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Identification and Seasonal Variations of Amino Acids in Birch Sap Used for Syrup Production

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ABSTRACT

The contents of the main free amino acids of birch sap (Betula pendula *Roth and* B. pubescens *Ehrh.), which will be used for syrup production in Finland, were analysed. Fifteen amino acids were monitored as their pentafluoropropionicanhydrideisopropyl esters with capillary–GC–MS. Glutamine, citrulline and glutamic acid were the main components in each tree studied. Together with isoleucine, valine and asparagine they comprised 92–96% of the amino acid pool. During the flow season their total content varied widely from 100 to 500 mg/litre, showing high deviations. The contents of the six main amino acids increased in April, and they at least doubled during the latter half of the month. The most efficiently increased amino acid was glutamine, which finally comprised about 40% of the entire pool. The amino acids take part in Maillard reactions, and thus affect the sensory properties of the heated syrup.*

INTRODUCTION

The major components and the measure of foodstuff quality of birch sap are the reducing sugars glucose and fructose (Kok, 1977; Kok *et al.*, 1978; Kallio *et al.*, 1985; Kallio & Ahtonen, 1987*a*; Kallio *et al.*, 1989), citric acid cycle intermediates (Schroeder, 1871; Nordal, 1944; Kallio *et al.*, 1985; Kallio & Ahtonen, 1987*b*) and the K-, Ca- and Mg-salts (Kok *et al.*, 1978; Ganns *et al.*, 1982). Free amino acids are, though present in only trace amounts, also of considerable importance. They take part in Maillard reactions producing flavour and colour when the sap is heated.

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The composition and concentrations of the free amino acids have been studied by some investigators previously. The methods were mainly paper chromatography (Reuter & Wolfgang, 1954; Barnes, 1963; Eglite & Oskaja, 1973; Ronkov & Lachkova, 1979) or automatic amino acid analyses (Sheldrake & Northcote, 1968). The quantitative methods have not always been very accurate. Results of these investigations are summarised in Table 1.

Reuter & Wolfgang (1954) named some tree species as *Fagus*, *Ulmus*, *Aesculus*, *Picea*, *Larix* and *Fraxinus* to be 'amide type' trees. This was based on the high contents of free glutamine and asparagine in the sap of the trees. Barnes (1963) tested sixty different tree species and in forty-one cases the major amino acid was either citrulline, glutamine or asparagine. Reuter & Wolfgang (1954) reported citrulline and glutamine to be the main components in the sap of *Betula pubescens*. Accordingly, Barnes (1963) reported one of the main components in *B. alba* and Sheldrake & Northcote (1968) in *B. populifolia* to be citrulline. In *B. verrucosa* and *B. verrucosa* Etr. f. *maserica* R. glutamine was also reported to be one of the major free amino acids even though aspartic acid was the number one (Eglite & Oskaja, 1973). Ronkov & Lachkova (1979) verified the importance of glutamine in birch sap.

The amino group carriers always have a central position among the amino acids in the spring sap of varying tree species, especially of *Betula*.

TABLE 1
Composition of Free Amino Acids in Birch Sap

Reference	*Species*	*Amino acid*
Reuter & Wolfgang (1954)	*B. pubescens*	Asn, Asp, Cit, Gln, Glu, Ile, Val
Barnes (1963)	*B. nigra*	Asp, Cit, Gln, Ser
Sheldrake & Northcote (1968)	*B. populifolia*	Ala, Arg, Asp, Cit, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Orn, Phe, Ser, Thr, Tyr, Val
Eglite & Oskaja (1973)	*B. verrucosa*, *B. verrucosa* Etr. f. *maserica* R.	Ala, Arg, Asp, Cys, Gln, Glu, Gly, Ile, Leu, Lys, Met, Ser, Thr, Tyr, Val
Ronkov & Lachkova (1979)	unknown	γ-Aba, Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Phe, Ser, Thr, Try, Val

Assimilation of nitrogen taken from the soil by roots is fast; nitrate and ammonium ions can hardly be recognised in the sap (Reuter & Wolfgang, 1954).

MATERIALS AND METHODS

The sap

The collection of sap took place once a week in April and in the beginning of May in 1984 in the natural park of Aulanko in Central Finland. The trees examined were three *Betula pendula* Roth. and three *B. pubescens* Ehrh. individuals, the same as were used for sugar and acid analyses (Kallio & Ahtonen, 1987*a*, *b*). The sap was stored at 20°C until analysed in 1984–85.

Isolation of amino acids

The internal standard (norleucine, 0·5 mg) was added to the 50 ml sap sample before the isolation procedure. The proteins were removed by alcohol precipitation (50 ml sap, 200 ml EtOH, 99·5%, overnight at −20°C). The precipitate was removed by filtering through a Millipore 0·45 μm filter. The filtrate was evaporated to dryness by rotatory evaporator at 40°C and dissolved in 0·1N HCl (5 ml). The solution was further cleaned by passing it through an ion-exchange column (id 1 cm, 2 ml wet Dowex 50 × 8, 200–400 mesh, Fluka AG, in H^+-form) as introduced by Näsi & Huida (1982).

Derivatisation of amino acids

The isolated amino acids were transformed to PFPA-(pentafluoropropionic-anhydride)-isopropyl esters (Frank *et al.*, 1977, 1978; Abe *et al.*, 1983). The evaporated residue was treated with 2N HCl in 2-propanol (0·5 ml, 1 h, 100°C). The HCl–2-propanol solution was made by bubbling gaseous HCl to the distilled 2-propanol. The gaseous HCl was obtained by adding HCl (conc.) dropwise to H_2SO_4 (conc.) (March, 1975). After esterification the solution was evaporated to dryness under a nitrogen stream at 40°C. CH_2Cl_2(0·2 ml) and PFPA-reagent (50 μl) were added. After one hour at room temperature the reagents were evaporated again with nitrogen stream and the remaining PFPA-isopropyl esters of the amino acids were dissolved in 0·2 ml redistilled $CHCl_3$.

Analysis of amino acid derivatives

The GC-analyses were carried out on a Varian Aerograph Model 3700 gas chromatograph equipped with a flame ionisation detector and a Hewlett Packard Model 3388A integrator. The 25 m long fused silica columns (i.d. 0·32 mm) were coated with either SE-30 (film thickness 0·15 μm) or Chirasil-Val (film thickness 0·23 μm) liquid phases. The flow rate of the nitrogen carrier was 1 ml/min and the injector split ratio was 1:70. The temperature of the injector was 180°C and that of the detector 290°C. Temperature was programmed from 90°C after 2 min isothermal period to 200°C at 4°C/min, and the final temperature level kept for 15 min. The correction factor of each amino acid was determined with authentic amino acid samples.

The GC–MS analyses (70 eV) of the major amino acid derivatives were performed on a VG-7070E mass spectrometer by using the Chirasil-Val column and the same conditions as in the GC-analyses mentioned above. The data system employed was VG-11-250.

RESULTS AND DISCUSSION

The GC-analysis of the amino acids as their PFPA-isopropyl esters was shown to be a rapid and reasonable method. It was necessary to remove the proteins, sugars and acids from the sap before the analysis. The repeatability of the method was especially affected by the timing of the derivatisation and of the entire GC-injection procedure. Usually the standard deviation of the analysis of the main amino acids ranged between 2% and 7% but in the case of citrulline it could sometimes be, for unknown reasons, as much as 20%.

The fifteen amino acids listed in Table 2 were identified and quantified in the spring sap of three *Betula pendula* and three *B. pubescens* individuals. Glutamine, citrulline and glutamic acid were the main components in each tree. Together with isoleucine, valine and asparagine they comprised 92–96% of the pool of the free amino acids.

In single samples, the total amount of amino acids varied between 20 and 700 mg/litre, always showing an increasing trend towards the end of the spring season. During the main flow in the second half of April, when the sap was collected for syrup production, the total content ranged widely from 100 to 500 mg/litre showing high variations between the trees (Table 3). These values clearly exceeded those analysed in birch syrup (Kallio *et al.*, 1989) when the concentration during evaporation has been taken into consideration.

The content of each of the six main amino acids increased in April and at least doubled during the latter half of the month. Most efficient was the

TABLE 2
Amino Acids in Birch Sap (mg/litre) in 1984

Free amino acid	*Date*									*Rate*[a]
	B. pendula					B. pubescens				
	9 April	*16 April*	*23 April*	*30 April*	*7 May*	*10 April*	*17 April*	*23 April*	*1 May*	
γ-Aba	0·7	0·7	1·0	1·2	2·5	0·8	0·8	2·1	3·5	4/4
Ala	0·2	0·1	0·2	0·2	2·0	0·2	0·2	0·3	0·5	10/3
Asn	0·4	0·9	2·1	5·4	6·7	1·8	2·1	8·3	10·1	17/6
Cit	36·9	32·7	54·5	76·8	107·2	68·2	105·0	85·6	163·0	3/2
Gln	10·3	15·3	30·9	90·2	188·4	23·8	50·3	131·5	177·5	18/7
Glu	5·8	7·0	17·2	30·7	62·8	9·1	14·4	41·3	41·4	11/5
Hse	tr	tr	tr	1·1	1·1	0·2	0·5	1·3	0·9	—/5
Ile	0·6	0·9	1·9	4·4	9·2	1·0	2·0	5·3	7·1	15/7
Leu	0·3	0·2	0·3	0·7	1·7	0·2	0·8	1·4	2·1	6/11
Lys	0·6	0·2	0·3	1·2	4·6	0·3	1·2	2·1	3·5	8/12
Phe	0·2	0·6	1·4	2·5	8·6	1·2	0·9	2·4	3·3	43/3
Pro	0·4	0·7	1·4	3·0	3·6	0·6	1·8	1·2	3·8	9/6
Ser	0·1	0·2	0·2	0·2	0·6	0·1	0·1	0·2	0·5	6/5
Tyr	0·2	0·2	0·4	0·7	6·8	0·5	0·4	1·4	1·0	34/2
Val	0·4	1·0	2·3	4·5	8·2	0·8	0·8	4·4	6·5	21/8

[a] Rate of increment of the amino acids in the sap of *B. pendula*/*B. pubescens*.

TABLE 3
Total Amino Acid Content (mg/litre) in the Sap of the Individual Trees in 1984

Tree	*Date*									*Liquid phase*
	9 April	*16 April*	*23 April*	*30 April*	*7 May*	*10 April*	*17 April*	*23 April*	*1 May*	
Betula pendula										
pe-1	24	41	126	192						SE[a]
pe-2	56	86	116	208	361					SE
pe-3	88	53	97	260	447					CV[b]
B. pubescens										
pu-1						115	255	485	432	CV
pu-2						109	233	301	743	SE
pu-3						99	52	68	82	SE

[a] SE-30 liquid phase
[b] Chirasil-Val liquid phase

increase in the concentration of glutamine, which at the end of the season comprised about 40% of the entire pool and thus exceeded the content of citrulline. This is in disagreement with the results of Eglite & Oskaja (1973). They did not find significant changes among the amino acids in the sap of *B. verrucosa* (= *B. pendula*). There again, Reuter & Wolfgang (1954) recognised some qualitative and quantitative changes in the amino acid pool during the flow season.

The trends of the contents of the amino acids in both birch species were analogical. The level of the pool was higher in *B. pubescens* through the whole spring, which might indicate the earlier break of the dormancy than in *B. pendula*. The flow of *B. pubescens* also terminated before the drying of *B. pendula*; the 5 May values in *B. pendula* and 1 May values in *B. pubescens* were close to each other (Table 2). The comparison between the species is, however, only coarse. One of the *B. pubescens* trees (pu-3) already showed the highest value of the total content of free amino acids on 10 April (Table 3).

Two trees of each birch species (pe-1, pe-2, pu-2 and pu-3) were analysed with SE-30 and one (pe-3 and pu-1) with Chirasil-Val phase (Table 3). The detection thresholds of the two columns were different. The minor components alanine, γ-aminobutryric acid, serine and phenylalanine could not be quantified in most of the pe-3 and pu-1 samples with Chirasil-Val phase. The analysis was possible in all the pe-1, pe-2, pu-2 and pu-3 samples with SE-30, because the detection threshold was somewhat lower with this phase. Conversely, the detection limits of homoserine and lysine were higher with SE-30. The analyses with the chiral stationary phase proved all the amino acids to be L-configuration as expected.

The high molarity of free amino groups in the sap at the end of the flow season affects the qualities of the syrup. Together with the reducing sugars (Kallio *et al.*, 1987) they accelerate the flavour and colour formation based on the Maillard reaction. This complicates the finishing of the birch syrup, and low temperature evaporation systems are needed (Kok *et al.*, 1978; Kallio *et al.*, 1985; 1987, 1989).

The overall content of sulphur containing amino acids was below the detectable limit as stated previously by Reuter & Wolfgang (1954). The dominant physiological role of the amino acid pool in the sap is to transport the active amino groups along the xylem channels to the developing cells all around the tree for the final synthesis of protein amino acids. This might be the explanation why the composition of the amino acids in the birch sap is not nutritionally well balanced. The same fact can be observed as a common feature in plants, e.g. in barley seeds, where the prolamins are the nitrogen reserves for the developing new generation. A high proportion of the nitrogen in such a protein, which is not a functional protein, is in amide form as glutamine (Shewry & Miflin, 1985).

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