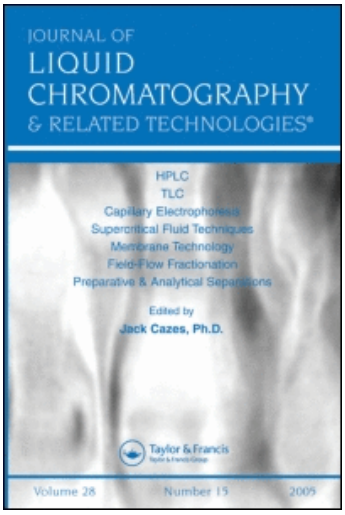

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A NOVEL SOLID-PHASE EXTRACTION-HPLC METHOD FOR THE ANALYSIS OF ANTHOCYANIN AND ORGANIC ACID COMPOSITION OF FINNISH CRANBERRY

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ABSTRACT

Spectrophotometric and liquid chromatographic methods were used to determine anthocyanin and organic acid content and composition of commercial Finnish cranberries (*Vaccinium oxycoccos* L.). Solid-phase adsorbents were used in the fractionation of juice components prior to chromatographic determination. In the freshly prepared cranberry juice the total amount of anthocyanins was 7.7 mg/100 mL juice determined spectrophotometrically at 510 nm. The most abundant individual anthocyanins were cyanidin-3-galactoside (19.2%), cyanidin-3-arabinoside (23.1%), peonidin-3-galactoside (21.5%) and peonidin-3-arabinoside (14.1%), as determined by RP-HPLC.

The total titrable acidity was 3.8g/100g. The main organic acids were citric and malic acids, as determined using an HPLC method. Only a few studies have been conducted on this cranberry species, but more is known about the composition of species *Vaccinium macrocarpon* Ait. However, to our surprise, the anthocyanin composition of the berries used in this study was different from those reported previously.

INTRODUCTION

Cranberry juice has become a popular drink due to its sourness and fresh taste and healthy components. In addition to vitamin C, the anthocyanins and other polyphenols, which are also present in cranberries, have been shown to be useful for human health. In several studies, the antioxidant activity of plant phenolics has been shown, and an inhibition of LDL oxidation by cranberry juice has been demonstrated *in vitro.*[1]

There are several *Vaccinium* species, which have the common name of cranberry. In Scandinavia, the main wild species are *V. oxycoccos* (cranberry) and more rare *V. microcarpum* (small cranberry). *V. oxycoccos* is tetraploid and supposedly formed from two diploidic species, *V. microcarpum* and American cranberry *V., macrocarpon* Ait. The latter is the only cranberry having a substantial economical value. It has also been grown in Finland, but so far with not much success due to too cold a climate. Both wild species grow in swamp and marsh in Finland, and sometimes they are difficult to distinguish from each other due to diverse phenotypes of *V. oxycoccos*. It has been suggested that the two wild species *V. oxycoccos* and *V. microcarpon* have produced sterile alloploidic (hexaploid) hybrids, which can be found in mixed growth areas, but coexisting as fertile hexaploidic plants, also In some areas of Finland, only hexaploidic cranberries have been found.[2] The species and their common nomenclature are still labile.[2,3]

Anthocyanins are responsible for almost all of the red, blue, and purple colors exhibited by flowers, fruits, and other plant tissues. Cranberries and cranberry juice products are popular because of their attractive red color and mildly astringent flavor which is mainly due to quite high concentrations of volatile organic acids. The anthocyanin and organic acid content of *V. macrocarpon* has long been studied,[4-8] while the contents of European species (*V. oxycoccos*, *V. microcarpum*) are not well documented.[9]

The aim of this study was to develop a rapid and convenient analysis protocol for the quantitative determination of organic acids and anthocyanins in berry samples for quality control purposes. In the present study cranberries of the species *V. oxycoccos* were used as an example, and the anthocyanin composition of commercial Finnish cranberries was analyzed with respect to the composition of *V. myrtillus*.

EXPERIMENTAL

Materials and Chemicals

Cranberries (*Vaccinium oxycoccos* L.) and bilberries (*V. myrtillus* L.) were bought from Finnish grocery stores and kept in the refrigerator (+5°C) before analyzing. HPLC-grade acetonitrile and methanol were purchased from

Rathburn Chemicals (Walkerburn, UK). Formic acid was purchased from Riedel-de Haen (Seelze, Germay), and potassium chloride from Sigma Chemicals (St Louis, MO). All the other salts, and concentrated sulfuric acid, were purchased from Merck (Darmstadt, Germany). Solid-phase extraction cartridges were from IST (Hengoed, UK).

Sample Preparation

Fresh cranberries were macerated to the juice which was then filtered through the gauze and through the Whatman no 1 filter paper. This cranberry juice was kept in the refrigerator, protected from light, prior to analysis.

Anthocyanin and Organic Acid Content of the Cranberry Juice

The total anthocyanins were determined by the method of Fuleki and Francis[4] as follows: two dilutions of freshly prepared juice were made by using different buffer solutions (pH 1 and pH 4.5). The absorbances of these samples were measured at 510 nm with LKB Ultrospec 4050 spectrophotometer (Wallac, Turku, Finland). The total amount of anthocyanins was calculated as cyanidine-3-galactoside using distinction of its extinction coefficients.

The total amount of organic acids in the juice was determined as a titriable acidity according to the AOAC method 22.058.

Isolation and Purification of Anthocyanins and Organic Acids from Juice

Anthocyanin fraction of the cranberry juice was separated through the use of a dual adsorbent extraction. This employs a non-polar mechanism (CH sorbent, 100 mg) and an anion-exchange mechanism (SAX sorbent, 100 mg). At first, both adsorbents were eluted with methanol and water (two adsorbent volumes). SAX sorbent was also eluted with a 0.2 M KH_2PO_4-buffer solution. After this, the juice sample (1 mL) was passed through the attached adsorbents (CH sorbent above SAX).

Carbohydrates were eluted with water and the adsorbents were separated. At the final stage, organic acids were eluted from SAX by using 0.5% H_2SO_4 (1 mL) and anthocyanins were eluted from CH by using 1% methanolic H_2SO_4 (2 mL). The eluates were then ready for HPLC-analysis.

High Performance Liquid Chromatography (HPLC) Methods

The anthocyanin eluate (10 μL) from dual SPE extraction was injected into a Shimadzu LC-9A HPLC system using a Rheodyne injector, model # 7125

with a 20 μL sample loop. A Shimadzu SDP-6 UV/VIS-detector (546 nm) was used for monitoring the anthocyanins. The data were recorded on a Merck & Hitachi integrator, model # D 2500. The HPLC-flow rate was 0.9 mL/min and the column utilized in this system was LiChroCart 125-4 Widepore RP-18, 300 Å, 5 μm (Merck, Darmstadt, Germany). The mobile phase consisted of water, acetonitrile and formic acid (81:9:10).

The identification of anthocyanins was obtained by matching their retention times to those of the anthocyanins present in an authentic sample of the bilberry juice. Bilberries were prepared and analyzed like cranberries. The anthocyanins of the bilberry samples were identified based on the previously published results.[10,11]

The organic acids were separated with a 300 x 7.8 mm Coregel 87H, H^+ form organic acid column (Interaction Chromatography, Inc., San Jose, CA, USA) using 0.01 M sulfuric acid at flow rate of 0.7 mL/min as an eluent. Organic acids were identified using pure compounds and quantitated with a spectrophotometric detector at 210 nm. The same instrumentation was used as in the determination of anthocyanins.

RESULTS AND DISCUSSION

The total amount of anthocyanins determined photometrically was 7.7 mg/100 mL juice, which is only 10% of the other *V. oxycoccos* result determined using the same method.[9] However, the anthocyanin content is reported to vary a lot during ripening and storage of the berries and the berry products,[7] and the loss during the storage was also observed in our laboratory (unpublished results). In addition, the chemically close group of compounds, flavonols, have been found during one season in variable concentrations and compositions in cranberries from different parts of Finland.[12]

Shown in Figure 1 is an HPLC-chromatogram of anthocyanins. The compounds representing numbered peaks and their abundances in the cranberry sample are listed in Table 1. The main anthocyanins of this variety of cranberry are cyanidin-3-arabinoside (23%), peonidin-3-galactoside (22%), and cyanidin-3-galactoside (19%).

According to Andersen, the main anthocyanins (80% of total content) of *V. oxycoccos* from Norway were cyanidin- and peonidin-3-glucosides, but also representative 3-galactosides and 3-arabinosides were identified.[9] This pattern differs from the anthocyanidin pattern of species *V. macrocarpon* and its cultivars.[5-7] The study of Hong & Wrolstad[8] confirms and concludes the results obtained previously, i.e., the two major compounds being 3-galactosides of cyanidin and peonidin (33% and 38%, respectively).

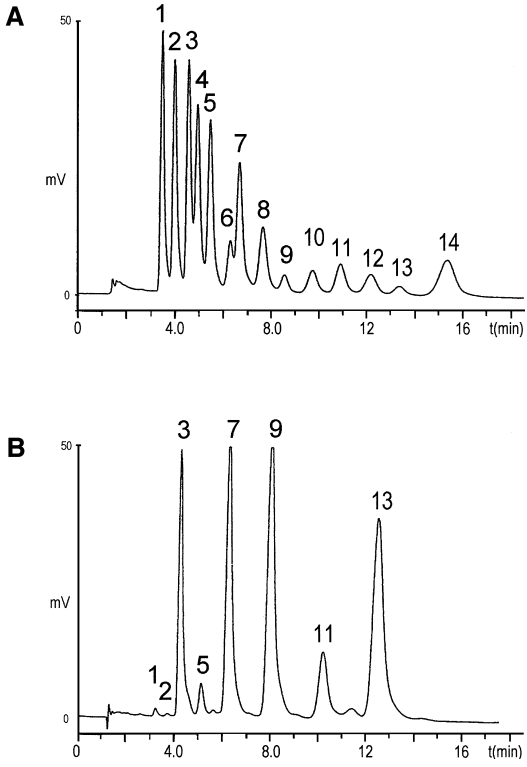

Figure 1. Separation of bilberry (A) and cranberry (B) anthocyanins by HPLC with UV/VIS-detection at 546 nm. Identification of peaks is presented in Table 1. HPLC-conditions: see materials and methods section.

Of the other cyanidin and peonidin derivatives, 3-glucosides were found in a lesser extent than the representative 3-arabinosides. In respect to these results, our variety/species of berries seems to be something between these two species reported earlier. Perhaps the berries analyzed were of hybrid species, but not much can be concluded before berries of different species with biologically sound identification have been analyzed.

The total titrable acidity of the cranberry juice, calculated as anhydrous citric acid, was 3.8 g/ 100 g juice. As compared with *V. macrocarpon*, the titrable acid content of our berry sample was somewhat higher.[6] The main organic acids found in the HPLC chromatograms were citric (0.94g/100 g) and malic acids (0.28 g/100 g). Quinic acid was not found, even though it has been sug-

Table 1

Identified Bilberry Anthocyanins Used as a Reference and the Relative Abundances of Anthocyanins Found in Cranberries*

Peak No.	Anthocyanin	% in Cranberry[a]
1	Delphidin-3-galactoside	2.7
2	Delphidin-3-glucoside	0.9
3	Cyanidin-3-galactoside	19.2
4	Delphidin-3-arabinoside	
5	Cyanidin-3-glucoside	8.6
6	Petunidin-3-galactoside	
7	Cyanidin-3-arabinoside	23.1
8	Petunidin-3-glucoside	
9	Peonidin-3-galactoside	21.5
10	Petunidin-3-arabinoside	
11	Peonindin-3-glucoside	8.6
12	Malvidin-3-galactoside	
13	Peonidin-3-arabinoside	14.1
14	Malvidin-3-glucoside	

* Peak numbering refers to Figure 1. [a] Abundances >0.5% shown.

gested to be responsible for the typical astringent flavor of cranberries, and it has been found to be the main acid in *V. macrocarpon*[6].

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REFERENCES

1. T. Wilson, J. P. Porcari, D. Harbin, Life Sciences, **62**, PL381-PL386 (1998).

2. H. Ahokas, Nordic Journal of Botany, **16**, 185-189 (1996).

3. A. Kurtto, T. Lahti, Pamphl. Bot. Mus. Univ. Helsinki, **11**, 1-163 (1987) (Accessible via internet: www.helsinki.fi/kmus).

4. T. Fuleki, F. J. Francis, J. Food Sci., **33**, 266-275 (1968).

5. A. L. Camire, F. M. Clydesdale, J. Food Sci., **44**, 926-927 (1979).

6. V. Hong, R. E. Wrolstad, J. Assoc. Off. Anal. Chem., **69**, 199-207 (1986).

7. G. M. Sapers, D. L. Hargrave, J. Amer. Soc. Hort. Sci., **112**, 100-104 (1987).

8. V. Hong, R. E. Wrolstad, J. Agric. Food Chem., **38**, 708-715 (1990).

9. ∅. M. Andersen, J. Food Sci., **54**, 383-384, 387 (1989).

10. A. Baj, B. Bombardelli, B. Gabetta, E. M. Martinelli, J. Chromatogr., **279**, 365-372 (1983).

11. J.-P. Goiffon, M. Brun, M.-J. Bourrier, J. Chromatogr., **537**, 101-121 (1991).

12. S. H. Häkkinen, S. O. Kärenlampi, I. M.Heinonen, H. M. Mykkänen, A. R. Törrönen, J. Agric. Food Chem., **47**, 2274-2279 (1999).

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