POLYPHENOLIC PROFILES OF BERRIES FROM DIFFERENT VACCINIUM SPECIES BY LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY

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INTRODUCTION

Vaccinium myrtillus is a spontaneous plant species native to mountain areas of Northern and Central Europe, widely diffused also in Italian Alps and Apennines. The fruit of this species has a large commercial importance, due to its consume, mainly in transformed products, as well as for the preparation of dietary supplements. The interest in this berry species is due to its high content of phenolic compounds, which are plant secondary metabolites, well-known for their health-protecting attributes, as anti-inflammatory, anti-hypertensive, anti-microbial and anti-cancer agents. Accordingly, many researches focusing on the characterization of selected phenolic compounds in *V. myrtillus* berry (i.e. bilberry) from different European countries (e.g. Italy, Slovenia, Serbia, Sweden and Finland) have been published in recent years, evidencing that the most abundant class of polyphenols in these berries are anthocyanins [1,2].
The composition of phenolic compounds of *V. myrtillus* berries has been found different from the one of other *Vaccinium* species – such as the widely commercialized *V. corymbosum* – indicating the potential use of phenolic profile for the chemotaxonomic discrimination of *V. myrtillus* fruits from other cultivated and wild species. This aspect is very important since *V. myrtillus* shows a nutraceutical value higher than that of *V. corymbosum* and the two species are not well-distinguished by consumers. However, to the best of our knowledge, no comprehensive investigation of the polyphenolic profiles of these *Vaccinium* species has been published. Moreover, the presence of a different *Vaccinium* species, namely *V. gaultherioides* (for which no data regarding the primary and secondary metabolic profiles are reported in literature) has been recently observed in the zones traditionally populated by *V. myrtillus*, such as Tuscan Apennines. In this regard, it should be underlined that the phenotype of *V. gaultherioides* berry is very similar to the one of *V. myrtillus* and the two berries can be confused by the harvesters involved in the production chain of transformed bilberry.

AIMS

- To obtain for the first time information concerning the polyphenolic composition of *V. gaultherioides* berries.
- To comparatively evaluate the polyphenolic profiles of *V. gaultherioides* and *V. myrtillus* berries, in relation to their nutraceutical value, as well as for the discrimination of both fresh fruits and transformed products.

FRUIT COLLECTION

Fully ripe *V. myrtillus* and *V. gaultherioides* berries were harvested in August 2014 in fifteen zones of Tuscan Apennines, characterized by different environmental conditions, such as altitude and solar exposure (Table 1). Fully ripe *V. corymbosum* fruits (cv. "Duke", "Berkely" and "Blue Crop") were obtained from a farm located in Cireglio (Tuscan Apennines, Italy).The fruits of each specie were frozen in liquid nitrogen within one hour from the collection and transported to the laboratory where they were freeze-dried and grinded in order to obtain a representative sample of each investigated specie in the growth area of Tuscan Apennines. The samples were finally stored at –20 °C until analyses were performed.

V. myrtillus

V. gaultherioides

V. corymbosum

RESULTS AND DISCUSSION

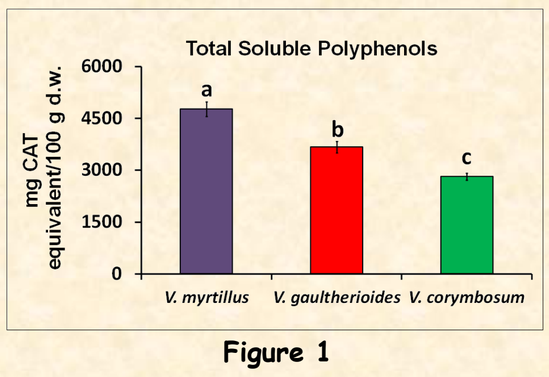

Figure 1

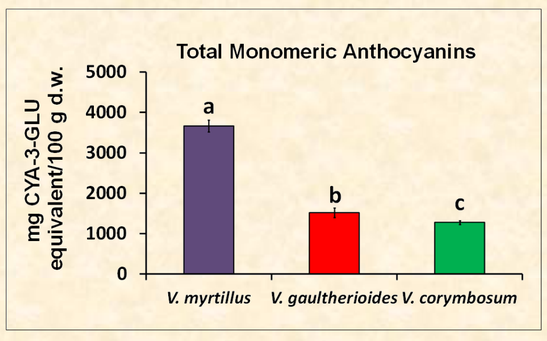

Figure 2

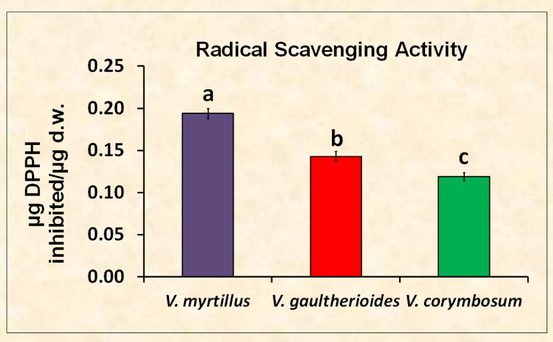

Figure 3

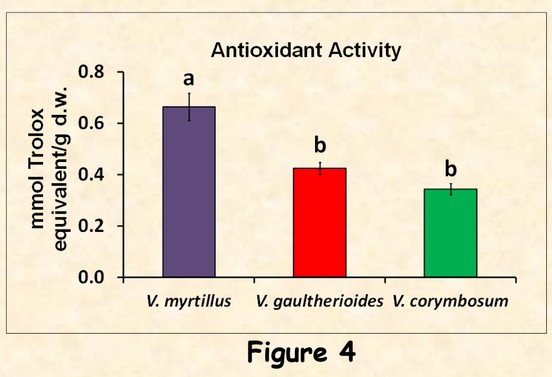

Figure 4

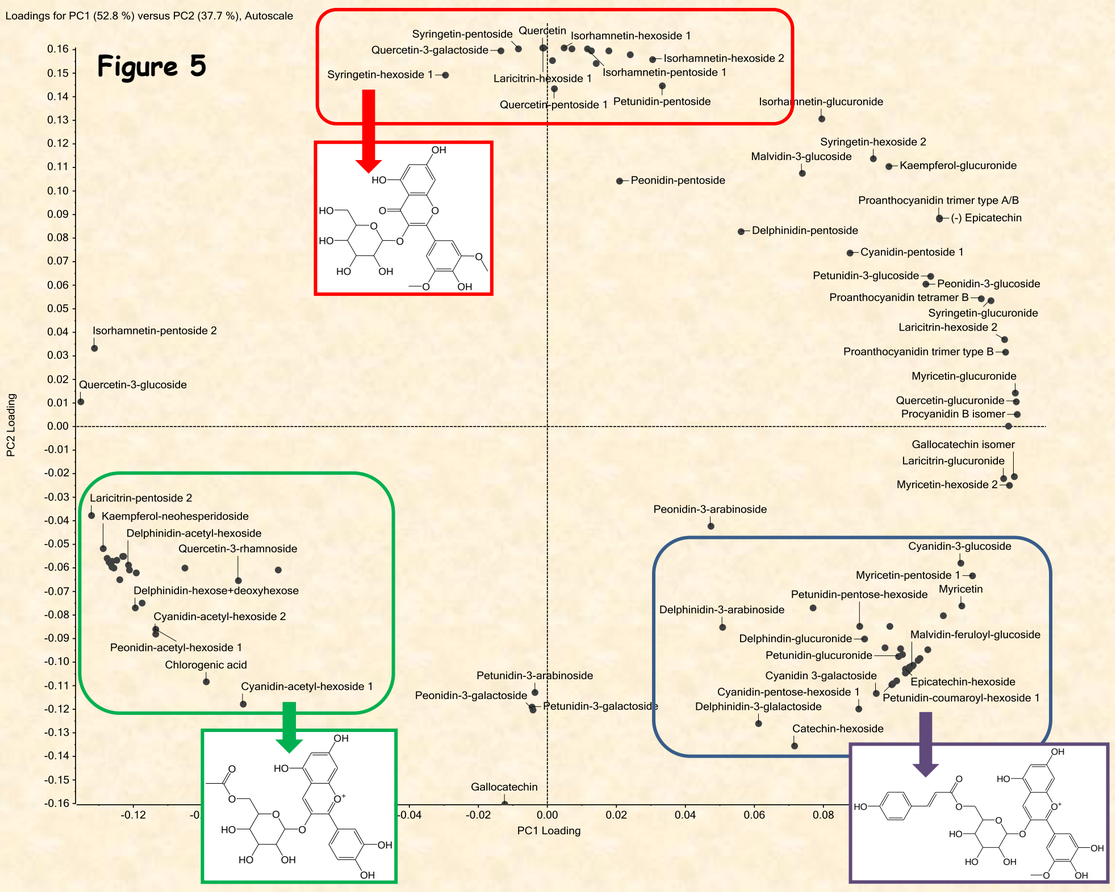

Figure 5

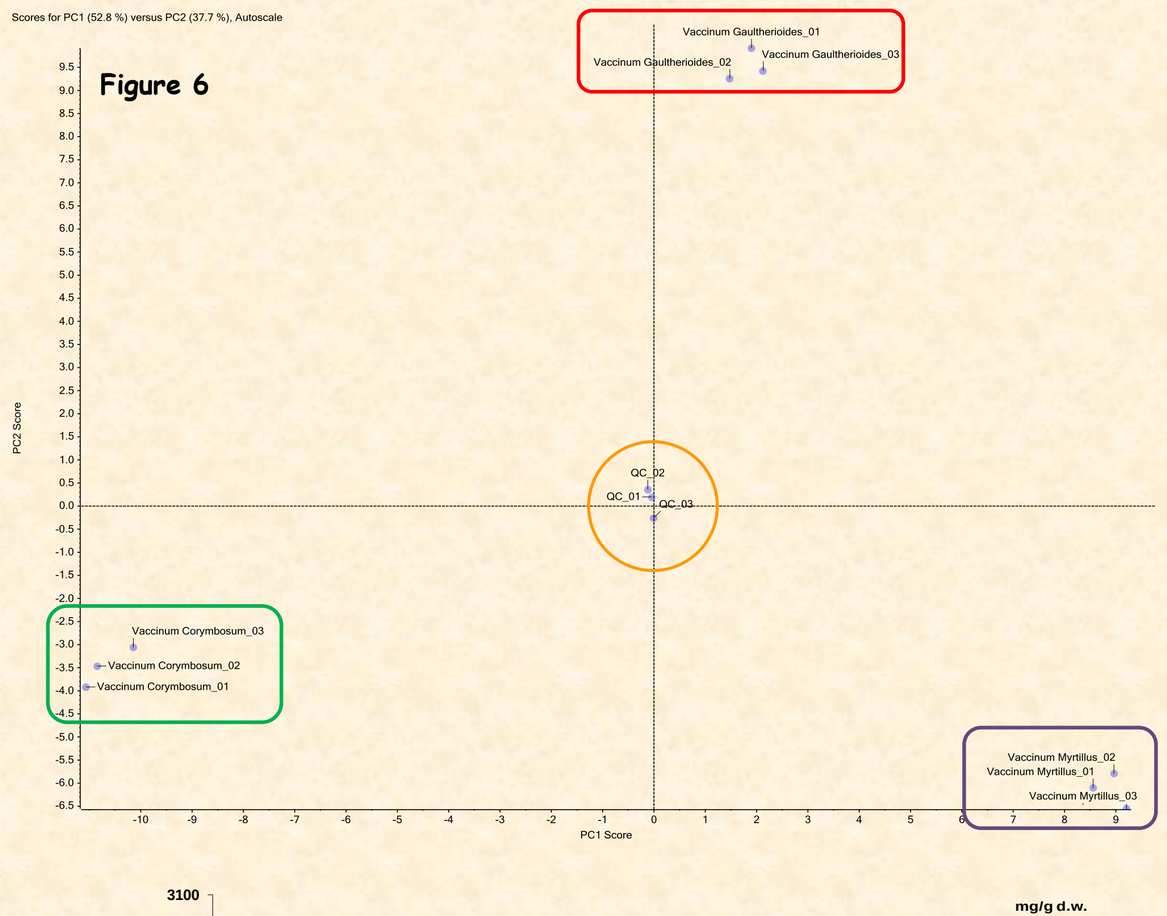

Figure 6

V. myrtillus berries are superior in terms of overall amount of Folin-Ciocalteu total polyphenols, total monomeric anthocyanins (as measured by the pH differential method), as well as antioxidant and antiradical properties (as measured by DPPH and FRAP tests), in respect to *V. gaultherioides* and *V. corymbosum* fruits (Fig. 1-4).

Principal Component Analysis (PCA) of individual polyphenols determined by liquid chromatography coupled with quadrupole-time of flight tandem mass spectrometry, gave rise to two significant PCs, accounting for 90.5% of the original variance of data (Fig. 5-6). Score plot (Fig. 6) evidenced a very good separation of the three investigated species in the PC space. *V. myrtillus* samples were mainly characterized by anthocyanin pentosides, hexosides and coumaroyl-hexosides, whereas, *V. gaultherioides* was associated to pentoside and hexoside derivatives of flavonols, such as Quercetin, Laricitrin, Isorhamnetin and Syringetin; in the *V. corymbosum* samples the distinctive presence of some acetyl derivatives was confirmed (Fig. 6) [3].

The comparison of the concentrations of selected polyphenols found in *V. myrtillus* and *V. gaultherioides* berries evidenced a very different composition. In particular, among the biomolecules investigated in negative ionization, CHL and QUE-3-GAL were the most abundant polyphenols in *V. myrtillus* and *V. gaultherioides* berries, respectively (Fig. 7), whereas in positive ionization the two prevalent anthocyanins were DEL-3-GLU and MAL-3-GLU (Fig. 8).

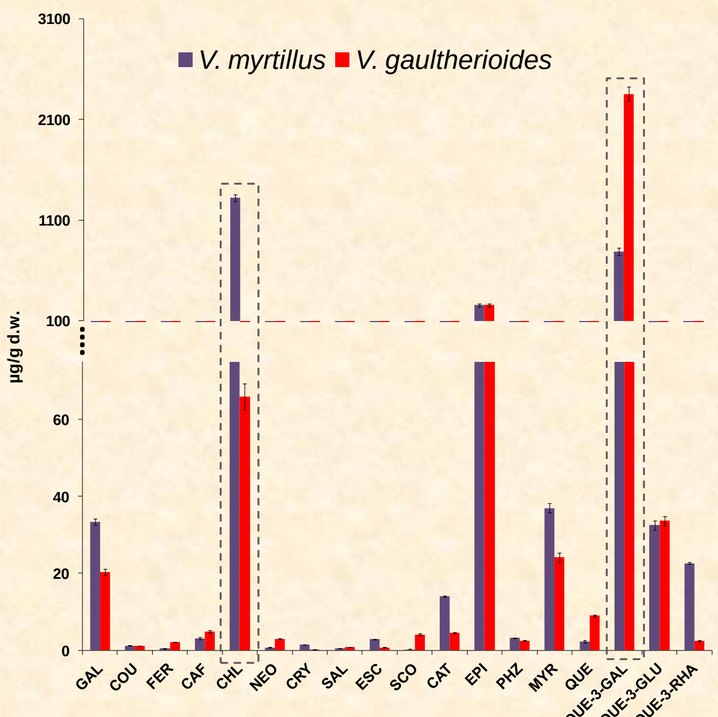

Figure 7

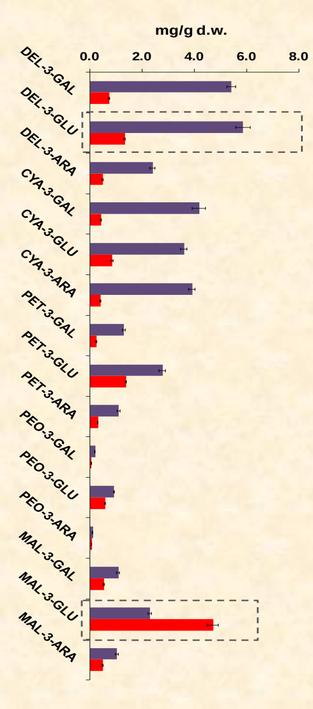

Figure 8

REFERENCES

[1] S. Moze et al, *J. Agric. Food Chem.* 59 (2011) 6998-7004.
[2] G. Giovanelli and S. Buratti, *Food Chem.* 112 (2009) 903-908.
[3] X. Wu and R.L. Prior, *J. Agric. Food Chem.* 53 (2005) 2589-2599.

CONCLUSIONS

V. myrtillus showed polyphenol concentrations and antiradical/antioxidant activities higher than *V. gaultherioides* and *V. corymbosum*. Efforts should be therefore done in order to maintain and if possible to increase the diffusion of this specie in Tuscan Apennines. The three species exhibited very different quali-quantitative profiles of phenolic substances and can be discriminated according to their polyphenolic compositions.

ACKNOWLEDGEMENTS

This research was co-funded by the Fondazione Cassa di Risparmio di Pistoia e Pescia.