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Sugar content in the sap of birches, hornbeams and maples in southeastern Poland

Research Article

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Abstract: There is very little data on the sugar content of tree sap in Central Europe. In order to fill this gap we measured sugar content in the spring sap of 55 trees from 8 species (six native and two introduced). Sugar content was measured chromatographically using an HPLC Dionex Ultimate 3000 system and CAD detection. The proportion of sugar was highly genus-specific: maples contained sucrose, birches a mix of glucose, fructose and sucrose and hornbeams glucose and fructose. *Acer saccharinum* had the sweetest sap (4.0%), followed by *A. platanoides* (3.2%), *A. pseudoplatanus* (3.2%), *A. negundo* (2.9%) and *A. campestre* (2.8%). Birches were slightly less sweet (*Betula pendula* 2.5% and *B. pubescens* 2.6%). *Carpinus betulus* sap contained only 0.9% sugar. The reported values for birches are higher than those reported in other European studies and the values for maples are similar to those for sugar maples in North America. As southeastern Poland has many large populations of maples and birches, a small-scale sugar syrup industry could become an additional source of income for forest owners.

Keywords: *Tree sap • Non-timber forest products • Glucose • Fructose • Sucrose • Sugar*

1. Introduction

Sugar maple sap extraction is an important part of the economy in North America and the sugar content of the American *Acer saccharum* L. and factors which affect it have been reported by several authors [1-4]. Fresh maple sap, which is later concentrated into syrup, contains mainly sucrose, from only 0.8% [5] to 5.5 % [4] or even 6% [2], but usually from 2.5 to 4 % (*e.g.* [4,6]). Birch syrup is also produced, but on a much smaller scale, mainly in Alaska [7]. However, much less is known about the chemical composition of the sap of European tree species. In Europe, it is mainly birch sap that has been extracted and used, either fresh, fermented or preserved. The sap of European maple species has been used in parts of central and northern Europe as well, usually fresh, only rarely concentrated into syrup or sugar [8]. In Poland birch sap (from *Betula pendula* Roth and *B. pubescens* Ehrh.) was commonly drunk fresh, and sometimes also fermented into a low alcohol drink. The drinking of fresh or, rarely, fermented *Acer platanoides* L. and *A. pseduplatanus* L. sap has also been recorded. Extremely locally, the sap of *Carpinus betulus* L., *Tilia* spp., and *Prunus avium* L. was also drunk [9,10].

At the end of the 19th century, the chemical content of the sap of birch, maple and hornbeam was studied in Germany [11,12], using low-specificity classical analytical

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methods, which may have distorted the results [11]. Over a hundred years later Essiamah [13] in his study of sap content in a few tree species in Germany used enzymatic methods to measure the sugar content. He reported very high maximum values of up to 4.3% glucose and 1.9% fructose in *Betula pendula*, and 3.6% sucrose in *Acer pseudoplatanus.* Unfortunately mean values and total sugar contents were not reported. The sap of *Quercus robur* and *Alnus glutinosa* contained less sugar and the author failed to extract the sap from *Fraxinus excelsior.* The content of birch sap in Europe using modern instrumental methods (gas chromatography) was studied in the research of Kallio and colleagues from Finland [14-16]. Birch sap content was also studied in Lithuania [17]. The works from both Finland and Lithuania reported an approximately 1% sugar content in birch sap. It has also been established that birch sap, both in European and in American species of birches, contains mainly fructose and glucose, as well as some sucrose [7,14].

The aim of our study was to look at the sugar content of some native tree species, as well as their exotic counterparts commonly cultivated in Poland. Our broader knowledge of sugar contents in European conditions (both concerning native and introduced tree species) may be important for developing the non-timber forest product sector, particularly in the heavily wooded Northern and Eastern Europe.

2. Experimental Procedures

2.1 Sampling

Sugar content was analyzed in the trees of six native species. i.e. silver birch *Betula pendula* Ehrh., downy birch *B. pubescens* Ehrh., European hornbeam *Carpinus betulus* L., Norway maple *Acer platanoides* L,. sycamore *A. pseudoplatanus* L. and field maple *A. campestre* L., as well as two exotic *Acer* species: boxelder *Acer negundo* L. and silver maple *Acer saccharinum* L. The genera of *Betula, Acer* and *Carpinus* were chosen as species from these genera have been the most common sources of tree sap in eastern Europe [8].

The sap was extracted in two regions in SE Poland, the Kolbuszowa Plateau and the Krosno region (in the Carpathian Foothills). On the Kolbuszowa Plateau, trees growing in the village of Werynia were studied, and in the Foothills trees were studied in the villages of Pietrusza Wola and Rzepnik, as well as in the town of Krosno (Figure 1).

For the commonest species we sampled five trees growing in both regions, in the same location, less than 100 m from each other. For some species, fewer trees were found on the study sites (Figure 1, Table 1). Altogether we sampled 10 trees of *B. pendula, A. platanoides, C. betulus*, 7 of *Acer negundo*, and, in the Krosno region only, 5 of *A. pseudoplatanus*, *A. campestre* and 4 of *B. pubescens, A. saccharinum*.

Trees were cut with a small axe at a 45º angle at the height of 50 cm. A copper pipe (12 cm long, 2 cm in diameter) was gently hammered into the base of the cut. Underneath each pipe, a sterile plastic tube was placed (50 ml volume). The tubes were closed instantly after collecting the sap (which usually took 5-20 min.) and instantly (within 20 min.) frozen at -20ºC to prevent conversion of carbohydrates. The samples from the more distant localities (Krosno region) were transported in a portable freezer.

The samples were collected between 9 and 14 April 2013, between late morning and early afternoon (10 am – 2 pm), roughly 2-4 days after the onset of intense tree sap flow. As spring appeared rapidly, with temperatures around 10–15ºC after a few week periods of frost, it was easy to establish the beginning of the sap flow (we checked it regularly) and all the trees started sap flow around the same day. We tried to collect the sap on 23 of April, but the trees were no longer producing it.

For chemical analysis, the samples frozen within a week were heated to 80ºC using a water bath and instantly analyzed.

2.2 HPLC chromatography

Sugar content was measured chromatographically using an HPLC Dionex Ultimate 3000 system (Dionex. Germering. Germany) consisting of: an UltiMate 3000 Solvent Rack SR-3000, a quaternary analytical pump LPG-3400SD, an autosampler WPS-3000, a column

Figure 1. Study area.

Species	L.	No of trees	Habitat	Date	Mean dbh (cm)	Min. and max. girth
Betula pendula	W	5	self-sown in municipal area	11.04	25	17-32
Betula pendula	P	5	self-sown in secondary forest	10.04	20	16-25
Betula pubescens	K	4	planted in gardens (plants originating from NE Poland)	11.04	20	17-25
Acer platanoides	W	5	historic park, remnant of native vegetation (TC)	11.04	60	14-118
Acer platanoides	P	5	sparsely wooded secondary vegetation	10.04	13	9-21
Acer pseuplatanus	R	5	natural deciduous forest (TC)	10.04	48	33-80
Acer campestre	R	5	natural deciduous forest (TC)	11.04	30	23-31
Acer negundo	W	5	female specimens, historic park, remnant of native vegetation (TC)	09.04	32	25-39
Acer negundo	P	2	male specimens in a private garden	10.04	29	29-29
Acer saccharinum	P	4	private garden	11.04	8	5-12
Carpinus betulus	W	5	self-sown in a field margin	14.04	33	17-42
Carpinus betulus	R	5	natural deciduous forest (TC)	13.04	24	18-29

Table 1. Characteristics of the trees studied.

heater TCC-3000 RS and a charged aerosol detector Corona Ultra RS (ESA. Chelmsford. MA. USA). Data processing was carried out with Chromeleon 6.8 software (Dionex). Chromatographic analysis was carried out at 25°C with 20 μl of injection volume. Samples were separated on a Grace Prevail Carbohydrate column packed with 5 μm shell particles (250 mm x 4.6 mm) and acetonitrile–water (20/80%, v/v) mobile phase at a flow rate of 1.00 mL min^{-1}. Nitrogen (99.99%) gas flow rate was regulated automatically at 35 psi and monitored by a CAD device. The total time of analysis amounted to 30 min.

The qualitative analysis of sugar content in the tree saps was performed by comparing retention times of fructose, glucose and sucrose standard solutions with the retention times of the substances present in the samples. Apart from the studied sugars we found one unidentified substance (in maple samples). The linearity of the detector response was determined by the square correlation coefficients of the two calibration curves. Because of the well-known nonlinear Corona CAD response [18,19] it was necessary to make the calibration curve into two ranges (0.01–0.25 and 0.25–5.0 g L^{-1} respectively), generated by the injections of standard solutions of the three studied sugars: sucrose, glucose and fructose. The calibration curves were obtained by plotting concentration (in g L^{-1}) against peak area. Responses obtained in the examined range were expressed by the linear equation $y = ax + b$.

HPLC-grade acetonitrile was obtained from J.T.Baker (Malinckrodt Baker B.V. Holland). Standards of D-(–)-fructose (99.9%), D-(+)-glucose (99.5%) and sucrose (99.5%) were purchased from Sigma-Aldrich Chemie (Steinheim. Germany).

2.3 Method validation

The system's stability was controlled using glycerol as a part of the samples (Chempur. Poland), a substance not recorded in tree saps by other authors, or during our control analysis. The repeatability of the detector's responses to known concentrations of the studied sugars were controlled by periodic injections of the kit of standards: fructose, glucose and sucrose [20].

2.4 Sample Preparation

The sample was thawed before the beginning of the chromatographic analysis to prevent the degradation of the studied sugars due to microbial activity (a separate trial showed that sugars in the sap were fully fermented only a few hours after thawing). The samples were then filtered with a teflon syringe filter (pore diameter 0.45 μm) and diluted 10 x with a water solution of glycerol (used as an internal standard).

3. Results

There were marked differences in the sugar content and sugar proportions between the genera. The difference in sugar content between the three studied genera was significant (both t-Student test and

Mann-Whitney-U-test, P<0.05). Maples had the sweetest sap, containing around 3% sugars, composed mostly of sucrose (Table 2, Figure 2-4). *A. saccharinum* had the sweetest sap (mean - 4.1%), followed by *A. platanoides* (3.24%), *A. pseudoplatanus* (3.18%), *A. negundo* (2.90%) and *A. campestre* (2.76%). Birches were slightly less sweet (Table 2, Figure 2-4; *B. pendula* 2.46% and *B. pubescens* 2.62%). The sap contained mainly fructose and glucose however sucrose also found in sufficient amounts. Hornbeam sap was the least sweet, with only 0.87% sugar (Table 2, Figure 2, 3-4), and consisted mainly of glucose and fructose. There was a large difference between the levels of sweetness of hornbeam sap from the two studied sites (Werynia – 0.51%, Rzepnik – 1.23%).

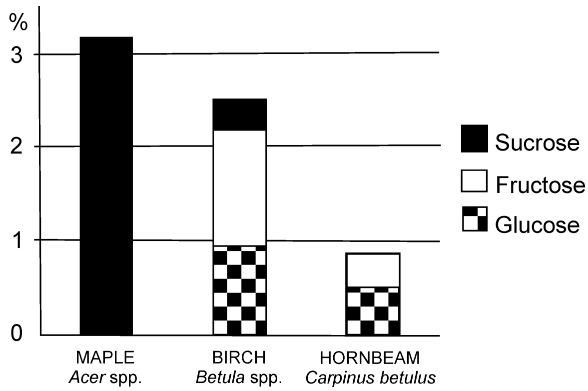

Figure 2. Proportions of sugars in the studied species.

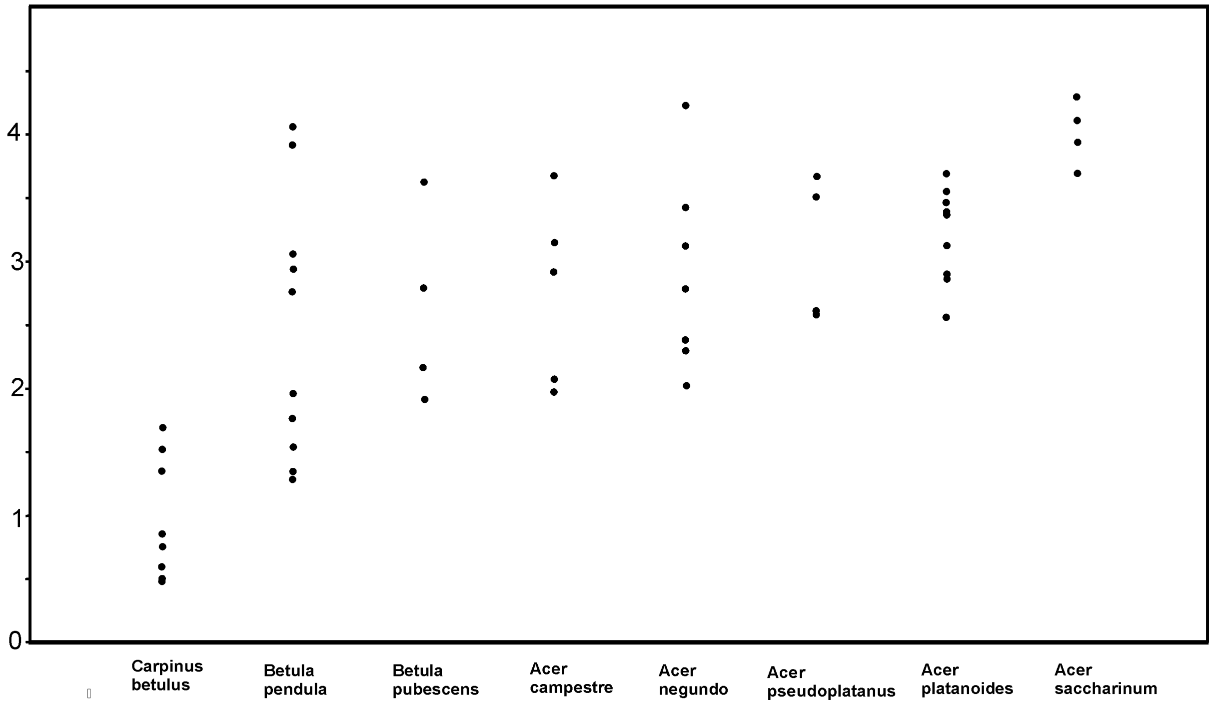

Figure 3. Variation in total sugar content (%) among individual samples.

	Glucose (%)	Fructose (%)	Sucrose (%)	Total sugar (%)
Acer campestre	0.0056 ± 0.0027	0.0040 ± 0.0020	2.74 ± 0.68	2.76 ± 0.73
Acer negundo	0.0045 ± 0.0021	0.0051 ± 0.0022	2.89 ±0.76	2.90 ± 0.76
Acer platanoides	0.002 ± 0.001	0.004 ± 0.003	3.23 ± 0.36	3.24 ± 0.36
Acer pseudoplatanus	0.003 ± 0.0005	0.002 ± 0.001	3.17 ± 0.50	3.18 ± 0.53
Acer saccharinum	0.002 ± 0.001	0.001 ± 0.001	4.01 ± 0.24	4.01 ± 0.26
Betula pendula	0.93 ± 0.39	1.21 ± 0.49	0.32 ± 0.24	2.46 ± 1.03
Betula pubescens	0.96 ± 0.29	1.35 ± 0.33	0.31 ± 0.14	2.62 ± 0.76
Carpinus betulus	0.49 ± 0.25	0.38 ± 0.22	0.02 ± 0.02	0.87 ± 0.47

Table 2. Sugar content (% weight) in the studied tree sap samples. Values reported are mean ± standard deviation.

4. Discussion

The sucrose concentrations found both in native Polish maples (*A. platanoides, A. pseduplatanus* and *A. campestre*) and in the exotic American species cultivated in SE Poland (*A. negundo*, *A. saccharinum*) were similar to mean sucrose concentrations in sugar maples in America (usually around 3-4% [1-5,21-24]).

Sugar proportions in birches were similar to those found in northern Europe (Finland, Lithuania, Russia) but the concentrations were higher; more than double [14-17,25,26]. For example, in Lithuania birch sap contained ca. 1.1% sugar [17] and in Russia *ca*. 1% [25,26]. In Finland, the observed concentrations of sap sugar were below 1% [14-16]. It was only Essiamah in Germany [13] who reported higher concentrations of sugar in the sap of European birches. It is not unlikely that the high sugar content in our study may have been a result of very good conditions of sap flow during sampling (sunny warm weather directly after a long period of frost), hence it may not be maintained on a yearly basis.

The amount of sugars in the sap of trees depends on many parameters and we are aware that our study gives only preliminary data on the sap sugar concentrations in Polish trees. The tree sap sugar concentrations can be affected by time of day, part of flow cycle (beginning/ end of the flow), tree size, age and soil fertility, basal area of ray tissue and weather conditions in the year, or even changes in gas contents in the atmosphere [1-4,21,23,24]. A variation between individual trees is attributed to genetic factors and was also documented [4,6,22,23]. It is not unlikely that the results from a similar study in other parts of Poland, *e.g.* with different soils may yield different sugar concentrations. On the other hand the relatively high values for the studied genera suggest that SE Poland may be a suitable place for developing tree sugar industry.

The observed proportions of glucose, fructose and sucrose seem to be genus-specific and are similar to those observed in North America (*e.g.* [2-4,6] for maples) and those observed in other European countries for birches [13-17].

Although the production of birch sap is common in some Eastern European countries (parst of the former Soviet Union), particularly in Belarus, Russia and Ukraine, maple syrup production is non-existent. The high sugar content found in Polish maples should encourage further studies on its production in this part of the world. Although pure maple stands are rare in central Europe, maples are a common admixture in forests, and locally *A. pseudoplatanus, A. platanoides* and *A. campestre* become subdominants. Attempts to produce maple syrup were made in 19th century Slovakia but were later abandoned [27]. In today's times, maple syrup production may offer a method of diversifying income for agritourist farms and owners of small forests.

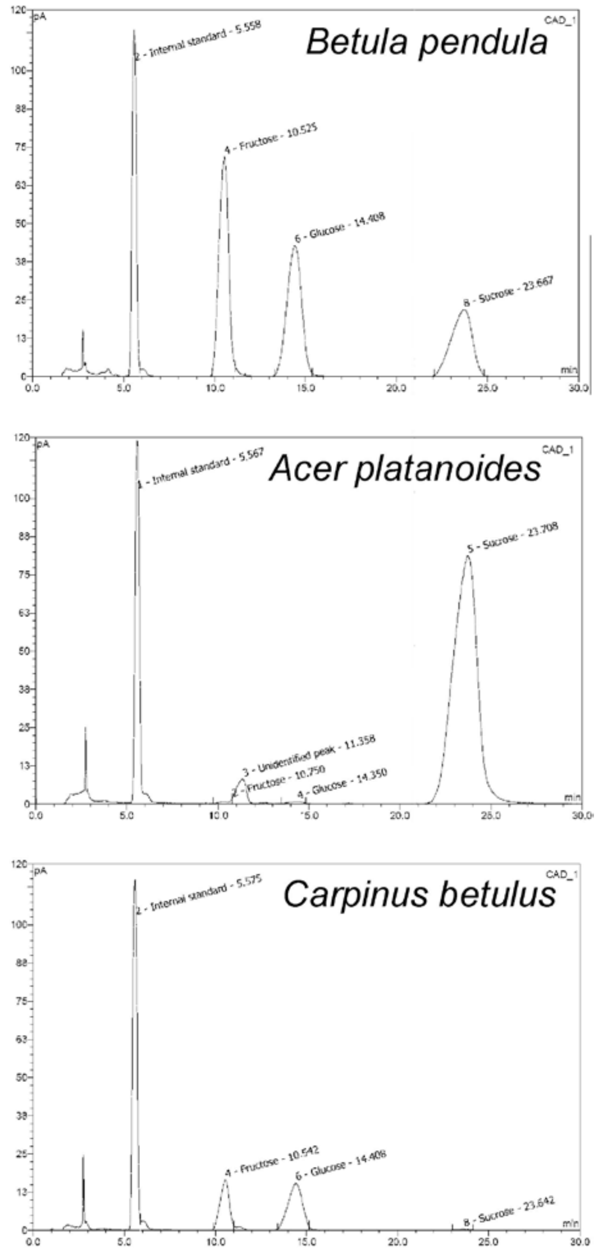

Figure 4. Representative chromatograms of sugar content for *Betula pendula* , *Acer platanoides* and *Carpinus betulus.*

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Authors' contribution

ŁŁ initiated the study, ŁŁ and KS collected the samples, MB carried out the chemical analyses (with the help of KS). The article was written together by all the authors.

Competing interests

The authors declare that they have no competing interests.

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