

Phenolic compounds in *Opuntia spp.* juices: preliminary studies by LC-MS/MS



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- Identification of phenolic compounds as flavonols (bioactive compounds)
- Compare phenolic composition of juices from different origins in Portugal

Methods

Characterization of bioactive compounds

Total phenolic compounds (TPC): Determined according to the modified Folin Ciocalteau colorimetric method^[1]. Results are expressed as gallic acid equivalents (GAE) in mg per gram or liter of extract and are a mean of 6 replicates.

HPLC-DAD-ED analysis: Was carried out using a Surveyor apparatus from Thermo, with a diode array detector and an electrochemical detector^[2] from Dionex.

HPLC-MS/MS: Was carried out in an HPLC in tandem with a triple quadrupole mass spectrometer (Micromass® Quattro MicroTM, Waters®) using an electrospray source (ESI) operating in negative mode.^[3]

Sample preparation

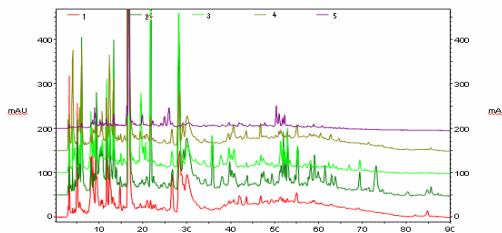
Cactus fruits (cactus pear) were collected in different parts of the country (Tramagal, Serpa, Marvão, Sines and Sesimbra). Fruits were washed, cut and were centrifuged. Juices obtained were filtrated before analysis.

Antioxidant activity

ORAC assay: The antioxidant capacity of the extracts towards peroxyl radicals was carried out by following the method described elsewhere^[4]. All data were expressed as micromoles of trolox equivalent antioxidant capacity (TEAC) per L of extract. Results are a mean of 6 replicates.

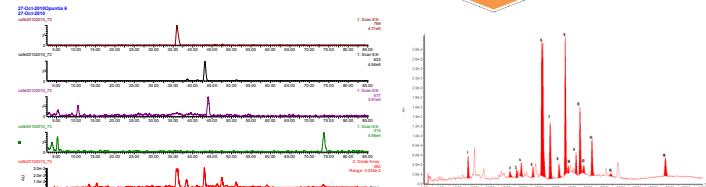
IDENTIFICATION OF COMPOUNDS

• Chromatograms at 280 nm of the fruit juices



Compounds presenting higher absorption values at 360 nm (peak filled) in sample 6:

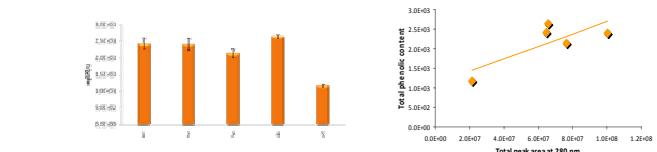
• Detection at different m/z



Peak	Compounds	Retention Time (min)	[M-H] ⁻	λ_{max} (nm)
1	Not identified	13.33	355	331
2	Not identified	26.10	383	315
3	Not identified	28.22	592	315
4	Not identified	29.57	903	340
5	Not identified	33.13	405	331
6	Isorhamnetin-3-O-(2,6-dirhamnosyl) glucoside Isorhamnetin/myricetin derivative	35.79 36.13	769 755	354 354
7	Rutin	38.38	410	331
8	Isorhamnetina 3-rutinoside	41.09	623	319
9	Isorhamnetina 3-rutinoside	42.99	623	351
10	Isorhamnetin 3-galactoside; Isorhamnetin 3-glucoside	43.93	477	323
12	Not identified	46.30	753	332
12	Not identified	47.49	767, 593	346
13	Not identified	48.69	621	319
14	Not identified	51.14	621	346
15	Not identified	56.97	-	319
16	Isorhamnetin	73.57	315	364

POLYPHENOLS AND ANTIOXIDANT ACTIVITY

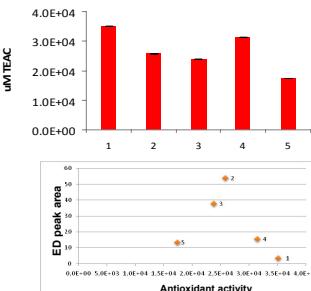
• Polyphe nols (TPC)



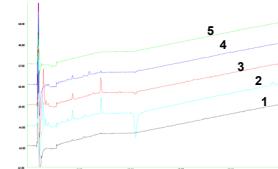
► *Opuntia ficus indica* juices from "Sines" have the highest total polyphenolic concentration and ORAC and values whereas the juices from "Sesimbra" fruits have the lowest phenolic concentration and antioxidant effect;

► A correlation was obtained between the total phenolic content of fruit juices and peak areas in the chromatogram at 280 nm ($R^2 = 0.61$).

• Antioxidant activity



• HPLC with Electrochemical Detection



► Higher antioxidant activity was obtained for juice from Tramagal;

► Although samples 2 and 3 present a higher number of compounds in the chromatogram their antioxidant activity is not as high as expected. These results are probably due to the negative peak detected in both samples.

► There is no correlation between the antioxidant activity of fruit juices and peak areas in the chromatogram obtained with ED.

CONCLUSION

- Isorhamnetin and derivatives were identified in the juices of *Opuntia*.
- The flavonols composition was different in type and number of compounds.
- More MS/MS experiments will be performed in order to try to identify more compounds;

ACKNOWLEDGEMENTS:

The authors acknowledge the financial support received from Fundação para a Ciéncia e Tecnologia (FCT) through PTDC/AGR-AAM/099645/2008 project and for the doctoral (SFRH/BD/19238/2004) and REDE/1518/REM/2005 for the LC-MS/MS equipment.

REFERENCES:

- [1] Singleton & Rossi (1965) Am. J. Enol. Vitic. 16: 144-158; [2] Bravo et al. (2006) Anal Chim Acta 563: 84-92; [3] Serra et al. (2010) J Supercr Fluids 55, 184-191; [4] Feliciano et al. (2009) Food Anal Methods 2: 149-161.