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The Bioactive and Mineral Compounds in Birch Sap Collected in Different Types of Habitats

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Grabek-Lejko, D.[*], Kasprzyk, I., Zagula, G. and Puchalski, Cz. 2017. The Bioactive and Mineral Compounds in Birch Sap Collected in Different Types of Habitats. *Baltic Forestry* 23(1).

Abstract

Birch sap is used as a traditional drink and in traditional medicine in many countries in the northern hemisphere. However, there are scarce data on the antioxidant properties, nutrients and the mineral content of birch saps. In this study, the above-mentioned properties were analyzed in two different birch species *Betula pendula* Roth (silver birch) and *Betula pubescens* Ehrh. (downy birch) in various areas (suburban, traffic and industrial). The current study evidenced significant differences between the antioxidant, nutrition and mineral content depending on the type of habitat, not on species. It was shown that higher antioxidant properties, sugar and protein content were detected for silver birch sap from industrial area, which may be due to the response of plants to environmental stress. Moreover, heavy metals presenting in soil were not detected or detected at low concentrations in the sap. Birch sap can be used as a valuable natural beverage, which is especially important nowadays, when there is a pressure to minimize the use synthetic and artificial food ingredients.

Keywords: Antioxidants, *Betula pendula*, *Betula pubescens*, birch sap, minerals, nutrients, heavy metals.

Introduction

In the olden days, the sap of spring trees was frequently used in traditional medicine in forested areas of northern hemisphere. In Europe, the main source of tree sap was birches: *Betula pendula* Roth. (silver birch), *Betula pubescens* Ehrh. (downy birch) (Jones 2011, Svanberg et al. 2012). Birch sap was known as valuable antidote against scurvy, kidney, stomach and liver diseases, for gall stones, skin diseases and as a nutritional drink. It was used also in veterinary and as a cosmetic product – hair conditioner. Nowadays, the birch sap has become more and more popular as natural probiotic, after fermentation (Semjonovs et al. 2014). Its tapping is carried out in Russia, Belarus, Ukraine, Latvia, Estonia and also in Korea, Japan and China at large scales (Svanberg et al. 2012, Kūka et al. 2013). The best time for the birch sap harvesting depends on geographical location, climate and weather course. In central Europe it is late March and early April, with later start toward the north. Generally, birch tree can secrete sap from two to four weeks and its yield depends on birch species (Kallio and Ahtonen 1989, Jiang et al. 2001, Peev et al. 2010). Birch sap is a transparent or a slightly opalescent fluid. It tastes similar to water and is slightly sweetish (Peev et al. 2010). It contains many bioactive compounds. The total amino acid concentration ranges from 100–500 $mg \cdot L^{-1}$. Among free amino acids glutamine, citrulline, glutamic acid, isoleucine, valine and asparagine are the most often detected. They represent 92–96 % of the total amino acid content. Their concentrations change during flow season (Kallio et al. 1985, Kallio and Ahtonen 1989). In Japan, the maximum of the total amino acid content of silver birch was above 50 $mg \cdot L^{-1}$ and reached this value at the end of the flow season (Jiang et al. 2001, Jeong et al. 2012).

The total sugar content oscillates from 1% in Finland and 2.5-2.6% in Poland. The dominant carbohydrates are glucose and fructose. Their concentrations range between 2-5 g L^{-1} in equal proportions each of them representing over 80% of total sugar. The content of sucrose is three to ten time less than fructose or glucose content (Kallio et al. 1985, Kallio and Ahtonen 1987, Kūka et al. 2013, Łuczaj et al. 2014).

Birch sap also contains valuable minerals. Calcium and potassium occur in the highest concentrations. In Latvian silver birch sap, the mean content of Ca ranged from

41 to 150 mg L^{-1} and K from 41 to 142 mg L^{-1} (Kūka et al. 2013, Vincēviča-Gaile 2014). In many samples, Zn, Mg, Mn, Cu, Cd, Fe and Na were detected (Jeong et al. 2012, Kūka et al. 2013, Vincēviča-Gaile 2014, Bilek et al. 2015a). In Polish samples, the contents of these minerals were lower and the variability between individual trees was much higher (Bilek et al. 2015a).

The problem of the chemical composition of tree sap was frequently investigated in the 80s and 90s of the 20th century. Much less attention has been paid to the bioactive properties (Klinger et al. 1989). Lee et al. (2009) observed a weak inhibitory effect on microbial growth, as well as phagocytosis-influencing, antiphlogistic and antipyretic activities. The question of impact of the type of habitat on the nutrient and mineral content was also very rarely investigated (Vincēviča-Gaile 2014). Due to the increasing use of birch sap, an exact knowledge of its properties seems to be justified.

This research was aimed to evaluate antioxidant properties, nutrient and mineral content of fresh sap of two birch species *B. pendula* Roth. and *B. pubescens* Ehrh. The goal of this study was to verify the preliminary hypothesis that the type of habitats (like soils with heavy metals content) and birch species affect the abovementioned properties. The purpose of this study was also to evaluate whether birch sap, so popular lately, can be a valuable natural beverage.

Materials and Methods

Sample collection

The study was conducted in three sites that differed in terms of the type of habitats: industrial area (steel mill, Ostrowiec), high traffic area (Rzeszów) and suburban area (Zalesie). Birch sap was collected from 4-5 randomly chosen individuals of *B. pendula* and *B. pubescens*. Soil samples were taken from one soil layer (0-20 cm) close the trees from which sap were harvested. The sampling was carried out at the end of March and the beginning of April 2015. At a height of 30 cm of the trunk, small 8 mm diameter holes were drilled, then, a plastic pipe (15 cm long and 8 mm in diameter) was put into the hole. Underneath each pipe, a sterile plastic tube was placed (volume of 50 mL). The tubes were closed after collecting the sap and then filtered with 0.45 µm filters and frozen at -80 °C till analysis.

Chemical analysis

Antioxidant activities

Antioxidant activities were determined by two methods: FRAP and ABTS. For ABTS determination, method of Re et al. (1996) was used. The results were expressed as µmoles of Trolox per 1L of birch sap.

A manual assay of ferric reducing/antioxidant power (FRAP) was used based upon the methodology of Benzie and Strain (1999). A standard Trolox solution was used for the calibration curve and the results were expressed as µmoles of Trolox equivalent per 1L of birch sap.

Total polyphenol content was measured using the Folin-Ciocalteu colorimetric method (Singleton and Rossi 1965). The results were expressed as mg of gallic acid equivalents (GAE) per 1L of birch sap.

Microbiological assay with *Enterococcus hirae* ATCC™ 8043 was used for folic acid determination according to Difco™ & BBL™ Manual, 2nd Edition. *E. hirae* was added to the medium used for this analysis (Folic Acid Assay Medium - FAAM). *E. hirae* cannot grow on FAAM without addition of external folic acid. The addition of folic acid in specified increasing concentrations gives a growth response that can be measured turbidimetrically. Briefly, night culture of *E. hirae* (previously washed tree times in order to remove all residues of folic acid from the medium) was used for inoculation of FAAM medium with increasing concentrations of folic acid (0-10 ng/10 ml for calibration curve) or with different concentrations of analyzed birch saps. After 24 h incubation at 37 °C, bacterial growth was determined turbidimetrically at OD 660 nm. Concentration of folic acid was calculated from the calibration curve.

Nutrients determination

Sugars (glucose, fructose, sucrose) were determined reflectometrically according to the appropriate manuals of Merck Reflectoquant®**.** Results were expressed as g of sugar per 1L of birch sap. Proteins were determined according to the Lowry method (Lowry et al. 1951). For the calibration curve, bovine serum albumin was used. Results were expressed as mg of protein in 1L of birch sap. Total calories were calculated as a sum of calories from sugars and proteins, where proteins possess 4 kcal g^{-1} and sugars 3.8 kcal g^{-1}.

Minerals detection

Mineral elements were analyzed by an inducted coupled plasma optical emission spectrometer (ICP-OES), ThermoiCAP Dual 6500 (USA). Each time, the dilution of 1 mL volume of birch sap filled up the tube to 10 mL with deionised water was performed. For each of the elements, 3-point calibration curves were created. Selection of appropriate length measuring line has been validated by method of standard additions in amount of 10 ppb to 100 ppb give the recovery on selected lines above 98.5% for each of the elements.

Soil samples were heated at 100 °C till constant weight. Then 0.2 g of soil samples were filled up with 6 mL of 40 % HCl + 2 mL of HNO_3. Microwave mineralization procedure with Milstone Ethos One Microwave

Digestion System was used. After mineralization, samples were filled up to 50 mL with demi water and used for mineral elements detection. Mineral elements were analyzed with the aid of inducted coupled plasma optical emission spectrometer (ICP-OES), ThermoiCAP Dual 6500 (USA). Results were expressed as g per kg of dry soil sample.

Data analysis

The comparison of the means of the analyzed parameters of two species was tested by the parametric Student's T-test. ANOVA or Kruskall-Wallis test (nonparametric) with appropriate post-hoc tests (Tukeya or Dunna respectively) were applied to compare the means from three sites. The type of statistical test was chosen after analysis of the data distribution using Shapiro-Wilk test. The statistical hypotheses were tested with $\alpha \leq 0.05$.

Results

Antioxidant activities

The *in vitro* antioxidant effect of the investigated extracts was evaluated by the ABTS assay as a capability of ABTS·+ - compound possessing and antiradical activity to scavenge free radicals and by the FRAP assay as a capability of antioxidants to reduce Fe(III) to Fe(II) (Liaudanskas et al. 2014). The results obtained from the study of the antioxidant properties are given in Table 1. It was shown that birch saps from Ostrowiec contained the highest levels of antioxidants in comparison with saps from Zalesie and Rzeszow. Antioxidant activities were higher by about 45-80% for the FRAP and 54-64% for the ABTS methods. Analysis of the variance allowed us to prove that there are statistically significant differences in antioxidant activities between different localities of silver birch trees. Antioxidant properties detected by FRAP and ABTS methods are different between saps from the industrial area (Ostrowiec) and the traffic area (Rzeszow), and also between the industrial region (Ostrowiec) and the suburban region (Zalesie). There are slightly but not significant differences in antioxidant activities between saps from Zalesie and Rzeszów (Table 1). Antioxidant properties were higher for silver birch sap, but the differences were not statistically significant.

Phenolic compounds

The total phenolic content of the birch saps was estimated by using the Folin-Ciocalteu reagent. Table 1 summarizes that average concentration of total phenolic compounds in birch saps varied widely ranging from 35.41 mg GAE·L^{-1} for silver birch from Zalesie to 55.15 mg GAE·L^{-1} for silver birch sap from Ostrowiec. When comparing the concentration of phenolic compounds depending on the locality, where sap was taken, there have been revealed statistically significant differences between the industrial region – Ostrowiec and the suburban region – Zalesie, which are in correlation with antioxidant activities (Table 1). Authors did not detect statistically significant differences in phenolic compounds concentration between the birch species.

Folic acid concentration

Folic acid is one of the vitamins, which also possess antioxidant activities. The highest values were obtained from silver birch sap from Ostrowiec, and values subsequently decreased in the following order: silver birch – Zalesie < silver birch – Rzeszow < downy birch (Table 1).

Nutrients

Among sugars, which were analyzed, the lowest glucose and fructose concentrations were detected for downy birch sap and the highest ones for silver birch sap from Ostrowiec. Differences between glucose concentrations are from 36% to 55%, and for fructose concentrations from 24% to 60%. The concentration of sucrose is also the lowest for the downy birch sap. Saps from silver birch trees localized in Rzeszow and Ostrowiec were the same in terms of the sucrose content and the highest sucrose concentration was detected for sap from Zalesie. However, it is worth to state that the standard deviation in these samples is very high. On the other hand, the highest protein concentration was detected for silver birch sap but differences between the protein concentrations are quite low, around 6 – 12% (Table 1). Comparing the saps of the two birch species, it can be stated that statistically significant differences were detected only in the content of glucose and sucrose (Student's *T*-test; $p = 0.0014$; Table 1). Birch sap is a low-calorie diet beverage. Calorie content of silver birch sap from suburban and industrial sites was in the range from 29.95 to 47.68 kcal L^{-1}, respectively, and the average calorie content for downy birch sap is 39.45 kcal·L^{-1}.

Minerals in the birch sap

Of all the investigated minerals, calcium presented the highest content especially in the traffic area, where its concentration was significantly higher than at the other sites (above 75%). In the industrial and suburban areas, its concentrations were similar oscillating around 165 mg per litre. Also, a quite high potassium content was noted. Its concentrations ranged on average from 100 to 180 mg L^{-1}. Authors stated significant differences in the potassium concentration at sites with the lowest content in the suburban area. Concentrations of manganese and zinc in silver birch sap were similar and on average ranged from 2 to 4 mg L^{-1}. It was stated that concentrations of certain microelements depended on the habitats. Mean concentration of sodium was the highest in the industrial area, and of iron and phosphorus in the traffic one. Copper concen-

trations differed at each of the habitats (Table 2). Silver and downy birches differed in phosphorus and iron content, which were higher for the latter species (Student's *T*-test; 0.046, $p = 0.0049$ respectively; Table 2). In downy birch sap, sodium was not detected. Concentrations of cadmium, chromium, lead, nickel and aluminium were below their levels of determination.

Table 1. Mean concentrations of chosen parameters of antioxidants and nutrients for Zalesie (Z), Rzeszów ($R_{a-B.\ pendula}$, $R_{b-B.\ pubescens}$) and Ostrowiec (O)

Species	Sites	FRAP Trolox (μmoles·L^{-1})	ABTS Trolox (μmoles·L^{-1})	Phenolic compounds Gallic acid (mg·L^{-1})	Folic acid (μg·L^{-1})
		Mean ± *SD*			
B. pendula	Z	41.10±18.89^{a}	313.03±67.95^{a}	35.41±19.82^{a}	5.79±2.49^{ab}
	R_a	49.45±8.38^{a}	305.92±60.18^{a}	40.39±9.91^{ab}	4.39±1.04^{a}
	O	71.95±15.51^{b}	481.36±73.83^{b}	55.15±15.89^{b}	7.10±2.43^{b}
B. pubescens	R_b	40.00±14.62	294.32±46.44	38.13±7.26	3.93±1.83
Species	Sites	**Glucose (g·L^{-1})**	**Fructose (g·L^{-1})**	**Sucrose (g·L^{-1})**	**Proteins (g·L^{-1})**
		Mean ± *SD*			
B. pendula	Z	5.23±3.47	5.36±2.95	0.63±0.71	0.272±0.105
	R_a	4.72±2.09	4.82±1.82	0.28±0.12	0.272±0.117
	O	5.38±1.11	6.23±3.11	0.28±0.19	0.287±0.130
B. pubescens	R_b	3.46±0.61	3.88±0.38	0.014±0.023*	0.307±0.073

Notes: (a, b *and* c mark statistically significant differences between the sites according to ANOVA or Kruskal-Wallis tests and post hoc tests, $\alpha \leq 0.05$;

* the statistically significant differences between species according to Student's *t*-test (with $\alpha \leq 0.05$).

Table 2. Mean concentrations of chosen minerals for Zalesie (Z), Rzeszów ($R_{a-B.\ pendula}$, $R_{b-B.\ pubescens}$) and Ostrowiec (O)

Species	Sites	Ca (mg·L^{-1})	K (mg·L^{-1})	Mg (mg·L^{-1})
		Mean ± *SD*		
B. pendula	Z	169.270±68.987^{a}	107.366±58.401^{a}	18.635±11.538^{a}
	R_a	212.767±16.238^{b}	174.569±53.820^{b}	25.307±4.6107^{a}
	O	162.587±29.356^{a}	179.136±60.641^{b}	31.196±12.646^{b}
B. pubescens	R_b	217.874±67.501	149.087±43.746	24.9627±6.642
Species	Sites	Zn (mg·L^{-1})	P (mg·L^{-1})	Na (mg·L^{-1})
		Mean ± *SD*		
B. pendula	Z	2.967±0.995^{a}	7.008±2.054^{a}	0.266±0.359^{a}
	R_a	4.115±1.72^{a}	23.522±7.447^{b}	0.158±0.279^{a}
	O	3.243±0.775^{a}	19.916±6.124^{b}	1.974±1.689^{b}
B. pubescens	R_b	4.498±1.608^{a}	34.992±8.44*	nd
Species	Sites	Cu (μg·L^{-1})	Fe (mg·L^{-1})	Mn (mg·L^{-1})
		Mean ± *SD*		
B. pendula	Z	0.048±0.019^{a}	0.198±0.042^{a}	2.955±2.363
	R_a	0.099±0.015^{b}	0.249±0.049^{b}	4.092±6.977
	O	0.138±0.021^{c}	0.203±0.034^{a}	1.929±1.910
B. pubescens	R_b	0.089±0.020	0.304±0.089*	7.923±3.61

Notes: (a, b *and* c mark statistically significant differences between the sites according to ANOVA or Kruskal-Wallis tests and *post hoc* tests, $\alpha \leq 0.05$;

* the differences between species according to Student's t-test with $\alpha \leq 0.05$;

nd stands for "not detected".

Minerals in soil

It can be stated that soil samples from Ostrowiec differs from soil samples from other places containing higher concentrations of some elements. They are richer in Ca, Zn, P, Ca, Cu, Fe and Na (Table 3). Among heavy metals, Pb, Cd and Cr concentrations are higher in soil from Ostrowiec too. Ni and Al concentrations are similar in all types of soils varying from 0.01 to 0.024 and from 4.1 to 6.38 g kg^{-1}, respectively. Pb concentration is 5-11 times higher than in soils from the traffic area, and more than 30 times higher than in soil from the suburban area. Cd concentration is 20 times higher than in suburban area and one soil from the traffic area was around 10 times higher than in the soil from other traffic area. Cr concentration is 1.5 times higher than in the traffic area, and 3.5 times higher than in the suburban area. Heavy metals (HM) like Ni, Pb, Cd, Cr, Al and Zn were determined in all analysed soil samples. But it can be stated that in most cases HM concentrations in soil from the industrial area (Ostrowiec) were higher than from ones of the other localities.

Discussion

Nowadays, there is a tremendous resurgence in the interest and use of natural healthy products, foods, medicinal plants etc. According to the World Health Organization (WHO), as many as 80 % of the world's people depend on traditional medicine for their primary healthcare needs (WHO 1993, Škrovánková et al. 2012). The number of consumers interested in healthy food continues to grow, which corresponds with an increasing number of health-food and specialty stores, in which different healthy products are available (Briskin 2000). Birch sap is an example of such a product that is becoming more and more popular in Poland. It is available in special healthy food stores but lately also in supermarkets, the advertising campaign was extended in the popular media, and moreover, there are more and more distributors and producers of this product.

The present study quantitatively determined the concentration of bioactive and nutrition compounds as well as mineral substances in saps obtained from two species of birch trees, *B. pendula* (silver birch) and *B. pubescens* (downy birch). We have attempted to find out if there are any differences in these parameters depending on the birch species and localities (between the traffic, suburban and industrial areas).

According to these studies, authors can easily detect that antioxidant properties of saps from the industrial region are significantly higher than those from the traffic and suburban areas both for FRAP and ABTS methods, respectively. It is known that different stress factors, en-

Table 3. Mean concentration of chosen minerals in soil from Zalesie (Z), Rzeszów ($R_{a-B.\ pendula}$, $R_{b-B.\ pubescens}$) and Ostrowiec (O)

Sites	Ca (g·kg^{-1})	K (g·kg^{-1})	Mg (g·kg^{-1})	Zn (g·kg^{-1})	P (g·kg^{-1})
			Mean ± *SD*		
Z	1.234±0.014^{a}	0.801±0.014^{a}	1.318±0.014^{a}	0.026±0.001^{a}	0.246±0.003^{a}
R_a	4.842±0.066^{b}	3.609±0.029^{b}	3.418±0.014^{b}	0.0797±0.000^{b}	0.607±0.014^{b}
O	12.284±0.014^{c}	1.220±0.014^{c}	2.635±0.043^{c}	0.806±0.014^{c}	8.091±0.052^{c}
R_b	1.600±0.054^{d}	2.309±0.052^{d}	2.510±0.025^{d}	0.056±0.001^{d}	0.632±0.014^{b}
Sites	Na (g·kg^{-1})	Cu (g·kg^{-1})	Fe (g·kg^{-1})	Mn (g·kg^{-1})	S (g·kg^{-1})
			Mean ± *SD*		
Z	0	0.007±0.000^{a}	7.404±0.052^{a}	0.2696±0.001^{a}	0.059±0.001^{a}
R_a	0.097±0.003^{a}	0.023±0.003^{b}	17.229±0.076^{b}	0.382±0.014^{b}	0.287±0.001^{b}
O	0.088±0.004^{b}	0.063±0.001^{c}	22.463±0.413^{c}	0.479±0.009^{c}	0.812±0.014^{c}
R_b	0.041±0.004^{c}	0.035±0.001^{d}	13.238±0.090^{d}	0.338±0.013^{d}	0.158±0.001^{d}
Sites	Ni (g·kg^{-1})	Pb (g·kg^{-1})	Cd (g·kg^{-1})	Cr (g·kg^{-1})	Al (g·kg^{-1})
			Mean ± *SD*		
Z	0.010±0.000^{a}	0.004±0.000^{a}	0.00009±0.000^{a}	0.015±0.001^{a}	4.100±0.025^{a}
R_a	0.024±0.001^{b}	0.029±0.001^{b}	0.0002±0.052^{b}	0.037±0.000^{b}	6.375±0.025^{b}
O	0.016±0.001^{c}	0.138±0.001^{c}	0.00193±0.000^{c}	0.053±0.001^{c}	5.350±0.025^{c}
R_b	0.018±0001^{c}	0.012±0.000^{d}	0.00008±0.000^{a}	0.030±0.001^{d}	6.300±0.05^{b}

Notes: (a, b *and* c mark statistically significant differences between the sites according to ANOVA or Kruskal-Wallis tests and *post hoc* tests with $\alpha \leq 0.05$;

* the statistically significant differences between species according to Student's *t*-test with $\alpha \leq 0.05$.

vironmental pollution etc. causing oxidative stress are connected with reactive oxygen species (ROS) formation. Living organisms like plants possess several antioxidative defense systems to scavenge toxic-free radicals in order to protect themselves from the oxidant stress (Zeneli et al. 2013). It was reported that the tolerance of plants for oxidative stressing conditions may be explained by the enhanced activity of antioxidative enzymes preventing cell and tissue damage (Shi et al. 2006). Higher antioxidant properties of saps from the industrial region may have reflected higher environmental stressing conditions in comparison with saps from other sites (Krishnaveni 2013). Moreover, it was reported that antioxidant properties may depend on the species or even individuals. Some plants, growing near cement factory and exposed to cement dust pollution, exhibited higher antioxidant properties than control plants, but other species have a lower defense system against cement dust pollution and lower antioxidant potential than appropriate control plants (Mutlu et al. 2009). Therefore, higher antioxidant capacities of birch sap from the industrial area can be explained as a high tolerance of birch to environmental pollution, manifested by an increased content of antioxidants protecting the plant against different environmental stresses. Therefore, in theory, variations in antioxidant responses throughout the life cycle occur, making the plant more or less susceptible to seasonal variations in diverse environmental stressors.

Variations in the levels of antioxidants may also occur during the aging of the plant (Ferreira and Domingos 2012). The authors chose the trees randomly so this can be an explanation for large differences in maximum and minimum values of these parameters at one location. It is difficult to compare results obtained in our study to others because there is little information about antioxidants in birch saps or researchers used different methods to estimate the antioxidant capacity with different standards, incubation time and concentrations. Kūka et al. (2013) detected the antioxidant concentration of Latvian birch saps at 0.35 mg L^{-1} of quercetin equivalent.

It is known that the concentrations of phenolic compounds in many plants are strictly correlated with their antioxidant properties (Piluzza and Bullitta 2011, Jeong et al. 2013). Observed differences between the phenolic content in analyzed saps showed that phenolic compounds were important antioxidant components. Concentration of folic acid along with antioxidant properties (Joshi et al. 2001) is much higher in saps from the industrial region, which means that in all methods used for antioxidant detection, authors observed statistically significant difference in their concentration in saps from the industrial region compared with saps from the traffic and suburban areas.

The formation and level of antioxidants may be seasonality marked in response to changes in the environmental conditions. This is plausible because the seasonality is reflected in solar irradiation, photoperiod, temperature, relative humidity and actions of other meteorological factors. Some authors also observed that antioxidants concentration can be a response to the meteorological characteristics of each season of the year (Ferreira and Domingos 2012). The differences in the antioxidant concentration between localities can be explained by some differences in the course of weather in 2015 during flow season in different regions of Poland as well as the microclimate (Meteoblue Weather 2016).

Analyzing the sugar concentration, authors observed that the saps contained mainly fructose and glucose, however, sucrose was also found in lower amounts. Sugar proportions were similar to those found in northern Europe and in Poland (Kūka et al. 2013, Łuczaj et al. 2014). Sugar, protein and mineral concentrations obtained by other authors were shown in Table 4.

These results are also similar to those detected by Korean researchers for white birch sap (Jeong et al. 2012). They detected that its sugar content varied from 3.6 to 4.3 g L^{-1} and from 4.9 to 6.6 g L^{-1} for glucose and fructose, respectively. In results presented in this paper, glucose concentration was slightly higher for silver birch sap from Zalesie and Ostrowiec. Polish silver birch sap contains more glucose and fructose than birch sap produced in Lithuania and in Finland (Kūka et al. 2013). Glucose and fructose concentrations in downy birch sap is approximately 30 % lower than in saps from Polish and

Table 4. Concentration of sugars, proteins and selected minerals in birch saps

Parameter	Object	Analytical results	Reference
Glucose (g·L^{-1})	*B. pendula*	3.6 to 4.3	Jeong et al. (2012)
	B. pendula	9.3±3.9	Łuczaj et al. (2014)
	B. pendula	3.55-4.96	Bilek et al. (2015b)
	B. pendula	4.46±0.04	Kûka et al. (2013)
	B. pubescens	1.99-3.016	Bilek et al. (2015b)
	B. pubescens	9.6±2.9	Łuczaj et al. (2014)
	B. platyphylla var. *japonica*	2.5	Jeong et al. (2013)

Fructose (g·L^{-1})	*B. pendula*	4.9 to 6.6	Jeong et al. (2012)
	B. pendula	5.39±0.05	Kûka et al. (2013)
	B. pendula	12.1±4.9	Łuczaj et al. (2014)
	B. pendula	4.03-4.77	Bilek et al. (2015b)
	B. pubescens	13.5±3.3	Łuczaj et al. (2014)
	B. pubescens	1.83-2.77	Bilek et al. (2015b)
	B. platyphylla var. *japonica*	3.3	Jeong et al. (2013)
Sucrose (g·L^{-1})	*B. pendula*	0.07	Jeong et al. (2013)
	B. pendula	0-1.509	Bilek et al. (2015b)
	B. pendula	0.58±0.01	Kûka et al. (2013)
	B. pendula	3.2±2.4	Łuczaj et al. (2014)
	B. pubescens	3.1±1.4	Łuczaj et al. (2014)
Protein (mg·L^{-1})	*B. pendula*	127±2	Kûka et al. (2013)
	B. platyphylla var. japonica	15-35	Jiang et al. (2001)
	B. verrucosa	15-28	Jiang et al. (2001)
Ca (mg·L^{-1})	*B. pendula*	41.0-53.3	Kûka et al. (2013)
	B.pendula	5.52 -17.28	Bilek et al. (2015)
	B. pubescens	15.12±4.74	Bilek et al. (2015)
	B. platyphylla var. japonica	25.82±0.12	Jeong et al. (2013)
	Betula sp.	42 – 150	Vincēviča-Gaile (2014)
Cu (mg·L^{-1})	*B. pendula*	0.15-0.39	Bilek et al. (2015)
	B. pendula	0-0.04	Kûka et al. (2013)
	B. pubescens	0.48±0.42	Bilek et al. (2015)
	B. platyphylla var. *japonica*	0.82±0.10	Jeong et al. (2013)
	Betula sp.	0.02-0.03	Vincēviča-Gaile (2014)
Fe (mg·L^{-1})	*B. pendula*	0-0.1	Kûka et al. (2013)
	B. platyphylla var. *japonica*	0.61±0.09	Jeong et al. (2013)
	Betula sp.	0.05-0.11	Vincēviča-Gaile (2014)
K (mg·L^{-1})	*B. pendula*	10.56-23.76	Bilek et al. (2015)
	B. pendula	41.1-66.4	Kûka et al. (2013)
	B. pubescens	18.08±18.85	Bilek et al. (2015)
	B. platyphylla var. japonica	30.10±4.81	eong et al. (2013)
	Betula sp.	54 – 142	Vincēviča-Gaile (2014)
Mg (mg·L^{-1})	*B. pendula*	4.42-14.36	Bilek et al. (2015)
	B. pubescens	13.82±5.55	Bilek et al. (2015)
	B. platyphylla var. *japonica*	11.90±0.15	Jeong et al. (2013)
	Betula sp.	0	Vincēviča-Gaile (2014)
Mn (mg·L^{-1})	*B. pendula*	0.5-0.52	Kûka et al. (2013)
	B. platyphylla var. *japonica*	2.36±0.02	Jeong et al. (2013)
	Betula sp.	0.11-6.16	Vincēviča-Gaile (2014)
Na (mg·L^{-1})	*B. pendula*	0.56-0.59	Bilek et al. (2015)
	B. pubescens	0.55±0.62	Bilek et al. (2015)
	B. platyphylla var. *japonica*	7.51±0.36	Jeong et al. (2013)
	Betula sp.	0	Vincēviča-Gaile (2014)
Ni (mg·L^{-1})	*B. pendula*	0-0.03	Kûka et al. (2013)
	Betula sp.	0.02-0.16	Vincēviča-Gaile (2014)
Zn (mg·L^{-1})	*B. pendula*	0.88-1.85	Bilek et al. (2015)
	B. pubescens	1.29±0.17	Bilek et al. (2015)
	B. platyphylla var. *japonica*	3.82±0.47	Jeong et al. (2013)
	Betula sp.	0.9-4.96	Vincēviča-Gaile (2014)
P (mg·L^{-1})	*Betula* sp.	3 - 41	Vincēviča-Gaile (2014)
S (mg·L^{-1})	*Betula sp.*	5 – 12	Vincēviča-Gaile (2014)
Cr (mg·L^{-1})	*Betula sp.*	0.02	Vincēviča-Gaile (2014)
Co (mg·L^{-1})	*Betula sp.*	0.05	Vincēviča-Gaile (2014)

Latvian silver birch (Table 4). In contrast to these results, Łuczaj et al. (2014) did not detect significant differences in sugar concentrations between silver birch and downy birch saps. It can be explained by the fact that the amount of sugars in tree saps depends on many parameters. The tree sap sugar concentrations can be affected by the time of day, stage of flow cycle (beginning/end of the flow), tree size, age and soil fertility, basal area of ray issue and weather conditions in the year, or even shifts in gas contents in the atmosphere.

Sucrose concentration in white birch sap detected by Jeong et al. (2013) was 0.07 g L^{-1}. For these sap, sucrose concentration was at least four times higher than for silver birch sap localized in Rzeszow and Ostrowiec, and almost ten times higher than for sap from Zalesie. However, authors observed a great variability between individuals. On the other hand, sucrose concentration for downy birch was five times lower than those detected by Jeong et al. (2013) (Table 4). For Latvian birch, the sucrose concentration was about 0.58 g L^{-1}, which is twice higher than for *B. pendula* from the industrial and traffic areas and slightly lower – around 10 % – than for sap from suburban area (Kūka et al. 2013). Statistically significant differences in glucose and sucrose concentrations between species are attributed to species factors.

Protein concentrations in our saps are at least twice higher than those detected for Lithuanian birch saps (Kūka et al. 2013). Japanese researchers also detected a very low protein concentration for saps from white and silver birches at a level of 15-35 mg L^{-1} depending on the harvesting time (Jiang et al. 2001) (Table 4). As Łuczaj et al. (2014) said, relatively high values of sugars in Polish saps suggest that Poland may be a suitable place for developing the tree sugar industry.

Birch sap contains over a dozen minerals (Harju and Hylden 1990). In many scientific works, the authors indicated that among minerals cooper reached the lowest concentrations, whereas calcium and potassium the highest ones (Kūka et al. 2013, Vincēviča-Gaile 2014, Bilek et al. 2015*a*) (Table 4). Harju and Hylden (1990) noted the concentrations of manganese, phosphorus and zinc exceeded 1 mg L^{-1}. Presented results are in accordance with them, however, we observed the great variability among individual trees as well as the sites. It concerned mainly sodium, what was also detected by Bilek et al. (2015*a*).

Bilek et al. (2015a) compared the mineral compounds of the sap with the recommended daily allowance for adults. According to their results, one liter of silver birch sap covers up to 40 % of daily copper requirements, zinc up to 17 %, calcium up to 2%, magnesium up to 3-4 % and sodium up to 0.04 %. The authors obtained different results such as about 11 %, 30 %, 18 %, 8 % and 0.05 %, respectively.

The high variability could be affected by environmental and anthropogenic factors. In polluted areas, concentrations of certain minerals in plants increases (Vincēviča-Gaile, 2014). The authors noted that the highest iron and phosphorus concentrations were in the sap harvested from the traffic and industrial sites. High concentrations of heavy metals (HM) may negatively affect living organisms, the ecosystems and human health. HMs presented in soils may be transported through the food chain to the human body and have a significant toxic effect to people (Butkus and Baltrėnaitė 2007, Yan et al. 2012). Harju and Hylden (1990) found increased concentrations of Pb, Zn, Ag and Cd in birch sap collected in polymetallic damps in south western Finland. Generally, in study areas the concentration of minerals was higher in soil collected near steel mill (Ostrowiec) and the lowest ones in soil from suburban area (Zalesie) but authors did not detect any connection between mineral concentrations in soils and in saps. Among HMs only a few ones were detected in saps. These results confirm that transport of many HMs from soil to plant is not very active (Kandziora-Ciupa et al. 2015). Lead is characterized by poor bioavailability and therefore it was not found in saps. Also very toxic cadmium was not transported from soils to saps. Copper and zinc as trace elements are essential for proper metabolism, growth and development of plants, but in the highest concentrations are toxic (Wierzbicka 2015). Authors noted that in soil these elements were presented but their concentrations in the saps were low. These results are very important for potential consumer because, regardless of the heavy metal soil contamination, birch sap is resistant to toxic minerals.

Conclusion

Taking into account its chemical composition, birch sap should be considered as a low-calorie diet supplement, a good substitute for water: medium and high mineralized, which should contain at least 150 mg per litre of macronutrients like Ca, K and Mg. In respect of minerals, antioxidant and nutrient properties the downy birch sap is just as valuable as the sap of silver birch. Variation in chemical compounds may be caused by the type of habitats and inter-individual variability. It seems that birch sap is quite resistant to heavy metals present in soil. Heavy metals concentrations in soil, as well as other environmental stresses such as pollutants of traffic origin may cause higher antioxidant activity of birch sap. When preparing to sap harvesting, the potential soil and air pollution in sampling site should be taken into account.

Acknowledgement

The work was partly supported by Cross-border Cooperation Programme Poland-Belarus-Ukraine 2007-2013, IPBU.03.01.00-18-452/11-00 and by the Polish Ministry of Science and Higher Education (No 3020/PBU/0755/11/13/2014/2). Responsibility for the content of this publication lies solely with the authors and cannot, under any circumstances, be considered to reflect the position of the European Union in any way.

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Received 19 March 2016
Accepted 30 September 2016