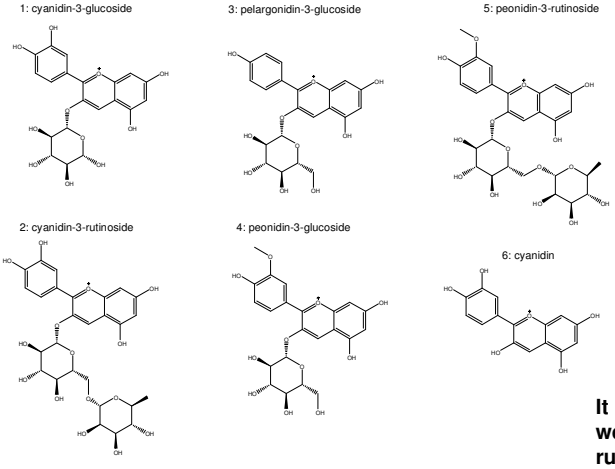


Fig 3: 520 nm chromatograms of anthocyanins obtained from MeOH fruits extracts



Anthocyanins	[M]$^+$ (m/z)	MS/MS (m/z)
cy-3-glu	449.10751	287.05481
cy-3-rut	595.16461	449.10721, 287.05466
pg-3-glu	433.11215	271.05969
pn-3-glu	463.12308	301.07053
pn-3-rut	609.18060	463.12305, 301.07056
cy	287.05457	241.04939, 213.05443

Table 1: Mass spectrometric data for identified anthocyanins in MeOH and MeOH 50% fruits extracts

- **UHPLC-DAD quantification of the main anthocyanins peaks (table 2) during ripening in MeOH and MeOH 50% fruits extracts**

Maturity stage	cy-3-glu (mg/g DEs*)	cy-3-rut (mg/g DEs*)	cy (mg/g DEs*)
1	n.q.	n.q.	n.q.
2	36.7	20.1	n.q.
3	38.8	9.9	4.2
4	73.0	32.9	5.4
5	59.0	36.4	n.d.
6	38.6	46.6	n.d.

Table 2: Variations of concentrations of de main anthocyanin during ripening. The compounds were detected at 515 (cy-3-glu and cy-3-rut) and 520 nm (cy) for the quantification.
*DEs: Dry MeOH+MeOH 50% extracts.
n.d.: not detected (under LOD).
n.q.: not quantified (under LOQ).

CONCLUSION

It was observed that the anthocyanin profiles in the extracts were relatively similar. Cyanidin-3-glucoside and cyanidin-3-rutinoside were the major constituents in all maturity stages, with similar proportions, except for the first maturity stage for which all anthocyanins were under the limit of quantification. However, in the last maturity stage, cyanidin-3-glucoside became less abundant than cyanidin-3-rutinoside. On the other hand, cyanidin decreased with maturation. The highest anthocyanin content was observed at stage 4.
This work may contribute to the selection of an optimal maturity stage for harvesting and will allow a rapid quality control of the fruits from the region analyzed.

REFERENCES

[1] H. Rogez (2000), Edupfa.
[2] Heinrich, M., et al., Phytochem. Lett. (2010), doi: 10.1016/j.phytol.2010.11.005

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