

DETERMINATION OF BIOACTIVE COMPOUNDS AND MINERAL SUBSTANCES IN LATVIAN BIRCH AND MAPLE SAPS

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*Birch and maple saps contain carbohydrates and organic acids, B complex vitamins and vitamin C, tannins, flavonoids, glycosides and mineral substances. The aim of the study was to quantitatively determine the concentrations of bioactive compounds and mineral substances in Latvian birch (*Betula pendula* Roth.) and maple (*Acer platanoides* L.) saps. Electrical conductivity was determined (629 and 967 S/cm in birch and maple saps, respectively) to characterise the total amount of mineral substances. In birch and maple saps the titratable acidity (0.50 and 0.70 mmol of NaOH per litre of sap, respectively) and formol number (0.25 and 0.20 mmol NaOH per litre of sap, respectively) were determined. The protein concentration was found to be higher in maple sap (171 and 127 mg/l, respectively). The antioxidant concentration, determined using quercetin as a standard, was 0.35 mg of quercetin equivalents (QE)/l in birch sap and 0.77 mg QE/l in maple sap. In conclusion, Latvian maple sap contains more bioactive and mineral compounds than birch sap. Latvian birch sap contains up to 20% more glucose and fructose than birch sap produced in Finland, but Latvian maple sap contains 10 to 40% less sucrose than sap produced in North America.*

Key words: birch sap, maple sap, bioactive compounds, mineral substances.

INTRODUCTION

Birch and maple saps are rich in carbohydrates, mineral salts, proteins, organic acids including aminoacids. These saps also contain B complex vitamins, vitamin C, phenolic compounds, tannins, flavonoids and glucosides. Birch and maple saps are pleasant and freshening dietary drinks and both are used to produce syrup. Both saps are also known to possess some curative properties. Saps have been shown to be effective in the treatment of stomach ulcer, bronchitis, radicular pain, arthritis, removal of some potentially unwanted substances from the body and stimulation of overall metabolism (Drozdova *et al.*, 1995; Deslauriers, 2000; Jiang, 2001; Sehm, 2007; Perkins and van den Berg, 2009). Extracts of maple sap and syrup have been shown to possess some antiproliferative effects on the colon cancer cells (González-Sarrías *et al.*, 2012).

In most studies birch and maple saps produced from trees growing in Finland and North America, respectively, have been used to characterise chemical composition and properties of the respective sap. In Finland birch sap is used to produce syrup (Kallio *et al.*, 1987; Kallio and Ahtonen, 1987b; Kallio, 1989; Kallio *et al.*, 1987; 1989; 2006). Several studies have described the chemical composition of birch sap: concentration of organic acids (Kallio *et al.*, 1985; Kallio and Ahtonen, 1987a), amino acids (Ahtonen and Kallio, 1989; Patzold and Bruckner, 2005), proteins

(Kallio *et al.*, 1995; Jiang *et al.*, 2001), carbohydrates (Kallio *et al.*, 1985; Kallio and Ahtonen, 1987b), and micro- and macroelements (Harju and Hulden, 1990). Similarly, the chemical composition of maple sap has been described: concentration of mineral substances (Robinson *et al.*, 1989), seasonal changes in amino acid concentration (Kozłowski and Pallardy, 1997), sucrose concentration varied from 0.5–10%, and malic acid was observed to be the most common organic acid (Gaucher *et al.*, 2003; Wong *et al.*, 2003; Perkins and van den Berg, 2009). Various phenolic compounds have been found in maple sap and the concentration of these compounds could reach up to 0.1 mg/l. Dominant compounds are sinapic and p-coumaric acids, and syringic and coniferic acids. In lesser amounts also vanillic, syringic, homovanillic and ferulic acids are present. Maple sap contains a number of flavonoids, like catechins, campherol and its glucosides, quercetine and its glucosides and others (Kermasha *et al.*, 1995; Deslauriers, 2000; Abou-Zaid, 2008; Perkins and van den Berg, 2009). Some studies have tested the antioxidant, antiradical and antimutagenic activities of maple sap and syrup (Therriault *et al.*, 2003; Legault *et al.*, 2010).

Although Latvia is known as a producer and consumer of birch and maple saps, up to date no studies have characterised chemical composition and properties of sap obtained from trees growing in Latvia. Previously we have character-

ised only the total content of phenolic compounds in birch and maple saps from Latvia. It was found that maple sap contains on average by 48% more total phenolic compounds than the birch sap (Kuka *et al.*, 2010). The aim of the present study was quantitative determination of bioactive compounds and mineral substances in birch (*Betula pendula* Roth.) and maple (*Acer platanoides* L.) saps collected from trees grown in Latvia.

MATERIALS AND METHODS

Saps were obtained from birch (*Betula pendula*) and maple (*Acer platanoides*) trees growing in sandy soil away from roads near towns of Ozolpils (56.685507N 23.731600E), Jelgava (56.649137N 23.651331E) and Tukums (56.8553437N 23.0115635E). Saps were collected in the early spring 2010 (end of March maple sap, beginning of April birch sap) during the most intense period of their production (first week of sap production).

Determination of titratable acidity and electrical conductivity. Titratable acidity was determined by potentiometric titration as described previously (Tanner, 1987). In brief, 25 ml of sap was titrated with 0.25 M NaOH solution till pH 8.1, as measured using electrode SenTix 97T for potentiometric titration connected to a pH-meter pH 538 from WTW. Titratable acidity was calculated as NaOH mmol/l of sap. Electrical conductivity was determined to characterise the total content of mineral substances using an electrode TetraCon 325 connected to a conductometer inoLab pH/Cond 720 from WTW.

Determination of content of mineral substances. Concentration of mineral substances was determined by microprocessor photometer MPM 3000 from WTW and commercially available reagent kits from MERCK according to manufacturer's protocol. The concentration of calcium ions was determined according to MERCK method Nr. 1.14815.0001. The concentration of potassium ions was determined according to MERCK method Nr. 14562. The concentration of iron was determined according to MERCK method Nr.14761/2. The concentration of copper (II) ions was determined according to MERCK method Nr. 14767. The concentration of manganese ions was determined according to MERCK method Nr. 14770. The concentration of nickel ions was determined according to MERCK method Nr. 14785. The concentration of ammonium ions was determined according to MERCK method Nr. 1.14752.0001 and the result was calculated as concentration of nitrogen in ammonia. The concentration of sulphate ions was determined according to MERCK method Nr. 1.14791.0001. The concentration of phosphate ions was determined according to MERCK method Nr. 1.14848.0001. The concentration of chloride ions was determined according to MERCK method Nr. 1.14897.0001. The concentration of nitrate ions was determined according to MERCK method Nr. 14556 and the result was calculated as concentration of nitrogen in nitrate ions. The concentration of nitrite ions was determined according to MERCK method Nr.

N4/25 and the result was calculated as a concentration of nitrogen in nitrite ions.

Determination of formol number and protein amount.

The formol number, which characterises the concentration of free amino acids, was determined as described previously (Tanner, 1987). In brief, to neutralise weak organic acids, 0.25 M NaOH solution was added to 25 ml of sap till pH 8.1, as determined by potentiometric titration. Then, formaldehyde solution (35%) was added and the solution was titrated with 0.25 M NaOH solution till pH 8.1 was reached. Results were calculated as the volume of 0.1 M NaOH in ml required to neutralise hydrogen ions liberated from 100 ml of sample solution treated with water solution of formaldehyde.

The protein amount was determined by Lowry procedure (Lowry *et al.*, 1951) using albumin as a standard. Results were calculated as mg of protein per litre of sap.

Determination of carbohydrate content by HPLC. Concentration of carbohydrates in sap was determined by high performance liquid chromatography (HPLC), (Shimadzu LC-20 prominence) as described previously (Kūka, 2008). In brief, calibration was made using standard solutions of fructose, glucose, sucrose and maltose at the following concentrations: 0.05 g/ml, 0.01 g/ml and 0.025 g/ml. Samples of sap (10 µl) were injected into the chromatograph and analysed using CH₃CN:H₂O (70:30 volume/volume, %) as a mobile phase by flow rate 1.3 ml/min.

Determination of ascorbic acid and antioxidant content.

The ascorbic acid concentration was determined using iodometry. 50 ml of sap were titrated with 0.01 M iodine solution in the presence of starch as an indicator. The results were calculated as mg of ascorbic acid per litre of sap.

The antioxidant concentration was determined with 2,2-diphenyl-1-picrylhydrazyl (0.02 mg/ml) measuring absorbance at 517 nm using UV/VIS spectrophotometer Jenway UV 6405. Quercetin was used to produce the standard curve (concentrations 0.5–4.0 µg/ml). The antioxidant content was expressed as quercetin equivalents (QE) – mg/l of sap (Theriault *et al.*, 2006).

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

RESULTS

Titratable acidity and electrical conductivity. Titratable acidity was determined using birch (Ozolpils) and maple sap (Ozolpils) samples obtained in the first week of sap production. Average titratable acidity in the maple sap samples was 40% higher than in the birch sap samples (Table 1). Electrical conductivity was determined in birch (Ozolpils, Jelgava) and maple sap (Ozolpils, Tukums) samples. The electrical conductivity in maple sap was higher than in birch

Table 1

VARIOUS PARAMETERS OF BIRCH (*Betula pendula* Roth.) AND MAPLE (*Acer platanoides* L.) SAPS

Sap	Titrateable acidity, NaOH mmol/l	Formol number, 0.1 M NaOH ml/100 ml	Protein, mg/l	Ascorbic acid, mg/l	Antioxidant content QE, mg/l
Birch	0.50 ± 0.01	0.25 ± 0.01	127 ± 2	3.2 ± 0.01	0.35 ± 0.03
Maple	0.70 ± 0.02	0.20 ± 0.01	171 ± 8	8.8 ± 0.03	0.77 ± 0.09

Data are presented as mean ± SD.

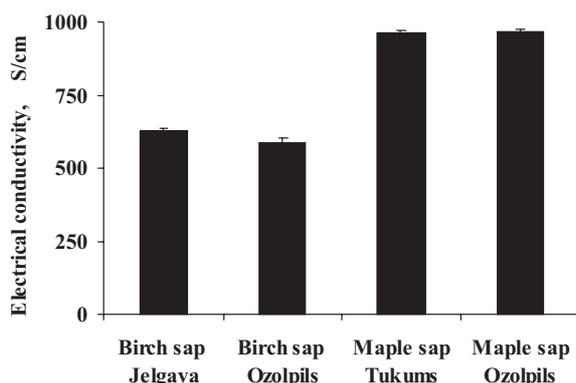


Fig.1. Electrical conductivity of birch (*Betula pendula* Roth.) and maple (*Acer platanoides* L.) saps. Data are presented as mean ± SD.

sap on average by 58% (Fig. 1), indicating greater concentration of strong electrolytes in maple sap samples.

Content of mineral substances. The predominant macroelements in the birch and maple saps were found to be Ca^{2+} and K^+ ions and the prevailing trace elements were Mn^{2+} ions, while the dominant anions were sulphate ions (Table 2). Nitrate and nitrite concentrations in both birch and maple sap samples were negligible.

Formol number and protein amount. The formol number, which characterizes the concentration of free amino acids, was determined in birch (Ozolpils) and maple sap (Ozolpils) samples. The concentration of free amino acids was similar and negligible in both birch and maple sap samples (Table 1). The concentration of proteins was by 34.6% higher in maple sap than in birch sap (Table 1).

Carbohydrate content. Carbohydrate concentration was determined using HPLC in birch (Ozolpils) and maple sap (Ozolpils) samples. The monosaccharides glucose and fructose and disaccharide sucrose were identified in sap samples. The concentration of sucrose in the maple sap was 31-fold higher than in birch sap, while the concentration of fructose and glucose was 10-fold and 3-fold higher, respectively, in birch sap than in maple sap (Table 3). Other carbohydrates were below the identification threshold.

Content of ascorbic acid and total content of antioxidants. Concentration of ascorbic acid was determined using birch (Ozolpils) and maple sap (Ozolpils) samples. Concen-

Table 2

CONCENTRATION OF MINERAL SUBSTANCES IN BIRCH (*Betula pendula* Roth.) AND MAPLE (*Acer platanoides* L.) SAPS

Mineral substances	Birch sap, mg/l		Maple sap, mg/l	
	Jelgava	Ozolpils	Tukums	Ozolpils
Ca^{2+}	53.3 ± 0.1	41 ± 1	61 ± 1	91 ± 1
K^+	41.1 ± 0.5	66.4 ± 0.1	70.1 ± 0.2	78.9 ± 0.1
Mn^{2+}	0.5	0.520 ± 0.006	-	0.24 ± 0.01
Ni^{2+}	-	0.03 ± 0	0.15 ± 0.02	0.13 ± 0
Cu^{2+}	0.04	-	-	-
Fe^{3+}	0.10	-	-	-
NH_4^+ -N	0.050 ± 0.001	-	0.080 ± 0.001	-
SO_4^{2-}	36.10 ± 0.06	34.0 ± 0.1	39 ± 1	62.6 ± 0.5
PO_4^{3-} -P	2.06 ± 0.01	2.36 ± 0.02	4.13 ± 0.09	-
Cl^-	-	9.0 ± 0.1	9.0 ± 0.1	12.0 ± 0.1
NO_3^- -N	3.40 ± 0.01	3.40 ± 0.01	3.40 ± 0.01	3.40 ± 0.01
NO_2^- -N	0.008 ± 0	0.012 ± 0	-	0.115 ± 0

Data are presented as mean ± SD.

Table 3

CONCENTRATION OF CARBOHYDRATES IN BIRCH (*Betula pendula* Roth.) AND MAPLE (*Acer platanoides* L.) SAPS

Sap	Fructose, g/l	Glucose, g/l	Sucrose, g/l
Birch	5.39 ± 0.05	4.46 ± 0.04	0.58 ± 0.01
Maple	0.55 ± 0.01	1.33 ± 0.03	17.95 ± 0.05

Data are presented as mean ± SD.

tration of ascorbic acid in maple sap was by 56% higher than in birch sap (Table 1).

Total concentration of antioxidants was characterised using quercetin equivalents and the obtained results show that maple sap samples contained around two times higher concentration of compounds with antioxidant properties than in birch sap samples (Table 1).

DISCUSSION

The present study quantitatively determined the concentration of bioactive compounds and mineral substances in saps obtained from birch (*Betula pendula*) and maple (*Acer platanoides*) trees growing in Latvia. The obtained results indicate that Latvian maple sap contains significantly more bioactive and mineral compounds than birch sap. The concentration of mineral substances in sap obtained from birch (*Betula pendula*) trees reflects the chemical composition of the soil where trees grow (Harju and Hulden, 1990). In addition, our results indicate that a similar tendency can be observed in sap obtained from maple (*Acer platanoides*) trees (Table 2). Overall, the concentration of mineral substances was higher in maple than in the birch sap (Table 2). These findings were in compliance with the results of electrical conductivity measurements (Fig. 1), which showed that maple sap contains higher concentrations of strong electrolytes

and thus the concentration of mineral substances must be higher. It should be noted that the concentration of mineral substances much varies in maple sap and increases during the period of sap production (Robinson *et al.*, 1989; Perkins and van den Berg, 2009). Dominant mineral substances are calcium and potassium. Maple sap is also rich in magnesium and manganese, while only small amounts of zinc, copper, iron, sodium and aluminium can be found. Birch sap mainly contains salts of potassium, calcium, magnesium, manganese and zinc (Harju and Hulden, 1990).

Although the overall concentration of bioactive compounds was higher in maple sap, the concentrations of free amino acids were similar and both birch and maple saps contained very low concentration of free amino acids (Table 1). This could be explained by the use of the first-week saps for the study. It has been shown that the concentration of free amino acids increases during the birch sap production period (up to 100–500 mg/l) and that the most prevalent amino acids are glutamine, citrulline and glutamic acid (Ahtonen and Kallio, 1989; Jiang *et al.*, 2001). In both birch and maple saps, the concentration of proteins increases in the last stage of the season (Kallio *et al.*, 1995; Kozłowski and Pallardy, 1997; Jiang *et al.*, 2001). Similarly, the total content of all organic acids found in the saps increases during the production period and the predominant acid in both birch and maple saps is malic acid (Kallio *et al.*, 1985; Kallio and Ahtonen, 1987a; Perkins and van den Berg, 2009). Glucose, fructose and sucrose were the only carbohydrates identified in the sap samples (Table 3). Although birch and maple saps contain other carbohydrates, their content is negligible and they were not quantified. It has been shown that carbohydrate content does not depend on species or variety of the tree and the amount of carbohydrates in birch sap decreases in the following order: glucose, fructose, sucrose and galactose (Kallio and Ahtonen, 1987b). Sucrose is the dominant carbohydrate in the maple sap, while other carbohydrates like glucose and fructose are in small quantities, but the concentrations of stachyose, raffinose and xylose are negligible (Wong *et al.*, 2003; Perkins and van den Berg, 2009). Latvian birch sap contains up to 20% more glucose and fructose (Table 3) than birch sap produced in Finland (Kallio and Ahtonen, 1987b), but Latvian maple sap contains up to 10 to 40% less sucrose (Table 3) than sap produced in North America (Kozłowski and Pallardy, 1997).

In conclusion, our results demonstrate that the overall content of Latvian maple (*Acer platanoides*) sap is richer in bioactive substances and mineral salts as compared to birch (*Betula pendula*) sap. The dominant carbohydrates in birch sap are glucose and fructose, while the sucrose is predominant in maple sap.

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BIOĻĢISKI AKTĪVO SAVIENOJUMU UN MINERĀLVIELU NOTEIKŠANA BĒRZU UN KĻAVU SULĀS

Bērzu un kļavu sulas satur ogļhidrātus un organiskās skābes, B grupas vitamīnus un C vitamīnu, flavonoīdus, glikozīdus un minerālvielas. Pētījuma mērķis – noteikt bioloģiski aktīvo savienojumu un minerālvielu saturu Latvijas bērzu (*Betula pendula* Roth.) un kļavu (*Acer platanoides*) sulās. Lai raksturotu minerālvielu kopējo daudzumu, tika mērīta elektrovadītspēja (bērzu un kļavu sulās atbilstoši 629 un 967 $\mu\text{S}/\text{cm}$). Minerālvielu saturs tika noteikts spektrofotometriski. Bērzu un kļavu sulās tika noteikti kopējais skābums (atbilstoši 0,50 un 0,70 mmol NaOH uz litru sulas) un formaldehīdskaitļi (atbilstoši 0,25 and 0,20 mmol NaOH uz litru sulas). Kļavu sula satur vairāk proteīnus nekā bērzu sula (atbilstoši 171 and 127 mg/l). Fruktozes, glikozes un saharozes saturs tika noteikts, izmantojot augstefektīvu šķidrums hromatogrāfiju. Askorbīnskābes saturs tika noteikts jodometriski. Kā standartvielu izmantojot kvercetinū, tika raksturots antioksidantu saturs un bērzu sulā tas bija 0,35 μg kvercetinā ekvivalentu (QE)/l, bet kļavu sulā 0,77 μg QE/l. Kopumā Latvijas kļavu sula satur vairāk bioloģiski aktīvo savienojumu un minerālvielu nekā bērzu sula.