Study on the bioactive content of an edible fruit from Cerrado, Brazil. *Butia capitata* Mart.

Mulinacci N, Piccini L., Innocenti M., Gallori S., Coran S.A.

Dipartimento di Scienze Farmaceutiche, Università di Firenze, via Ugo Schiff 6, I-50019 Sesto Fiorentino (Florence) Italy

Butia capitata fruits

Introduction

Coquinho-azedo is the name both of the edible fruit and the tree of Butia capitata (Palmae).

The use of Butia capitata fruit is attested in Cerrado region, Brazil since 2500 years. Due to the really nice taste of its mesocarpus both fresh or in jelly or as **fruit juices** and **ice-creams**, also nowadays the local people consume this fruit widely. The fruit peels are also used for **antielmintic preparation**.

Some previous studies on the fruit of coquinho [1-5], report an **high content of antioxidant compounds like polyphenols and flavonoids**. Moreover, the frozen pulp largely used by local population, maintains good levels of antioxidants like vitamin C and flavonoids, particularly quercetin, kaempferol derivates and chlorogenic acid [6]. These data support the value of this fruit, commonly used in Brazil as snack during school break or as energy drink for athletes [1,4].

Aims of this work

a) to apply the HPTLC video-densitometric approach for obtaining a fingerprint of the phenolic and flavonoidic compounds;

b) to highlight the antioxidant molecules by the DPPH reagent;

c) to use this matrix as model to study both of the UV-Vis and the mass spectra of flavonoids and cynnamoyl compounds measured *in situ* and after their desorption from the stationary phase.

Experimental section

Materials. Freeze-dried pulps of the fruits : *Butia capitata* var capitata yellow (BCY) and *Butia capitata* var capitata red (BCR), collected in Montes Claros City, Minas Gerais State, Brasil, and freeze-dried by EmBraPA according to the Programme on biodiversity Brazil-Italy.

Extraction. 250 mg of freeze-dried pulp were suspended in 2 mL of MeOH, or MeOH/H_2O 7:3 or MeOH/acidic H_2O 7:3. After 15 minutes in ultrasounds bath (40°C), the samples were centrifuged and the solutions were then analysed.

SPE-C18. A fractionation of the total MeOH extract both from BCR and BCY was carried out by SPE on C18 cartridges (3 mL) according to the following elution steps each with 2,5 mL of solvent: SPE-A) 7/3 of HCOOH (0,1%)/ MeOH; SPE-B) 1/1 of HCOOH (0,1%)/MeOH ; SPE-C) 3/7 of HCOOH (0,1%)/MeOH; SPE-D) MeOH.

HPTLC apparatus. HPTLC wettable silica gel 60 F254S plates; optimized elution system: ethyl acetate/MeOH/HCOOH/H_2O 50:4:5:6

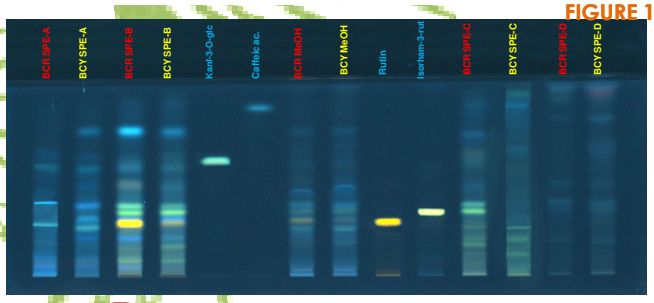
From CAMAG: a) Linomat 5 for semi-automatic sample application; b) Reprostar 3 for image storing; c)Automatic Developing Chamber ADC 2; d) TLC Scanner 3 for densitometric evaluation and dipping device for plates derivatization; e) software WinCATS for data storage and evaluation; f) TLC-MS Interface was used for online transfer into ESI-MS detector.

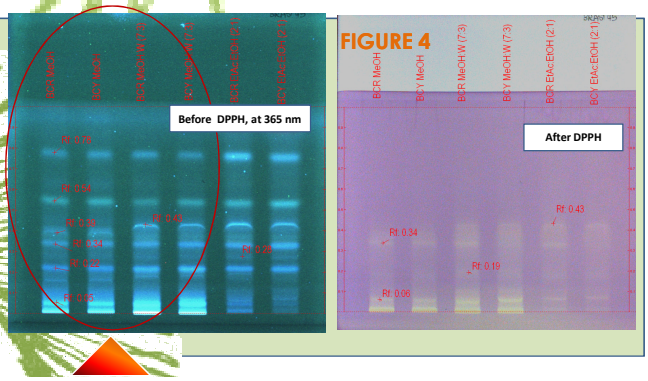
Results and discussion

To recover all the main phenolic components from the pulp, MeOH and MeOH/H2O 7:3 were preliminary tested, applying the same extractive ratio. After the HPTLC and HPLC/DAD tests, the MeOH was chosen as the most suitable solvent for our aims. The best results in terms of resolution were achieved with wettable HPTLC silica plates and an acidic elution mixture.

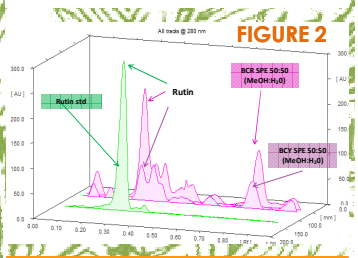
To improve the sensitivity and the resolution of this method, the total MeOH extract was fractionated on C18 cartridges by SPE. The FIGURE 1 shows the total MeOH extracts compared with their correspondent fractions from SPE and some pure standards at 366 nm after the use of the NP/PEG, a typical reagent for cynnamoyl derivatives and flavonoids. These two classes of constituents were revealed as the main phenols of the pulp of this fruit (FIGURE 2). The applied HPTLC method has highlighted almost the same phenolic profile for the two varieties, but showing an higher concentration in BCR with respect to the yellow one.

FIGURE 1



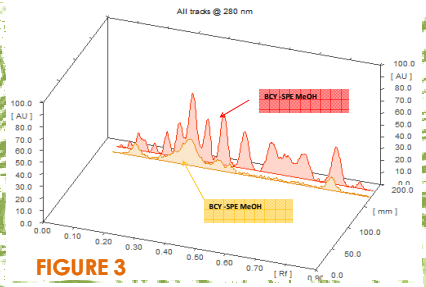


Among the flavonoids, the rutin was the more aboundant of BCR while isorhamnetin 3-O-rutinoside resulted the most abundant for BCY.



MeOH:H_2O 50:50 from SPE of BCR and BCY vs Rutin std, scanning at 280 nm

MeOH eluate from SPE of BCR and BCY, scanning at 280 nm



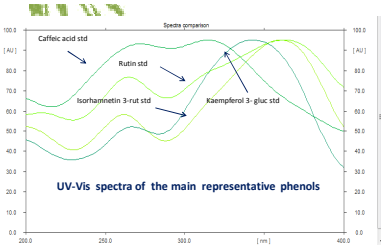
Comparing the MeOH fractions from SPE, several minor phenols were detected in BCY but not in BCR. This fraction can be thus a very efficient tool, a fingerprint, useful to distinguish the two varieties.

For a rapid evaluation of the antioxidant potency of these extracts, the DPPH was selected as high sensitivity reagent (FIGURE 4) to *in situ* detect the presence of orto-diphenolic groups in the total MeOH extracts and in some fraction from SPE on C18 cartridge.

Rutin, isorhamnetin 3-O-rutinoside, kaempferol 3-O-rutinoside, caffeic acid and its analogous have been identified in the two varieties of coquino.

The shape of the *in situ* UV-Vis spectra of the flavonols and cynnamoyl derivatives are strongly dependent by their concentration and are different from those in alcohol solutions.

Comparative analyses by HPLC/DAD/MS, have confirmed the structures of these phenolic compounds.



Conclusions

•The proposed HPTLC approach allows to obtain a fingerprint of the phenolic constituents of the pulp and to differentiate the yellow variety from the red one.

•It was pointed out that the shape of the in situ UV-Vis spectra are strongly affected by their concentration. Moreover the quality of mass spectra, obtained after desorption by Camag interface, have been modified by the possible interactions between the analyte and the silica stationary phase .

References

Faria J.P., et al., *Chemical characterization of pulp of Butia capitata var capitata.* Rev Bras Frutic 30(3) (2008): 827-829 [a]

Faria J.P.,et al., *Chemical characterization of nut of Butia capitata var capitata.* Rev Bras Frutic 30(2) (2008): 549-552 [b]

Da Silva P.A.D., *Ecologia populacional e botânica econômica de Butia Capitata (Mart.) Beccari no cerrado no norte de Minas Gerais.* 2008, Thesis (Doctor Degree). Agraria - Istituto di Scienze biologiche, Università di Brasilia

Schwartz E. *Yield, phenology and quality of fruits of Butia capitata in populations of Santa Vitória do Palmar*. 2008. 92 f. Thesis (Doctor Degree) – Program of Post-Graduation in Agronomy. Universidade Federal de Pelotas, Pelotas.

De Moura R.C., et al., *Fruit and seed biometry of Butia capitata (Mart.) Beccari (Arecaceae), in the natural vegetation of the north of Minas Gerais, Brazil*. Biota Neotropica 10(2) (2010): 415-419

Genovese M.I., et al., *Bioactive compounds and antioxidant capacity of exotic fruits and commercial frozen pulps from Brazil*. Food Science and Tecnology Int 14(3) (2008): 207-214.

Acknowledgements

This research was developed in cooperation with EmbraPA within the biodiversity Brazil-Italy programme