



Sugars, acids, ethyl β -D-glucopyranose and a methyl inositol in sea buckthorn (*Hippophaë rhamnoides*) berries

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ARTICLE INFO

Article history:

Received 29 February 2008

Received in revised form 2 April 2008

Accepted 14 May 2008

Keywords:

Sea buckthorn

Hippophaë rhamnoides

°Brix

pH

Sugars

Acids

Ethyl β -D-glucopyranose

Methyl inositol

Harvesting time

Subspecies

Annual variation

ABSTRACT

Sea buckthorn berry is a rich source of nutrients and bioactive components beneficial for human health. Sugars and acids play an important role in determining the sensory properties of the berry. Sugars, acids, ethyl β -D-glucopyranose and a methyl inositol were analysed in berries of three subspecies (*Hippophaë rhamnoides* ssp. *sinensis*, *rhamnoides* and *mongolica*) collected from China, Finland and Russia over four consecutive years. Fructose and glucose were the major sugars, and the dominating acids were malic and quinic acids. Origin and harvesting date have significant impacts on sugars, acids and sugar/acid ratio in the berry. During the harvesting period, the sugar content followed different changing patterns in berries of different subspecies. Ethyl glucose dominated in the sugar fraction of ssp. *rhamnoides* but existed only in trace amounts in the other two subspecies. In ssp. *rhamnoides*, the level of ethyl glucose increased during the harvesting period; the increase was accompanied by a decrease in glucose content, indicating the presence of a biochemical pathway converting glucose into its derivatives. A methyl inositol was identified for the first time in sea buckthorn with higher levels found in ssp. *sinensis* than in the other two subspecies. The levels of ethyl glucose and methyl inositol may be important sensory and nutritional quality factors of sea buckthorn berry. The data presented by this study provide important chemotaxonomic information characterising different subspecies of sea buckthorn and useful guidance for breeding, harvesting, and industrial utilisation of sea buckthorn.

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1. Introduction

Sea buckthorn (*Hippophaë rhamnoides* L.) berry is rich in a wide range of lipophilic and hydrophilic bioactive components, such as essential fatty acids, phenolic compounds, vitamins, and plant sterols (Beveridge, Li, Oomah, & Smith, 1999; Guliyev, Gul, & Yildirim, 2004; Kallio, Yang, & Peippo, 2002; Rösch, Bergmann, Knorr, & Kroh, 2003; Rösch, Krumbein, Mügge, & Kroh, 2004; Yang, Carlsson, Oksman, & Kallio, 2001; Yang & Kallio, 2002). Beneficial effects of the berry and berry fractions on human health have been extensively investigated and substantiated by studies, suggesting a great potential of the berry for maintaining and promoting human health (Cheng et al., 2007; Gao, Ohlander, Jeppsson, Bjork, & Trajkovski, 2000; Johansson, Korte, Yang, Stanley, & Kallio, 2000; Rösch, et al., 2003; Shukla et al., 2006; Suleyman et al., 2002; Vijayaraghavan et al., 2006; Yang & Kallio, 2002, 2005).

Wild sea buckthorn, naturally distributed in Europe and Asia, belongs to various subspecies of *H. rhamnoides* (Rousi, 1971). A

number of varieties/cultivars have been developed and cultivated in different parts of the world. Chemical composition of the berry varies considerably among the subspecies, cultivars and varieties. Harvesting time and year have a significant impact on the quality of the berries. In addition, the influence of harvesting time seems to vary with the compounds studied, as well as the genetic background of sea buckthorn (Kallio et al., 2002; Raffo, Paoletti, & Antonelli, 2004).

Sugars and fruit acids are important components influencing the sensory properties of sea buckthorn. Both the absolute content and the relative abundance of these components play a crucial role in determining the flavour and the consumer acceptance of the berry and berry products (Tiitinen, Hakala, & Kallio, 2005; Tiitinen, Yang, Haraldsson, Jonsdottir, & Kallio, 2006a). Sugars and acids in some commercial cultivars from Germany and Russia have been reported (Raffo et al., 2004; Tiitinen et al., 2006a). Significant difference was found in the content and composition of sugars, acids and sugar/acid ratio among the cultivars studied. The sugar content followed different changing patterns in three German cultivars during the ripening period of the berries (Raffo et al., 2004). Systematic knowledge is lacking on the general profile of sugars and acids of different subspecies of sea buckthorn and the variation among harvesting dates and years. The aim of this study was to compare the content

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and composition of sugars and fruit acids in the berries of sea buckthorn of three major subspecies (*H. rhamnoides* ssp. *sinensis*, *rhamnoides*, and *mongolica*). The effects of harvesting time and annual variation on the content and composition of sugars and fruit acids of these subspecies were also studied.

In a previous publication, we reported the presence of a new sugar derivative, ethyl glucose, in sea buckthorn berry (Tiitinen et al., 2006a). In the present study, a methyl inositol was identified in the sugar fraction of the berry for the first time. The contents of these two compounds were studied in the wide range of samples belonging to three subspecies of *H. rhamnoides*. The changing patterns in the contents of these compounds during the harvesting period were also studied in two subspecies.

The data presented by this paper provide useful guidance for breeding, berry harvesting and industrial utilisation of sea buckthorn. In addition, the profiles of sugars, acids and sugar derivatives are important chemotaxonomic features characterising different subspecies of sea buckthorn.

2. Materials and methods

2.1. Sea buckthorn berries

Cultivated berries of *H. rhamnoides* ssp. *rhamnoides* were grown at the Research Station at Satakunta (Kokemäki, Finland), Agrifood Research Finland. These were selections of the Horticultural Research Institute (Piikkiö, Finland), Agrifood Research Finland. S3003 and S3006 were two individuals selected from seedlings of X-ray-irradiated seeds of wild Finnish berries. 74006003 and 74006005 were two crosses between a wild Finnish male bush (*H. rhamnoides* ssp. *rhamnoides*) and a wild German female bush (*H. rhamnoides* ssp. *rhamnoides*). Raisa is a cross between a wild Finnish female bush and a wild male bush (*H. rhamnoides* ssp. *caucasica*) from the Caucasus Mountains in Iran. Berries were collected from these selections in four years from 1996 to 1999. During the same years, wild berries of *H. rhamnoides* ssp. *sinensis* were collected from China. In addition, wild berries of *H. rhamnoides* ssp. *rhamnoides* were picked from the Baltic coast in south-western Finland in 1999 (Table 1). Berries of seven commercial cultivars of *H. rhamnoides* ssp. *mongolica* were picked from Novosibirsk, Russia (Ruet, Luchezarnaya, Dar Katuni, Vitaminaya and Maslichnaya) in 1997 and from Riihimäki (Oranzevaya and Tsuisakaya), Finland, in 1999 (Table 1). In order to follow the changes during the harvesting period, wild berries of ssp. *sinensis* from two natural growth sites were collected at different harvesting dates during the period from August to November 1998 and 1999. In addition, four Finnish cultivated bushes (74006003, 74006005, S3006, S3006) were collected at different harvesting dates from the end of August to the end of November 1998. The origins, locations and collection dates of the berry samples are listed in Table 1.

Berries were loosely frozen immediately after picking and kept at -20°C prior to being analysed within one year after the collection. Berries of each sample were pulled from a 5 kg lot following a sample partitioning procedure.

2.2. Reagents

Reference compounds, D-glucose and L-malic acid, were purchased from Fluka (Buchs, Switzerland), D-fructose and ascorbic acid from Sigma–Aldrich Finland Oy (Helsinki, Finland), quinic acid from Chem Service (West Chester, PA, USA) and citric acid from J.T.Baker (Devanter, Holland). The reference compound for methyl inositol purchased was 1L-2-O-methyl-*chiro*-inositol (Alexis Corporation, Läufelfingen, Switzerland). Ethyl β -D-glucopyranose was synthesised and purified at the University of Turku. Internal stan-

dards, L-tartaric acid and D-sorbitol, were obtained from Merck (Darmstadt, Germany).

2.3. Sample preparation

Thirty grams of sea buckthorn berries were thawed in a microwave oven (225 W) with 30% power, twice over 15 s, and shaken out in between. The berries were crushed with a spoon and juice was pressed and filtered with cheesecloth. The filtered juice sample was used for the determination of °Brix and pH and for the fractionation of sugars and acids.

2.4. Determination of °Brix and pH of berry juice

Brix values of berry juice were measured with Atago hand-held refractometers N-1 and N-10 (Atago Co. Ltd., Tokyo, Japan). The pH values were determined using a PHM 80 portable pH meter (Radiometer, Copenhagen, Denmark).

2.5. Fractionation of sugars and acids

Sugars and acids were fractionated and analysed according to the method applied earlier in this laboratory with slight modification (Kallio, Hakala, Pelkkikangas, & Lapveteläinen, 2000). One millilitre of filtered juice sample was taken, followed by addition of 1 ml of a water solution of sorbitol (2.0 g/100 ml) and tartaric acid (2.5 g/100 ml) as the internal standards, 6 ml of 0.1 N NaOH, and 11 ml of Milli Q water. For fractionation of sugars and acids, 350 μl of the dilution was applied on a preconditioned anion-exchange Isolute SAX column (International Sorbent Technology, Hengoed, UK). From the SAX column the sugars were eluted with 2 ml of Milli Q water and the organic acids were eluted with 2 ml of 15 N formic acid. Both fractions were diluted to a final volume of 3 ml, of which a sample of 1–2 ml was taken and evaporated to dryness, followed by a further drying process overnight in a desiccator. Trimethyl silyl (TMS) derivatives of sugars and acids were prepared in screw-cap glass vials sealed with butyl Teflon septa. 200 μl of the reagent Tri-Sil (trimethyl chlorosilane and hexamethyl disilazane in pyridine, Pierce, Rockford, IL) were added to the vial; the vial was tightly closed and shaken vigorously on a Vortex (Vortex-Genie, Springfield, MA) for 5 min. Thereafter, the vial was incubated at 60°C for 30 min, followed by storage at room temperature overnight before GC analysis.

2.6. Identification of sugars, acids and sugar derivative and sugar alcohol

The TMS derivatives were identified by gas chromatography–mass spectrometry (GC–MS), using a Shimadzu 17 A gas chromatograph equipped with a QP 5000 MSD detector (Shimadzu, Japan) and controlled by a Class-5000 software. A Supelco Simplicity-1 fused silica capillary column (30 m, id 0.25 mm, film thickness 0.25 μm) (Bellefonte, PA) was used for the analysis, with helium as the carrier gas. Temperature programme of the column was as follows: hold at 90°C for 2 min, increase to 275°C at a rate of $4^{\circ}\text{C}/\text{min}$, hold at 275°C for 10 min. The temperature of the injector was 210°C , and the temperature of the interface was 290°C . The scan range was m/z 40–400. The sugars, acids, sugar derivative and sugar alcohol were identified by comparing the retention times and mass spectra with those of the reference compounds.

2.7. Quantification of sugars, acids and sugar derivative and sugar alcohol

The TMS derivatives in sugar and acid fractions were analysed with a Varian 3300 gas chromatograph equipped with a flame

Table 1Brix value, pH, sugars, acids, ethyl β -D-glucopyranose and a methyl inositol (g/100 ml juice), as well as sugar/acid ratio, in berries of three subspecies of sea buckthorn

Subspecies	Origin/cultivar	Harvest. date	$^{\circ}$ Brix	pH	Malic	Quinic	Total acid	Fructose	Glucose	E. G ^a	M. I. ^b	Total sugar	Sugar/acid
<i>sinensis</i>	Wenshui, Shanxi	October 23, 96	15.8	2.6	4.5	2.2	6.7	5.2	5.7	tr ^c	0.5	10.9	1.6
		October 4, 97	16.5	2.6	4.1	5.5	9.6	3.1	3.3	tr	0.6	6.4	0.7
		September 15, 99			3.6	2.2	5.8	1.6	1.6	tr	0.3	3.2	0.6
		October 15, 99			3.8	2.3	6.1	2.4	2.5	tr	0.4	4.9	0.8
		November 15, 99			3.6	1.9	5.5	6.5	7.1	tr	0.6	13.6	2.5
<i>sinensis</i>	Fuxian, Shanxi	October 23, 96	15.4	2.6	5.2	2.6	7.8	4	4.8	tr	0.6	8.8	1.1
		October 5, 97	24.5	2.7	4.8	7.2	11.9	3.6	5.2	tr	1.4	8.8	0.7
<i>sinensis</i>	Wangtao, Shanxi	October 29, 96	13.4	2.3	7.7	2.9	10.6	1.5	2.6	tr	0.6	4.1	0.4
		October 27, 97	21.4	3	2.3	4.7	7.1	7.3	8.1	tr	0.9	15.4	2.2
<i>sinensis</i>	Kelan, Shanxi	October 30, 96	16.5	2.6	5.9	1.6	7.5	3.8	7.4	tr	0.7	11.2	1.5
		October 29, 97	18.5	2.8	3.9	2.1	6	5	8.2	tr	0.7	13.2	2.2
<i>sinensis</i>	Youyu, Shanxi	November 6, 96	15.0	2.5	6.4	4.6	11	3.9	4.5	0.1	0.8	8.4	0.8
		November 7, 97	20.0	2.9	3.7	3.5	7.1	4.7	5.8	tr	0.9	10.5	1.5
<i>sinensis</i>	Xixian, Shanxi	October 12, 96	17.0	2.4	9.2	2.5	11.6	2.5	4.4	tr	0.8	6.9	0.6
		October 17, 97	21.0	2.7	5.4	3.8	9.2	3.6	4.8	tr	1.0	8.4	0.9
		September 15, 99			4.4	2.5	6.9	3.9	4.7	tr	0.9	8.6	1.2
		October 15, 99			3.9	2.6	6.5	6.5	6.1	tr	0.8	12.6	1.9
		November 15, 99			7.7	1.1	8.8	2.1	3.9	tr	0.8	6.0	0.7
<i>sinensis</i>	Heshun, Shanxi	November 20, 96	19.0	2.7	4.8	2.9	7.7	5.9	6.5	tr	0.7	12.4	1.6
		November 5, 97	21.0	2.9	3.3	3.2	6.5	4	6.6	tr	0.8	10.6	1.6
<i>sinensis</i>	Xunyi, Shaanxi	November 11, 96	19.4	2.3	7.6	4.6	12.2	3.2	4.9	tr	0.9	8.1	0.7
		November 3, 97	26.0	3	2.6	6.3	8.9	5.7	6.4	tr	1.1	12.1	1.4
<i>sinensis</i>	Wuzai, Shanxi	November 15, 96	19.2	2.5	6	2.8	8.8	5.6	7.9	tr	0.5	13.5	1.5
		November 26, 97	22.0	2.9	1.9	3.2	5.1	6.9	7.8	tr	0.5	14.7	2.9
<i>sinensis</i>	Youyu, Shanxi	November 29, 96	17.5	2.8	3.7	3.5	7.1	4.7	7.5	0.1	0.9	12.2	1.7
		November 23, 97	19.0	3	3.5	2.9	6.4	6.1	6.1	tr	1.3	12.2	1.9
<i>sinensis</i>	Wutai, Shanxi	November 27, 96	16.8	2.6	6.1	3.1	9.2	4.7	5.5	tr	0.9	10.2	1.1
		November 21, 97	15.0	3	2.6	2.7	5.3	3.6	5.2	tr	0.5	8.8	1.7
<i>sinensis</i>	Yongshou Shaanxi	November 28, 96	16.6	2.6	5.8	3.8	9.6	4.4	4.9	tr	1.0	9.3	1.0
		November 21, 97	22.5	3	3.2	5	8.2	5.8	6	tr	1.3	11.8	1.4
		October 27, 97	18.0	2.8	4.3	2.8	7.1	4.7	5.4	tr	0.5	10.1	1.4
<i>sinensis</i>	Datong, Qinghai	November 7, 97	21.0	2.6	5.4	3.4	8.8	5.7	6.9	tr	0.7	12.6	1.4
<i>sinensis</i>	Longde, Ningxia	November 20, 97	34.0	2.9	4.5	7.5	12	11.7	12.5	tr	1.6	24.2	2.0
<i>sinensis</i>	Dingxi, Gansu	November 9, 97	25.0	2.8	4.5	5.1	9.6	5.9	6.9	tr	1.1	12.8	1.3
<i>sinensis</i>	Zhongyang, Shanxi	October 15, 98			3.4	1.6	5	3.6	5.3	tr	0.7	8.9	1.8
		October 15, 99			3.3	1.3	4.6	5.4	5.9	tr	0.5	11.3	2.5
		October 15, 99			3.1	0.7	3.8	3.8	6.5	0.1	0.5	10.3	2.7
<i>sinensis</i>	Mean		19.5 x	2.7 x	4.6 x	3.3 x	7.9 x	4.6 x	5.8 x	tr x	0.8 x	10.5 x	1.4 x
		Stdv.	4.3	0.2	1.6	1.6	2.2	1.8	1.9	0.3	0.3	3.6	0.6
<i>rhamnoides</i>	74006003 (SAT ^d)	September 4, 96	7	2.8	3.2	1.8	5	0.1	0.9	0.2	0.2	1.0	0.2
	74006003 (TUO ^e)	August 25, 97	8	2.9	3.7	2.1	5.7	0.2	1.3	0.2	0.4	1.5	0.3
	74006003 (SAT)	September 17, 99			3.2	0.8	4	0.3	0.9	1.7	0.2	1.2	0.3
<i>rhamnoides</i>	74006005 (SAT)	September 4, 96	8.5	2.7	4.3	1.6	5.9	0.2	1.1	0.2	0.1	1.3	0.2
	74006005 (TUO)	August 25, 97	9	2.8	4.3	1.9	6.1	0.4	1.9	0.3	0.4	2.3	0.4
	74006005 (SAT)	September 17, 99			4	0.7	4.7	0.3	1.3	1.1	0.2	1.6	0.3
<i>rhamnoides</i>	S3003 (SAT)	September 5, 96	8	2.7	4.6	1.2	5.7	0.2	0.8	0.1	0.2	1.0	0.2
	S3003 (SAT)	August 28, 97	7	2.8	4.7	1.2	5.9	0.1	0.6	0.2	0.2	0.7	0.1
	S3003 (SAT)	September 17, 99			4.2	0.7	4.9	0.2	1.1	0.6	0.2	1.3	0.3
R \times C ^f	Raisa (SAT)	September 1, 96	9.4	2.8	3.1	4.3	7.4	0.3	1.1	0.5	0.2	1.4	0.2
	Raisa (PTL ^g)	October 16, 97	10	3.1	2.3	2.2	4.4	0.5	1.3	1.5	0.2	1.8	0.4
<i>rhamnoides</i>	S3006 (SAT)	September 10, 96	8	2.8	3.9	1.7	5.6	0.2	1	0.1	0.2	1.2	0.2
	S3006 (SAT)	August 28, 97	7	2.8	4.5	1.7	6.2	0.1	0.5	0.1	0.2	0.6	0.1
	S3006 (SAT)	September 17, 99			4	1	5	0.2	1.3	0.2	0.2	1.5	0.3
<i>rhamnoides</i>	72004004 (SAT)	September 17, 99			3.2	2.1	5.3	0.6	1.3	1.6	0.2	1.9	0.4
<i>rhamnoides</i>	Pyhämaa, Finland	August 30, 99			3.7	1.6	5.3	0.3	2.9	0.7	0.4	3.2	0.6

(continued on next page)

Table 1 (continued)

Subspecies	Origin/cultivar	Harvest date	°Brix	pH	Malic	Quinic	Total acid	Fructose	Glucose	E. G ^a	M. I. ^b	Total sugar	Sugar/acid
<i>rhamnoides</i>	Pyhärinta, Finland	August 30, 99			3.8	1.3	5.1	0.2	2.3	0.2	0.4	2.5	0.5
<i>rhamnoides</i>	Siikajoki, Finland	September 3, 99			3.5	1	4.5	0.1	1.3	0.5	0.3	1.4	0.3
<i>rhamnoides</i>	Vaasa, Finland	September 3, 99			3.3	1.4	4.7	0.4	2.8	tr	0.2	3.2	0.7
<i>rhamnoides</i>	Pyhämaa, Finland	September 8, 99			4	1.4	5.4	0.3	0.8	1.9	0.5	1.1	0.2
<i>rhamnoides</i>	Pyhämaa, Finland	September 8, 99			2.9	0.8	3.7	0.4	2.9	1.2	0.2	3.3	0.9
<i>rhamnoides</i>	Mean		8.2 y	2.8 x	3.7 x	1.5 y	5.3 y	0.3 y	1.4 y	0.6 y	0.3 y	1.7 y	0.3 y
	Stdv.		1	0.1	0.6	0.8	0.8	0.1	0.7	0.6	0.1	0.8	0.2
<i>mongolica</i>	Ruet	August 25, 97			1.2	1.6	2.8	4.3	5.8	0.1	0.2	10.1	3.6
<i>mongolica</i>	Luchezarnaya	August 25, 97			1.1	2.6	3.6	2.8	7	0.1	0.2	9.8	2.7
<i>mongolica</i>	Dar Katuni	August 25, 97			1.3	1.8	3.1	1.5	4.3	tr	0.2	5.8	1.9
<i>mongolica</i>	Vitaminaya	August 25, 97			1.4	1.3	2.7	1.3	5.8	tr	0.3	7.1	2.6
<i>mongolica</i>	Maslichnaya	August 25, 97			0.8	1.8	2.6	0.9	7.2	0.3	0.2	8.1	3.1
<i>mongolica</i>	Oranzevaja	August 23, 99			2.3	1.3	3.6	2.8	3.5	0.1	0.3	6.3	1.8
<i>mongolica</i>	Tsuiskaya	August 23, 99			2.7	1.4	4.1	3	3.5	tr	0.2	6.5	1.6
<i>mongolica</i>	Mean		9.1 y	3.2 y	1.5 y	1.7 y	3.2 z	2.4 z	5.3 x	0.1 z	0.2 y	7.7 x	2.5 z
	Stdv.		1.6	0.1	0.7	0.4	0.6	1.2	1.6	0.1	0.0	1.7	0.8

Different letters (x, y, z) following the values in the same column indicate statistically significant difference ($P < 0.05$) between the values.

^a Ethyl β -D-glucopyranose.

^b Methyl inositol.

^c < 0.05 .

^d Agrifood Research Finland, Research Station at Satakunta (Kokemäki, Finland).

^e Agrifood Research Finland, Research Station at Tuorla (Tuorila, Piikkiö, Finland).

^f *H.rhamnoides* ssp. *rhamnoides* (♂) X *H.rhamnoides* ssp. *caucasica* (♀).

^g Agrifood Research Finland, Research Station at Piikkiö (Piikkiö, Finland).

ionisation detector (Varian, Limerick, Ireland). The column, column temperature programme and injector temperature were the same as those used in the GC–MS analysis. The split ratio of the injector was 1:20, and the detector temperature was 290 °C. The quantification of the sugars was carried out using sorbitol as an internal standard. Different correction factors were applied in the calculation of the sugars, namely 0.71 for fructose, 0.84 for glucose, and 0.66 for sucrose. The sugar derivative and sugar alcohol were quantified as sorbitol without application of correction factors. The acids were quantified using tartaric acid as an internal standard. The correction factors used were 0.88 and 1.06 for malic and quinic acids, respectively.

2.8. Statistical analysis

Statistical analysis was carried out using the statistical software SPSS for Windows version 11.0. Independent samples' *T*-Test and Mann–Whitney Test were used for the comparison of the three subspecies. Differences reaching a minimal confidence level of 95% were considered as being statistically significant.

3. Results and discussion

3.1. °Brix and pH

Brix value of the berry juice of ssp. *sinensis* varied widely from 13 to 34, whereas the corresponding values of the ssp. *rhamnoides* and *mongolica* berries were within a narrower range of 7–11 (Table 1). pH values of the juice samples were in the range 2.3–3.2, the average value of ssp. *mongolica* being slightly higher than those of the other two subspecies (Table 1). As shown in Fig. 1, during the harvesting period from late August to late November, 1998, the Brix values of the juice of the ssp. *sinensis* berries increased considerably (from 10 to 16 in berries of Xixian, and from 12 to 22 in berries of Wenshui). In contrast, the Brix value of the berry juice of ssp. *rhamnoides* remained rather constant during the whole period (Fig. 1). pH of the juices of the two subspecies followed analogous changing patterns during this harvesting period with an increasing trend from late August to mid October, followed by a decrease thereafter. The highest pH values were found at mid October (Fig. 1).

3.2. Identification of sugars, acids, ethyl β -D-glucopyranose and a methyl inositol

Fig. 2 presents the GC–FID chromatograms of the sugar and acid fractions from berries of three different subspecies of sea buckthorn. Fructose and glucose are major sugars in the berries of all the three subspecies (Fig. 2A–C). Fructose appeared as a three-peak group, representing a mixture of α - and β -furanose and β -pyranose. The two glucose peaks are the α - and β -anomers of pyranose. Sucrose was found only at trace level in most of the samples analysed. The identification of ethyl β -D-glucopyranose in the sugar fraction of sea buckthorn has recently been reported by researchers of this laboratory (Tiitinen et al., 2006a). In the present study, a previously unknown compound in the sugar fraction was identified to be a methyl inositol (Fig. 2A–C). The GC retention time and mass spectrum were identical with those of the reference compound, 1L-2-O-methyl-*chiro*-inositol (Fig. 3).

In the acid fraction, malic acid and quinic acid were the major compounds (Fig. 2D–F). In berry juice of ssp. *sinensis* and *rhamnoides*, the content of malic acid was higher than that of quinic acid (Fig. 2D and E); in the juice of the five cultivars of ssp. *mongolica* from Russia, the situation was *vice versa*. The relative abundance of malic acid to quinic acid may be used as one of the composi-

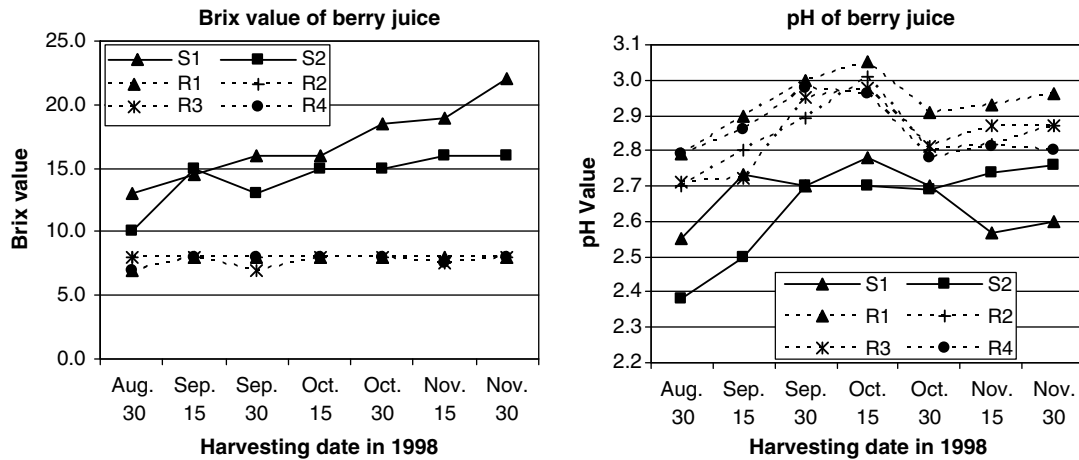


Fig. 1. Changes in °Brix and pH values of juice of sea buckthorn during the harvesting period in 1998. S1, wild berries of *ssp. sinensis* from Wenshui, Shanxi, China; S2, wild berries of *ssp. sinensis* from Xixian, Shanxi, China. R1, R2, R3, and R4 are cultivars selected from wild *ssp. rhamnoides*. R1, S3003; R2, S3006; R3, 74006003; R4, 74006005.

tional characteristics differentiating berries of different origins and cultivars. Vitamin C and some minor unidentified components were also seen in the acid chromatograms but not quantified.

3.3. Sugars

Table 1 summarises the sugar contents of the samples collected in 1996, 1997, 1999 and two Chinese samples collected in 1998. The sum of fructose and glucose in the samples analysed varied widely from 0.6 g/100 ml in juice of cultivated berries of *ssp. rhamnoides* (S3006 from Satakunta, Finland) to 24.2 g/100 ml in juice of wild berries of *ssp. sinensis* (from Longde, Ningxia Province, China) collected in 1997. The average contents of fructose and glucose in the Chinese (*ssp. sinensis*) berry juice were 15 and 4 times, respectively, as high as the corresponding values in the Finnish (*ssp. rhamnoides*) berries (fructose, 4.6 vs. 0.3 g/100 ml of juice; glucose, 5.8 vs. 1.4 g/100 ml of juice; $P < 0.001$). Therefore the sum of the two sugars in the berry juice of *ssp. sinensis* was significantly higher than that of *ssp. rhamnoides* (10.5 vs. 1.7 g/100 ml of juice, $P < 0.001$). The berries of *ssp. mongolica* had a similar level of glucose but less fructose than had the Chinese berries (2.4 vs. 4.6 g in 100 ml of juice, $P < 0.01$). Generally, the sum of fructose and glucose in the three subspecies followed the following order: *ssp. sinensis* (10.5 g/100 ml of juice) > *ssp. mongolica* (7.7 g/100 ml of juice) > *ssp. rhamnoides* (1.7 g/100 ml of juice), the level in *ssp. rhamnoides* being statistically lower than the corresponding levels in the other two subspecies ($P < 0.001$). Some annual variations were recognised in the sugar content among berries collected in different years. This could be explained by the slight variation in the collecting dates and the weather conditions over the years.

From the end of August to the end of November 1998, the levels of fructose and glucose in the Chinese (*ssp. sinensis*) berry juice clearly increased, whereas the corresponding levels in the Finnish (*ssp. rhamnoides*) berry juice decreased (Fig. 4). In the study of Raffo et al. (2004), three German cultivars, Askola, Herko and Leikora, showed different changing patterns in the contents of fructose and glucose during the ripening and harvesting period from late July to late September. The content of fructose decreased in all three cultivars; the level of glucose decreased in Herko and Leikora and was rather stable in Askola (Raffo et al., 2004). These results suggest the influence of genetic background on the changing pattern of sugar content of sea buckthorn berries during the harvest period.

3.4. Acids

Table 1 shows the content of the acids in samples collected in the years of 1996–1999. In the Chinese (*ssp. sinensis*) berries collected from different natural growth sites in the years 1996–1999, malic acid content varied widely from 1.9 to 9.2 g/100 ml of juice. The corresponding level in the *ssp. rhamnoides* samples ranged between 2.3 and 4.7 g/100 ml of juice. The average contents of malic acid in the two subspecies (average values, 4.6 vs. 3.7 g in 100 ml of juice of *ssp. sinensis* and *rhamnoides*, respectively) were not statistically different. The malic acid content in the commercial varieties of *ssp. mongolica*, 0.8–2.7 g/100 ml of juice (average 1.5 g/100 ml of juice), was significantly lower than the levels found in the other two subspecies ($P < 0.001$).

The content of quinic acid varied widely from 0.7 to 7.5 g in 100 ml of juice, the average being higher in the berries of *ssp. sinensis* (3.3 g/100 ml of juice) than in *ssp. mongolica* (1.5 g/100 ml of juice) and *rhamnoides* (1.7 g/100 ml of juice) ($P < 0.001$). The sum of malic and quinic acids in the berry juice of *ssp. sinensis* (7.9 g/100 ml of juice) was significantly higher than the corresponding values in the berry juice of the two other subspecies (5.3 and 3.2 g in 100 ml of juice of *ssp. rhamnoides* and *mongolica*, respectively, $P < 0.001$).

From late August to mid October, 1998, the content of malic acid in berry juice decreased, followed by a slight increase during the rest of the period (Fig. 5). The content of quinic acid and the sum of the two fruit acids followed a similar trend during this period (Fig. 5), which was in agreement with the changes in pH values shown in Fig. 1. Compared with the sugar content, the acid content varied to a smaller extent with harvesting dates and years.

Acid content correlates negatively with the sweetness of sea buckthorn berries (Tiitinen et al., 2005). High malic acid content is an important contributor to the sour and astringent tastes of sea buckthorn berries and juice (Tiitinen et al., 2005). Malolactic fermentation may be a potential technology for decreasing the malic acid content and improving the flavour of sea buckthorn juice (Tiitinen, Vahvaselkä, Hakala, Laakso, & Kallio, 2006b). Due to differences in chemical composition, berries of different sea buckthorn subspecies/varieties may respond differently to malolactic fermentation (Tiitinen, et al., 2006b). Thus the technology needs to be carefully optimised for different types of raw materials prior to industrial applications.

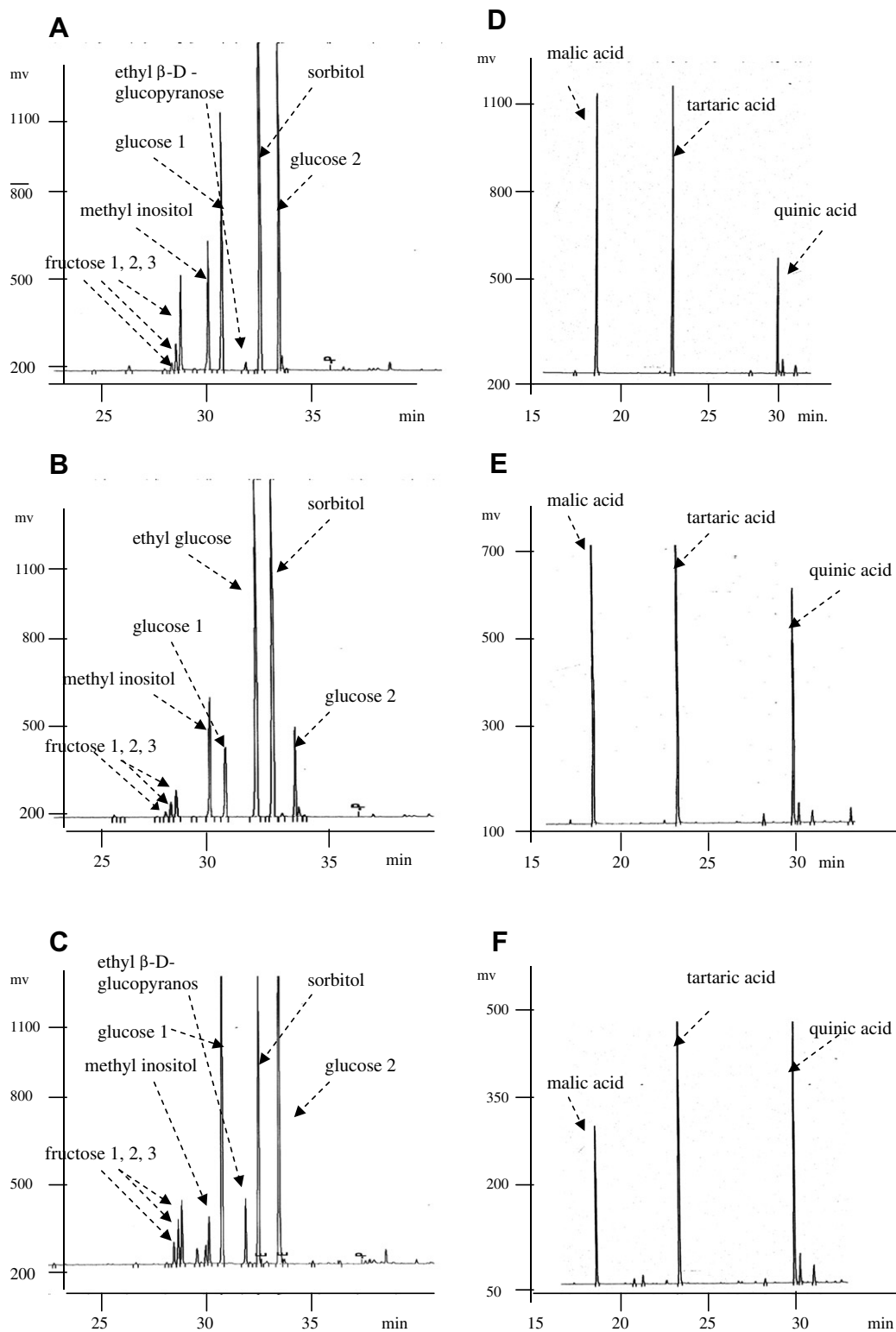


Fig. 2. Gas chromatography–flame ionisation detection (GC–FID) chromatograms of sugar and acid fractions of sea buckthorn berries of different subspecies. (A) Sugars of wild sea buckthorn from a natural growth site in Wenshui, Shanxi Province, China (*ssp. sinensis*); (B) sugars of wild sea buckthorn from the Baltic coast of Pyhämaa in south-western Finland (*ssp. rhamnoïdes*); (C) sugars of cultivar Maslichnaya (*ssp. mongolica*) from Novosibirsk, Russia; (D) acids of wild sea buckthorn from a natural growth site in Shanxi Province, China (*ssp. sinensis*); (E) acids of wild sea buckthorn from the Baltic coast of Pyhämaa in south-western Finland (*ssp. rhamnoïdes*); (F) acids of cultivar Maslichnaya (*ssp. mongolica*). The two glucose peaks are the α - and β -anomers of pyranose. Fructose appeared as a three-peak group, representing a mixture of α -, and β -furanose and β -pyranose. Sucrose (retention time: 46 min) as a minor component appeared in the sugar chromatograms of some samples.

3.5. Sugar/acid ratio

Sugar/acid ratio is an important factor determining the sensory properties of berries and fruits (Poll, 1981; Tang, Kälviäinen, &

Tuorila, 2001). The sugar/acid ratio was highest in the berries of the commercial cultivars of *ssp. mongolica* (average value 2.5) and lowest in the berries of *ssp. rhamnoïdes* (average value 0.3). The corresponding value in the wild berries of *ssp. sinensis* (average

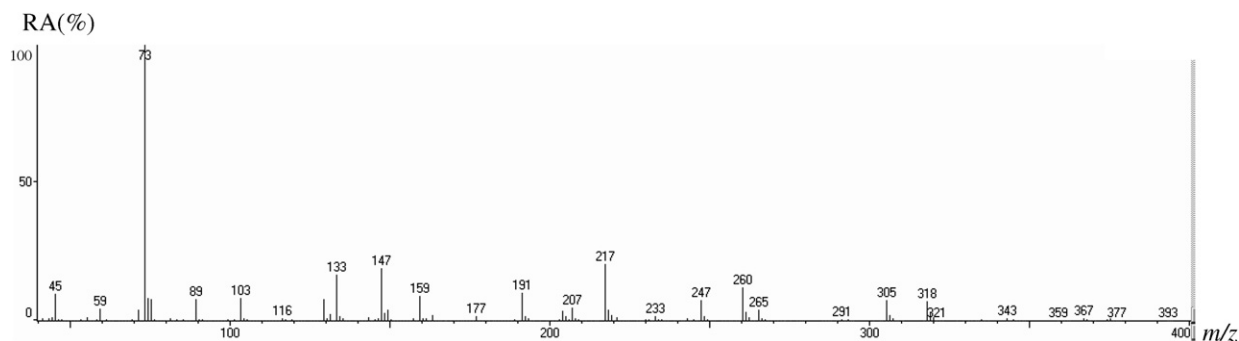


Fig. 3. Mass spectrum of trimethyl silyl ether (TMS-derivative) of methyl inositol in sea buckthorn. RA, relative abundance of ions.

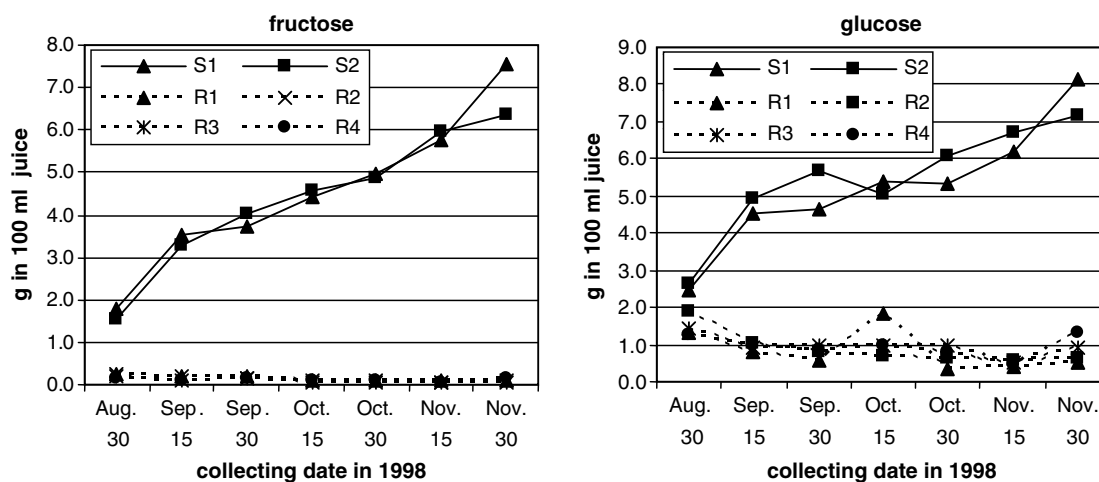


Fig. 4. Changes in content of fructose and glucose in berries of two subspecies of sea buckthorn during the harvesting period in 1998. S1, wild berries of *ssp. sinensis* from Wenshui, Shanxi, China; S2, wild berries of *ssp. sinensis* from Xixian, Shanxi, China. R1, R2, R3, and R4 are cultivars selected from wild *ssp. rhamnoides*. R1, S3003; R2, S3006; R3, 74006003; R4, 74006005.

value 1.4) fell between those of the two other subspecies (Table 1). During the harvesting period from late August to late November, 1998, the sugar content in the Chinese berries increased considerably whilst the acid content decreased slightly. These resulted in a clear increasing pattern in the sugar/acid ratio in these berries during this harvesting period (Fig. 5). In most of the Finnish berries, the sugar/acid ratio decreased during this period, mainly due to the decrease in glucose content (Fig. 5). In addition to the biological difference between the two subspecies, the different local climates among the growth locations may have contributed to the difference in the sugar/acid ratio in the berries.

Total sugar content and sugar/acid ratio correlate positively with the sweetness and the fruity flavour of sea buckthorn berries (Tang et al., 2001; Tiitinen et al., 2005). Compared with sugar content, sugar/acid ratio has been shown to be a stronger indicator of the sweetness of the berries (Tiitinen et al., 2005). The higher sugar content and sugar/acid ratio indicate that the wild berries of *H. rhamnoides ssp. sinensis* are generally sweeter than the berries of *H. rhamnoides ssp. rhamnoides*.

The high sugar content and sugar/acid ratio, together with the lower malic acid content, are important compositional factors contributing to the sweet and fruity tastes of the berries of the commercial varieties of *ssp. mongolica* (Tiitinen et al., 2005). These compositional characteristics of the commercial cultivars of *ssp. mongolica* may not represent the general situation of wild *ssp. mongolica*. Instead, it may have been the result of some specific

selection and breeding programmes, where agreeable sensory properties are often among the main targets.

3.6. Ethyl β -D-glucopyranose and a methyl inositol

Only a trace amount of ethyl β -D-glucopyranose was found in the berries of *ssp. sinensis* and *mongolica* (Fig. 2A and C), whereas the sugar derivative occurred at much higher levels in the berries of *ssp. rhamnoides* (Fig. 2B). The dominance of this compound in the sugar fraction and the lower contents of fructose and glucose were clear characteristics of *ssp. rhamnoides* compared with the two other subspecies (Fig. 2 A–C and Table 1). Whilst the content of ethyl glucose in Chinese berries remained at trace level during the whole of the harvesting period, the content of this sugar derivative increased 6–8 fold in the Finnish berries (Fig. 6). In *ssp. rhamnoides*, the increase in the content of ethyl glucose was accompanied by a decrease in the content of glucose (Fig. 4). Strong negative correlation was found between the levels of the two compounds (correlation coefficients, -0.633 in S3003, -0.945 in S3006, -0.934 in 74006003 and -0.953 in 74006005) during the harvesting period from late August to mid November. During the second half of November, the contents of both glucose and ethyl glucose increased in these berries, probably due to some temperature change during this period. In our previous study, negative correlation between the content of glucose and that of ethyl glucose was also found in the berries of several cultivars grown in Finland

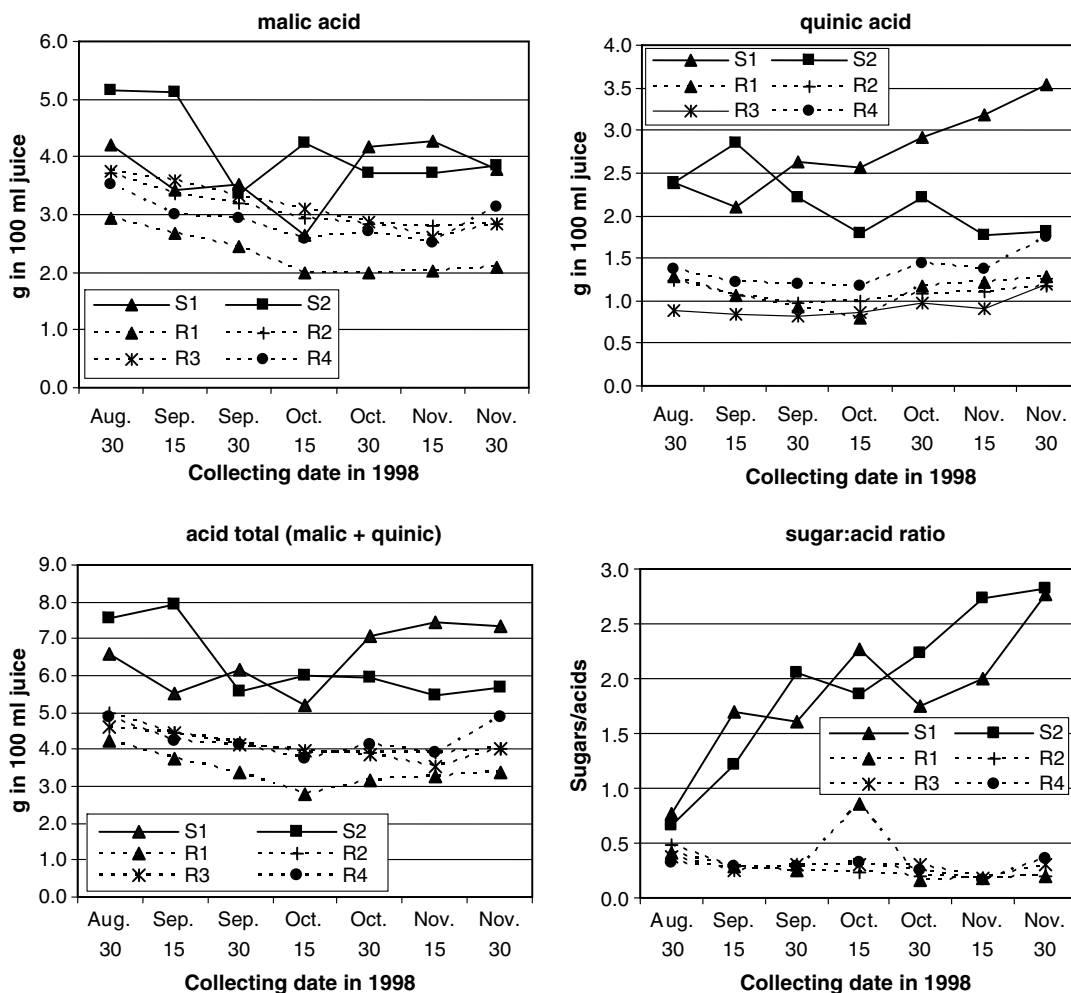


Fig. 5. Changes in contents of acids and sugar/acid ratio in berries of two subspecies of sea buckthorn during the harvesting period in 1998. S1, wild berries of *ssp. sinensis* from Wenshui, Shanxi, China; S2, wild berries of *ssp. sinensis* from Xixian, Shanxi, China. R1, R2, R3, and R4 are cultivars selected from wild *ssp. rhamnoides*. R1, S3003; R2, S3006; R3, 74006003; R4, 74006005.

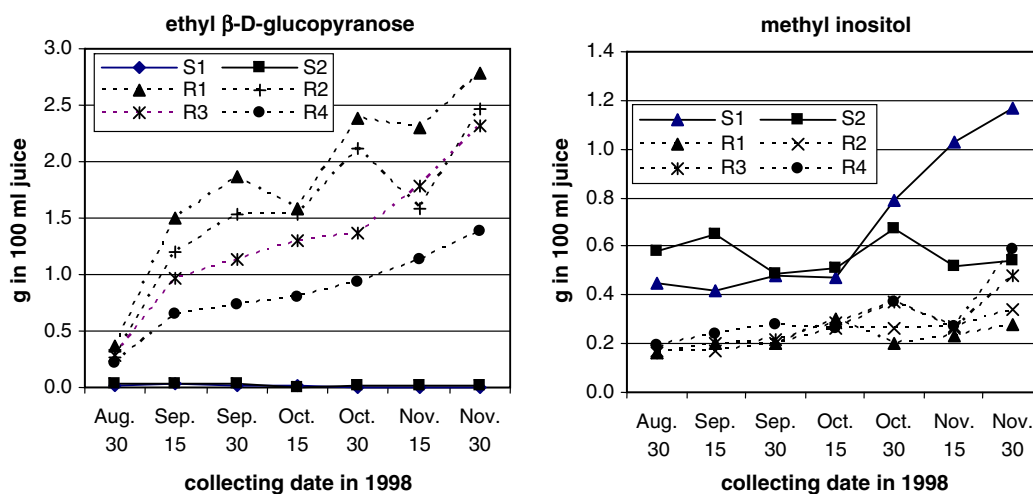


Fig. 6. Changes in contents of ethyl glucose and methyl inositol in berries of two subspecies of sea buckthorn during the harvesting period in 1998. S1, wild berries of *ssp. sinensis* from Wenshui, Shanxi, China; S2, wild berries of *ssp. sinensis* from Xixian, Shanxi, China. R1, R2, R3, and R4 are cultivars selected from wild *ssp. rhamnoides*. R1, S3003; R2, S3006; R3, 74006003; R4, 74006005.

(Tiitinen et al., 2006a). These results indicate the active role of a biochemical pathway converting glucose into its derivatives in some sea buckthorn subspecies and cultivars.

A previously unidentified S2 compound was present in the sugar fraction (Fig. 2 A–C) of all three subspecies. This compound was identified to be a methyl inositol according to the retention time

and the mass spectrum (Fig. 3). Sea buckthorn is a hardy plant, resistant to cold, drought and salt. Accumulation of the methyl inositol may be important for the plant to survive the stress of difficult growth conditions, such as water deficiency (Pattanagul & Madore, 1999). The content of this compound was generally higher in the wild Chinese berries than in the wild and cultivated berries from Finland. This difference may be explained by genetic backgrounds and/or the adaptation to different growth conditions in Finland and China. The level of this cyclitol increased in both Chinese and Finnish berries during the harvesting period that was followed in 1998 (Fig. 6), indicating increased synthesis and/or transport of this compound to the berries.

Cyclitols such as 1-D-1-O-methyl-muco-inositol, pinitol (3-O-methyl-D-chiro-inositol and ononitol (1-D-4-O-methyl-myo-inositol), have been reported to be effective scavengers of hydroxyl radicals and detoxifying compounds in biological systems (Orthen, Popp, & Smirnoff, 1994; Pharr et al., 1995). Thus, the methyl inositol may be an important natural antioxidant previously unknown in sea buckthorn. Determination of the isomeric structure of this compound by NMR analysis is important in order to understand its role in the physiology and the health benefits of sea buckthorn.

Compositional information is important for evaluating the berry quality of different sea buckthorn subspecies and cultivars. This study is the first report on the sugars and acids in the berries of three major subspecies of *H. rhamnoides*, on the basis of a large number of samples collected at different harvesting dates and in different years. The relatively high sugar content and sugar/acid ratio were clear characteristics of the berries of wild ssp. *sinensis* and the cultivars of ssp. *mongolica*, indicating more pleasant and fruitier flavour of these berries than the berries of ssp. *rhamnoides*. In addition, the cultivated berries of ssp. *mongolica* contained less malic acid than the berries of the other two subspecies. During the harvesting period, sugar content and sugar/acid ratio increased in ssp. *sinensis* but decreased in ssp. *rhamnoides*. These, together with the relative abundance of ethyl glucose and methyl inositol, are important chemotaxonomic features characterising different subspecies of *H. rhamnoides*. Many health benefits have been shown for sea buckthorn berry in scientific investigations, believed to be a result of synergy among many different bioactive components in the berry. Further studies on the health effects and sensory properties of ethyl glucose and methyl inositol of sea buckthorn will lead to a better understanding of the importance of these compounds for the quality and health benefits of sea buckthorn berries.

Acknowledgements

The author would like to express sincere thanks to Dr Saila Karhu (Horticultural Research Institute Piikkiö, Agrifood Research Finland) and Mr Zhou Xueren and He Yicai (Shanxi Forestry Research Institute, China) for providing the berry samples. The authors thank Mrs Tatjana Saarinen, Mrs Marika Lassila, and Mr Pekka Peippo for their help in the analytical work.

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