Analysis of Secondary Bioactive Metabolites from Corinthian Currants Produced in Messinia: a Comparative Study with Sultanas and California Raisins.

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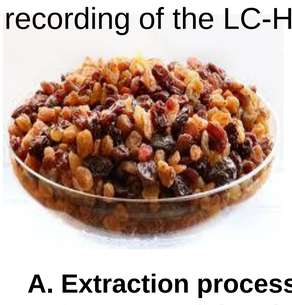
Acknowledgements: Authors would like to thank the "CAPTAIN VASSILIS FOUNDATION" & "PAPADIMITRIOU S.A." for the financial support of the project and Dr. N. Lemonakis for the recording of the LC-HRMS/MS spectra.



Introduction: Corinthian currants are dried vine products, almost exclusively produced in Southern Greece. Other types of dried vines that are widely consumed worldwide are Sultanas, mainly produced in Turkey and raisins, mainly produced in California. Previous phytochemical investigations show the presence of anthocyanins, flavonoids and phenolic acids.

The aim of the present work was the comparative phytochemical analysis of the three kinds of dried vines. The work was focused in the comparative qualitative analysis of the lipophilic content, as well as the phenolic extract of the Corinthian currents, Sultanas and California raisins.

A. Extraction processes

I. Recovery of the phenols and lipophilic ingredients

Raisins from all three types were initially extracted with dichloromethane for, the recovery of the more lipophilic constituents, and subsequently with ethanol for the recovery of the more hydrophilic constituents, including phenols. All experiments were triplicated for the confirmation of the yields. The initial amount of product extracted was 100g. Corinthian current gave the highest yield of ethanolic extract (20.3g) followed by the california raisins (12.3g). In order to condense the phenols and remove the extracted sugars, the ethanolic fractions were further enriched by SPE cartridges (C18). This procedure was also triplicated. Corinthian current also gave the highest yield in phenols (10mg per 500mg of ethanolic extract), while the respective yields for Sultanas and Californian raisins were 4 and 3 mg. The total phenolic content, expressed as Gallic acid equivalets (Folin Ciocalteau method), was similar for all the above mentioned fractions.

Summarizing the extraction results, the total yield of the phenolic fraction, after the SPE enrichment was 2.4% for Corinthian currents, and 0.6% for the other two species.

Corinthian currents yielded 4 times more phenolic extract in comparison to sultanas and California raisins.

Extraction yiels in 100g initial plant material	Corinthians	Sultanas	Californias
CH_2Cl_2 extract (mg)	500	370	540
Total EtOH extract (g)	20.3	7.5	12.3
SPE enriched extract (mg/500mg ethanolic extract)	10	4	3
% Yield of Phenolic fractions	2.4	0.6	0.6
% Total Phenolic Content (Folic Ciocalteau method)	5.65	4.53	5.25
% Yield of Anthocyanins	0.56	-	-

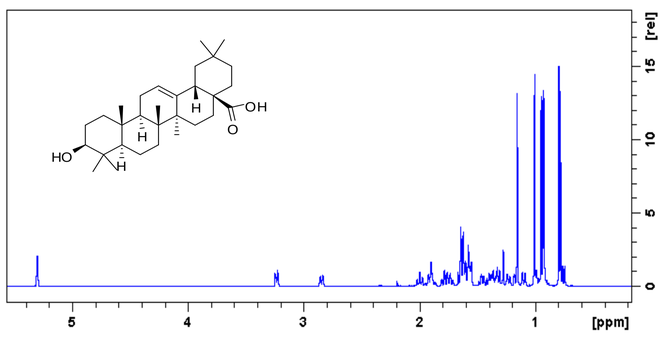
II. Recovery of Anthocyanins

For the recovery of the anthocyanins, corinthian currents were extracted with MeOH, 0.1%HCl. The extract was also treated with SPE C18 cartridge using acidified MeOH (0.1% HCl) as an eluent. The extraction yielded 2.8mg from 500mg of initial plant material (0.56% extraction yield)

B. Analysis of the dichloromethane extracts

The lipophilic extracts were similar. In terms of yields. The GC-MS analysis came up with the identification of several molecules, such as Ethyl hexadecanoate, 8-Octadecenoic acid, Methyl linoleate, 9-Octadecenoic acid (*Z*)-ethyl ester, Ethyl octadecanoate, Dotriacontane, Pentacosane, Eicosane, 1-Eicosanol, Hexadecanal, 1-Hexacosanal.

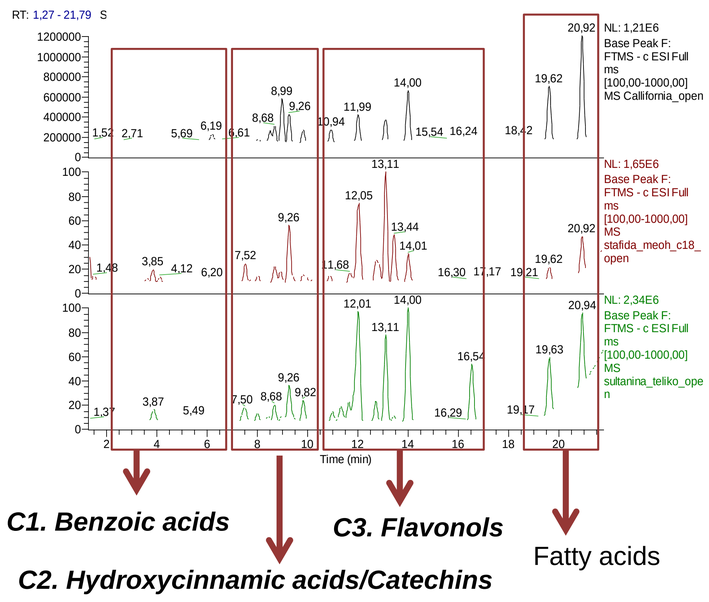
In addition, preparative work was realized in a corinthian currant dichloromethane extract and two major triterpenes were isolated. Their structures were elucidated by means of NMR spectroscopy, and they were identified as oleanolic acid and triterpenic ester derivative. Oleanonic acid (1H NMR spectrum presented below) was the major constutuent of the dichloromethane extract (20% of the total extract).



C. Analysis of the ethanolic extract

The ethanolic extracts were qualitatively analyzed by the HPLC-LTQ-Orbitrap platform at negative ionization mode. The exact mass measurements allowed the identification of several constituents in the extracts. Picture below shows the Base Peak comparative chromatograms of the three extracts.

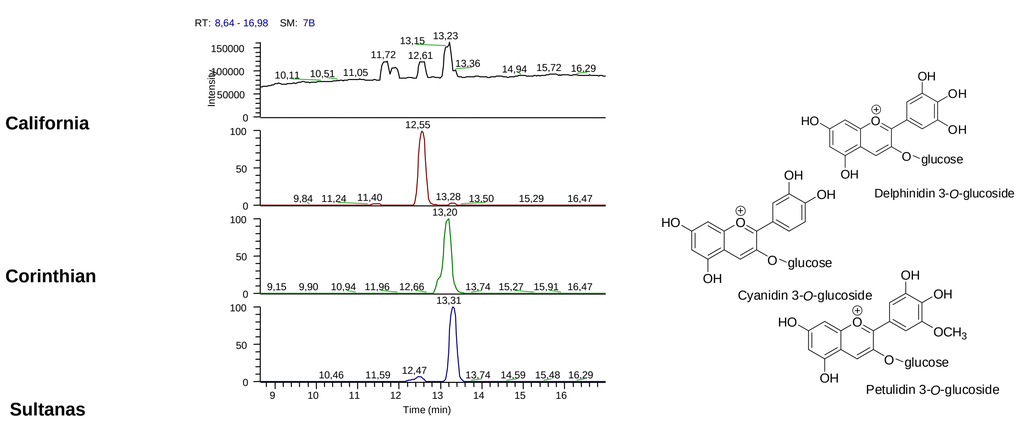
Comparative chromatograms for the three species



D. Analysis of Anthocyanins

The anthocyanin extract of Corinthian currants was also analyzed by the HPLC-LTQ-Orbitrap platform at positive ionization mode. Standard compounds were utilized for the identification of the constituents. The extract was found to contain Delphinidin, Cyanidin and Ferulidin glucosides, as presented in the picture below.

HPLC-HRMS/MS, positive mode- Comparison with standard compounds



C1. Benzoic acids/ Small bicarboxylic acids

Small phenolic acids are absent from the California raisin extract, where *p*-hydroxybenzoic acid was only present. **However, they are major constituents of Corinthian currents extract. Protocatechuic acid glucoside is a major constituent in Sultanas. Succinic and malic acis were only found in Corinthian currants**

Rt 1.27 & 1.48 min Succinic acid, Malic acid

Rt 3.61min

Protocatechuic acid

Rt 3.85min

Protocatechuic acid hexoside

Rt 4.12 min

Vanillic acid hexoside

Rt 6.19min

***p*-hydroxybenzoic acid**

C2. Hydroxycinnamic acids/ catechins

Compounds of this category, such as ferulic and coumaric acids were detected in all extracts, as shown in figure below.

Some peaks of this area remain unidentified, e.g. peaks at Rts 8.70, 8.99 and 9.26 min

Rt 7.52min

Catechin

Rt 8.01min

***p*-coumaric acid hexoside**

Rt 9.03 min

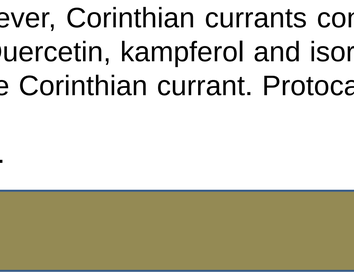
Ferulic acid

Rt 10.94min

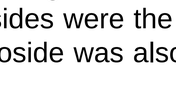
***p*-coumaric acid**

C3. Flavonols

Flavonols were the most abundant class of compounds in the phenolic extracts and several were identified. Different extracts were qualitatively similar, however there were some important quantitative differences in specific flavonols, as shown in the comparative chromatogram below. In several cases the identification was based on the comparison with isolated pure compounds from the chemical library of UoA.

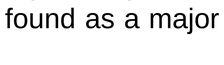


Rt 13.44min Isorhamnetin hexoside



Rt 16.01min Quercetin

Rt 14.00 min



Azelaic acid
Compound produced during stress
-Antimicrobial
- Anti-inflamatory
- Tyrosinase inhibitor

Rt 11.68min

Quercetin 3-*O*-rutinoside

Rt 12.71min

Kaempferol *O*-rutinoside

Rt 12.74 min

Kaempferol hexoside

Rt 12.05min

Quercetin 3-*O*-glucoside

Rt 13.11min

Kaempferol 3-*O*-glucoside

Rt 11.62min

Rutin

Conclusions

- CH_2Cl_2 extracts from all three dried vines were of approximately the same yield. Oleanolic acid was the major component (approx. 20% of the dried extract).
- On the other hand, the yields of the ethanolic extracts differentiated. The black currant yielded almost two times more than the Califonian raisin and three times more than the Sultanas.
- The phytochemical analysis showed that Corinthian currants and Sultanas had the most intense flavonol peaks. However, Corinthian currants contained a bigger range of flavonol structures and higher relative quantity in comparison to the other groups. The California raisins contain the least flavonol structures. Quercetin, kampferol and isorhamnetin glycosides were the major compounds identified. Small phenolic acids and catechin were absent from California raisin, while they are major constituents in the Corinthian currant. Protocatehuic acid glycoside was also found as a major constituent in Sultanas extract.
- Concerning anthocyanins, cyanidin, delphinidin and petulidin glucosides were detected in the Corinthian currant extract.