Material Science and Applied Chemistry
doi: 10.7250/msac.2014.001
2014/30

Bioactive Compounds in Latvian Wild Berry Juice

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Abstract **– Fructose is the dominant monosaccharide in bilberry juice and red bilberry juice, but glucose is dominant in the cranberry juice. Dominant polyphenols are catechine and chlorogenic acid. In the bilberry and red bilberry juices, the most common acids are citric, quinic and tartaric acids, in the cranberry juice – citric, quinic and malic acids. When bilberry juice is heat-treated for 30 min at 98 °C ± 1 °C, HMF content is 2-fold higher than in samples treated at 60 °C ± 1 °C. In solutions from lyophilisates of wild berry juice, turbidity and translucent coloration have been determined.**

Keywords **– bioactive compounds, 5-(hydroxymethyl)furfural, wild berry juice.**

I. Introduction

Inclusion of the organic food in the diet may help to improve health. Latvian wild berries are usually gathered in the least polluted areas – forests and bogs – and can be considered an uncontaminated source of vitamins, various polyphenols, carbohydrates and organic acids. Although the food market is overflowed with a large variety of juices and canned berries, research on healthier products without added preservatives, antioxidants and other synthetic substances continues. The unique properties of wild berries have encouraged scientists all over the world to study their properties and potential use [1–4].

Two major species of cranberry are the American cranberry (*Vaccinium macrocarpon*) spread in the USA and Canada and the European cranberry (V*accinium oxycoccos*), which is widely spread in Latvia. Up to date, most studies have used cultivated American cranberry [1, 5–9] and less attention has been devoted to European cranberry [10-13], and only few studies have examined wild cranberry [13]. The mostly studied compounds that are present in cranberries are flavonols, flavonones, anthocyanins and flavanols [14–19]. In addition, cranberry is an exceptional source of various organic acids [6]. Many cranberry phytochemicals, including various polyphenols and anthocyanins, are absorbed in humans [5], [20–22]. Several of these phytochemicals can improve the human health in case of urogenital and cardiovascular diseases (CVD) [1], [14].

Bilberry is rich in anthocyanins, which are believed to be responsible for most of the effects on health improvement attributed to bilberry [3], [23], [24]. Most well-known effects are related to vision improvement, but some effects on blood glucose lowering and lipid lowering may prove to be beneficial in treatment of diabetes and CVD [25]. Red bilberry is rich in proanthocyanidins and anthocyanins [2], [26], which are absorbed in humans [27]. Red bilberry is best known for its antibacterial and anti-inflammatory properties [26].

In order to obtain various food products (jams, pasteurized drinks), berries are heat-treated. During the processing of berries, 5-(hydroxymethyl)furfural (HMF) may be formed from monosaccharides and organic acids found in the berries. Due to the potential carcinogenic activity of HMF [28–30], its content should be monitored and could serve as an indicator of food product quality. In order to prevent seasonality in berry juice production, it is purposeful to use juice concentrates or rapidly soluble dried concentrates; nevertheless, new techniques to process berries and measurements to monitor the quality of products are needed.

The aim of the study is to quantitatively determine the content of bioactive compounds and HMF in juice and juice lyophilisates from Latvian bilberries (*Vaccinium myrtillus* L.), cranberries (*Oxycoccus palustris* Pers.) and red bilberries (*Vaccinium vitis-idaea* L.).

II. Materials and Methods

Juice samples from wild bilberries (*Vaccinium myrtillus* L.), cranberries (*Oxycoccus palustris* Pers.) and red bilberries (*Vaccinium vitis-idaea* L.) gathered in Latvian forests and bogs were used in the study. Bilberries were harvested in the first part of August, red bilberries in the second part of September in Jelgava region, cranberries in the first part of October 2010 in the bog near Kalnciems. Berries were processed using cold press to obtain juice that was immediately analyzed.

The content of polyphenols, carbohydrates, organic acids, ascorbic acid and HMF were determined by high performance liquid chromatography (HPLC) (Shimadzu LC-20 prominence) using the calibration curve method.

Concentration of carbohydrates in wild berry juice was determined by HPLC as described previously [31]. In brief, calibration was made using standard solutions of fructose, glucose, sucrose and maltose at the following concentrations: 0.05 g L^{-1}, 0.025 g L^{-1} and 0.001 g L^{-1}. Juice samples (10 μL) were injected into the chromatograph and analyzed using acetonitril: water (70:30 volume/volume, %) as a mobile phase by flow rate 1.3 mL min^{-1} and Alltech NH_2 column (4.6 mm x 250 mm), detector RID–10A.

Concentration of phenolic compounds in wild berry juice was determined by HPLC. Calibration was made using standard solutions of gallic acid, catechine, epicatechine, caffeic acid, syringic acid, chlorogenic acid, coumaric acid, sinapic acid, ferulic acid, rutine and 4-hydroxybenzoic acid. Juice samples (10 μL) were injected into the chromatograph and analyzed using methanol:glacial acetic acid (20:80 volume/volume, %) as a mobile phase by flow rate 1.3 mL min^{-1} and a PerkinElmer C18 column (4.6 mm x 250 mm), detector DAD SPD-M20A (278 nm).

Concentrations of organic acids in wild berry juice were determined by HPLC. Calibration was made using standard solutions of oxalic, tartaric, quinic, malic, citric, fumaric, succinic, ascorbic acids. Juice samples (10 μL) were injected into the chromatograph and analyzed using acetonitril:0.05 M KH_2PO_4 (1:99 volume/volume, %) as a mobile phase by flow rate 1.25 mL min^{-1} and Alltech C18 column (4.6 mm x 250 mm), detector DAD SPD-M20A (210 nm).

In addition, for the comparison the concentrations of organic acids were also determined in black currant (Titania), red currant (Laila), quince (Cido), lemon (Eureka) and apple (Antonovka) juices.

Concentration of HMF in wild berry juice was determined by HPLC. Calibration was made using standard solutions of HMF at the following concentrations: 20 mg L^{-1}, 30 mg L^{-1} and 40 mg L^{-1}. Juice samples (15 μL) were injected into the chromatograph and analyzed using acetonitrila: water (10:90 volume/volume, %) as a mobile phase by flow rate 1.3 mL min^{-1} and Alltech C18 column (4.6 mm x 250 mm) connected to a UV/VIS detector (SPD-20A) set at 280 nm.

To obtain lyophilisates, juice samples were freeze-dried using Christ Freeze Dryer Alpha 1-2 LD plus at -50 °C and 6.4 Pa. Solutions were prepared from lyophilisates and used to measure turbidity with WTW photometer MPM 300 (λ = 800 nm) (DIN 27027/ISO 7027) and translucent coloration number with WTW photometer MPM 300 (λ = 445 nm, λ = 520 nm, λ = 620 nm) (EN ISO 7887:1994).

The results are presented as the mean ± standard deviation (SD). Data analysis was performed using in-built analysis of Microsoft Excel 2007.

III. Results and Discussion

The present study was performed to determine the content of polyphenols, carbohydrates, organic acids, ascorbic acid and HMF in juice from wild bilberries, cranberries and red bilberries gathered in Latvian forests and bogs.

Only the monosaccharide glucose and fructose were found in berry juice samples, but no sucrose and maltose were identified. Fructose was the dominant monosaccharide in bilberry and red bilberry juices, but glucose was dominant in the cranberry juice (Table 1).

TABLE 1

Content of Carbohydrates in Wild Berry Juice

Carbohydrate	Bilberry	Cranberry	Red bilberry
Glucose, mg L^{-1}	23.63	33.15	32.41
Fructose, mg L^{-1}	39.20	18.44	36.17

The content of polyphenols in the analyzed juice samples varied in a wide range up to 147.4 mg L^{-1} (Table 2). Dominant polyphenols in bilberry, cranberry and red bilberry juices were catechine (147.4 mg L^{-1}, 130.2 mg L^{-1} and 72.3 mg L^{-1}, respectively) and chlorogenic acid (59.6 mg L^{-1}, 46.7 mg L^{-1} and 11.4 mg L^{-1}, respectively). The content of polyphenols in Latvian cranberry juice is higher than the content of polyphenols found in American cranberry (*Vaccinium macrocarpon*) [1].

TABLE 2

Content of Phenol Compounds in Wild Berry Juice

Phenols	Bilberry	Cranberry	Red bilberry
Catechine, mg L^{-1}	147.4	130.2	72.3
Chlorogenic acid, mg L^{-1}	59.6	46.7	11.4
4-Hydroxybenzoic acid, mg L^{-1}	33.6	12.2	14.6
Gallic acid, mg L^{-1}	2.73	0.61	0.67
Caffeic acid, mg L^{-1}	15.0	35.3	62.8
Syringic acid, mg L^{-1}	1.23	1.10	7.65
Epicatechine, mg L^{-1}	0.57	0.78	0.01
Coumaric acid, mg L^{-1}	0.48	20.4	0.07
Sinapic acid, mg L^{-1}	5.86	2.87	6.58
Ferulic acid, mg L^{-1}	32.10	2.28	2.67
Vaniline, mg L^{-1}	1.16	2.25	0.46
Rutine, mg L^{-1}	30.10	7.36	8.86

To compare wild berry juice to black currant, red currant, quince, lemon and apple juices, the content of the following organic acids was determined: tartaric, quinic, malic, citric, fumaric, succinic acids (Table 3). Dominant acid in the juices, except for the quince and apple juices, was citric acid ranging from 12.83 g L^{-1} (bilberry juice) to 117.3 g L^{-1} (lemon juice). The 3 most common acids in the bilberry and red bilberry juices are citric, quinic and tartaric acids. The 3 most common acids in the Latvian cranberry juice are citric, quinic and malic acids, similar to the American cranberry [6]. Meanwhile, the content of ascorbic acid in cranberry, bilberry and red bilberry juices was 0.580 g L^{-1}, 0.115 g L^{-1} and 0.038 g L^{-1}, respectively.

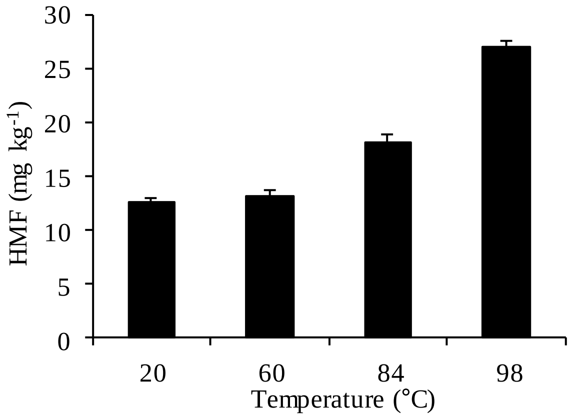

Fig. 1. Content of HMF found after 30 min in Latvian bilberry juice depending on the temperature used to process juice. Results are presented as the mean ± SD.

When bilberry juice was heat-treated for 30 min at 98 °C ± 1 °C, HMF content was 2-fold higher than in the samples treated for 30 min at 60 °C ± 1 °C. No difference in HMF content was found between samples treated at 20 °C ± 1 °C and 60 °C ± 1 °C (Fig. 1). Therefore, if heat treatment is required it is preferable to use 60 °C ± 1 °C instead of 98 °C ± 1 °C.

TABLE 3

CONTENT OF ORGANIC ACIDS IN WILD BERRY AND FRUIT JUICES

Organic acids	Bilberry	Cranberry	Red bilberry	Black currant	Red currant	Quince	Lemon	Apple
Tartaric acid, g L^{-1}	1.94	0.664	1.75	1.54	2.61	0.265	0.095	3.27
Quinic acid, g L^{-1}	4.24	9.39	9.33	0.079	0.956	8.71	0.426	0.710
Malic acid, g L^{-1}	1.43	6.24	0.304	2.56	4.84	33.7	2.37	13.5
Citric acid, g L^{-1}	12.83	24.5	35.5	64.2	27.8	0.248	117.3	0.122
Succinic acid, g L^{-1}	0.535	0.222	0.296	0.512	0.386	0.003	3.64	0.012

The yield for lyophilisates on average was 11% of the original mass. The solutions produced from liophilisates of cranberry, red bilberry and bilberry juices at a concentration of 10 g L^{-1} had pH values of 2.8, 3.0 and 3.2, respectively as determined by using electrode Sen Tix 97T connected to a pH-meter 538 from WTW. Meanwhile, the pH values of cranberry, red bilberry and bilberry juices were 2.5, 2.7 and 3.0, respectively. Together, the pH values of lyophilisates are only slightly higher (less acidic) than pH values for respective juices.

Solutions from lyophilisates of wild berry juice were used to measure turbidity expressed as formasin turbidimetric units (FTU) and to measure translucent coloration number expressed as m^{-1}. Bilberry juice had the highest turbidity, while cranberry juice had the lowest one (Fig. 2). Nevertheless, unlike the drinking water, turbidity of juice is not regulated, and commercially produced juice often contains bits of berries and fruits.

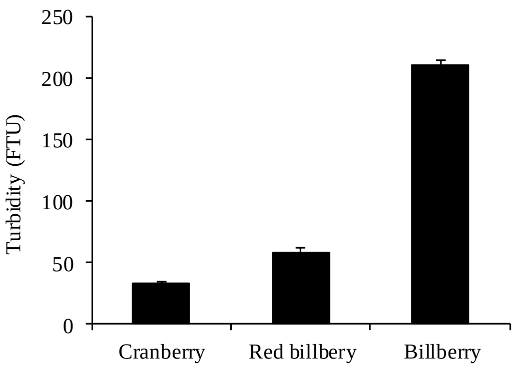

Fig. 2. Turbidity of solutions from lyophilisates of wild berry juice. Results are presented as the mean ± SD.

The main compounds that determine the translucent coloration number of wild berry juice are anthocyanins, but to some extent also organic acids may affect coloration. As can be seen in Fig. 3, the translucent coloration number of the juice tested increases in the following order: red bilberry, cranberry and bilberry. Tested colourful solutions from lyophilisates absorb various wavelengths of visible light in a distinct manner. The least coloration numbers and, therefore, the highest absorption was observed at wavelength λ=620 nm.

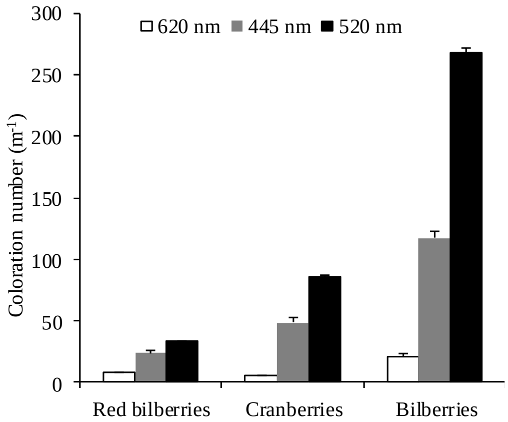

Fig. 3. Translucent coloration number of solutions from lyophilisates of wild berry juice. Results are presented as the mean ± SD.

IV. CONCLUSIONS

Latvian wild berry juice is rich in various bioactive organic compounds. Freeze drying is a valid method to be used during processing of wild berry juices and can be used as an alternative to pasteurization. If heat treatment for juice processing is a must, then temperatures should be kept below 60 °C.

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Ilze Čakste, Māra Kūka, Ingrīda Augšpole, Ingmārs Cinkmanis, Pēteris Kūka. Latvijas savvaļas ogu sulu bioloģiski aktīvie savienojumi

Savvaļas ogu unikālais saturs rosina zinātniekus pētīt to īpašības un potenciālo pielietojumu. Lai no ogām iegūtu dažādus pārtikas produktus, ogas tiek termiski apstrādātas. Šajā procesā no ogās esošajiem monosaharīdiem un organiskajām skābēm var veidoties 5-(hidroksimetil)furfurols (HMF).

Pētījuma mērķis ir bioloģiski aktīvo savienojumu un HMF satura analīze Latvijas melleņu (*Vaccinium myrtillus* L.), dzērveņu (*Oxycoccus palustris* Pers.) un brūkleņu (*Vaccinium vitis-idae* L.) sulās un sulu liofilizātos.

Polifenolu, ogļhidrātu, organisko skābju, askorbīnskābes un HMF saturs noteikts ar augsti efektīvo šķidruma hromatogrāfiju (AEŠH), izmantots hromatogrāfs "Shimadzu LC – 20 Prominence". Salīdzināšanai organisko skābju saturs noteikts arī *Titania* šķirnes upeņu, *Laila* šķirnes jāņogu, *Cido* šķirnes cidoniju, *Antonovka* šķirnes ābolu un *Eureka* šķirnes citronu sulās. Liofilizāti tika iegūti, sasaldētas svaigi spiestas sulas vakuumā izžāvējot "Christ Freeze Dryer Alpha 1-2 LD plus" iekārtā. No liofilizātiem iegūtajiem šķīdumiem noteikta duļķainība un krāsojuma skaitļi.

Dominējošie polifenoli melleņu, dzērveņu un brūkleņu sulās bija katehīns (atbilstoši 147,4 mg L^{-1}; 130,2 mg L^{-1} un 72,3 mg L^{-1}) un hlorogēnskābe (atbilstoši 59,6 mg L^{-1}; 46,7 mg L^{-1} un 11,4 mg L^{-1}).

Tika pētīts un veikts organisko skābju satura salīdzinājums savvaļas ogu un upeņu, jāņogu, cidoniju, citronu un ābolu sulās. Pētītajās sulās, izņemot cidoniju un ābolu sulas, dominējošā skābe bija citronskābe, kuras saturs bija robežās no 12,83 mg L^{-1} (melleņu sula) līdz 117,3 mg L^{-1} (citronu sula).

Askorbīnskābes saturs dzērveņu, melleņu un brūkleņu sulās tika noteikts atbilstoši 0,580 mg L^{-1}; 0,115 mg L^{-1} un 0,038 mg L^{-1}.

Ogļhidrātu masas lielāko daļu mellenēs, dzērvenēs un brūklenēs veidoja glikoze (atbilstoši 23,63 mg L^{-1}; 33,15 mg L^{-1} un 32,41 mg L^{-1}) un fruktoze (atbilstoši 39,20 mg L^{-1}; 18,44 mg L^{-1} un 36,17 mg L^{-1}).

Paaugstinātā temperatūrā melleņu sulā veidojās HMF, pie kam pēc 0,5 h karsēšanas (98 ± 1) °C temperatūrā HMF saturs bija divas reizes lielāks nekā pēc 0,5 h karsēšanas (60 ± 1) °C temperatūrā izturētajos sulu paraugos.

Veiktais pētījums apliecināja, ka Latvijas savvaļas ogu sulas ir bagātas ar dažādām bioloģiski aktīvām organiskām vielām. Sulu liofilizācija ir ieteicams kā maigs apstrādes paņēmiens un šo metodi var lietot kā alternatīvu pasterizācijai.

Илзе Чаксте, Мара Кука, Ингрида Аугшполе, Ингмарс Цинкманис, Петерис Кука. Биологически активные соединения соков дикорастущих ягод Латвии

Уникальный характер дикорастущих ягод способствует исследованию учеными их свойств и возможностей применения. При производстве из ягод пищевых продуктов ягоды подвергают термической обработке. Они содержат моносахариды и органические кислоты, из которых при переработке может получаться образование 5-(гидроксиметил)фурфурола (ГМФ).

Цель исследования – изучение биологически активных соединений и ГМФ в соках и лиофилизатах соков латвийской черники (*Vaccinium myrtillus* L.), клюквы (*Oxycoccus palustris* Pers.) и брусники (*Vaccinium vitis-idae* L.).

Методом высокоэффективной жидкостной хроматографии (ВЭЖХ) на хроматографе Shimadzu LC–20 Prominence исследовано содержане полифенолов, углеводов, органических кислот, аскорбиновой кислоты и ГМФ. Содержание органических кислот определено также в соках чёрной смородины сорта *Титаниа*, красной смородины сорта *Лаила*, цидонии сорта *Цидо*, яблок сорта *Антоновка* и лимонов сорта *Эурека*. Лиофилизаты получены при высушивании в вакууме в приборе «Christ Freeze Dryer Alpha 1-2 LD plus» свежевыжатых замороженных соков. Из лиофилизатов приготовлены растворы и определена их мутность и числа окрашивания.

В изученных образцах содержание полифенолов колебалось в широком интервале. Доминирующими полифенолами в соках черники, клюквы и брусники были катехин (соответственно 147.4 мг $л^{-1}$, 130.2 мг $л^{-1}$ и 72.3 мг $л^{-1}$) и хлорогеновая кислота (cоотвественно 59.6 мг $л^{-1}$, 46.7 мг $л^{-1}$ и 11.4 мг $л^{-1}$).

В соках дикорастущих ягод и в соках чёрной смородины, красной смородины, цидонии, яблок и лимонов исследовано и проведено сравнение содержания органических кислот. В исследуемых соках, кроме цидониевого и яблочного, доминирующей являлась лимонная кислота, содержание которой колебалось в пределах от 12.83 мг $л^{-1}$ (сок чёрной смородины) до 117.3 мг $л^{-1}$ (лимонный сок).

В соках клюквы, черники и брусники установлено содержание аскорбиновой кислоты соответственно 0.580 мг $л^{-1}$, 0.115 мг $л^{-1}$ и 0.038 мг $л^{-1}$.

В соках черники, клюквы и брусники основную массу углеводов создавали глюкоза (соответственно 23.63 мг $л^{-1}$, 33.15 мг $л^{-1}$ и 32.41 мг $л^{-1}$) и фруктоза (соответственно 39.2 мг $л^{-1}$, 18.44 мг $л^{-1}$ и 36.17 мг $л^{-1}$).

При повышенной температуре в соке черники выявлено образование ГМФ, при этом после нагревания сока 0.5 час. при температуре 98 °C ± 1 °C содержание HMF было в два раза выше по сравнению с образцом, выдержанным 0.5 час. при температуре 60 °C ± 1 °C.

Исследования показали, что в Латвии дикорастущие ягоды богаты различными биологически активными органическими веществами. Лиофилизация соков является рекомендуемым способом нежной обработки соков, и этот метод можно применять как альтернативу пастеризации.