

Concentrations of Organic Acids and Soluble Sugars in Juices from Nordic Berries

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Comprehensive information was gathered on acid and sugar concentrations of six wild berries (bilberry, lingonberry, cranberry, cloud-berry, red raspberry and black crowberry) and five cultivated berries [blackcurrant, whitecurrant, redcurrant, gooseberry (red) and strawberry], all grown in Finland. The main acids of the berry juices were invariably citric and malic acids, even though their concentrations varied widely from one berry to another (2.9–16.2 and 3.3–24.7 g l⁻¹, respectively). In addition, juices of lingonberry, cranberry, cloud-berry and black crowberry contained benzoic acid (0.1–0.7 g l⁻¹). The main sugars of the investigated berry juices were fructose (18.0–57.2 g l⁻¹) and glucose (22.2–50.0 g l⁻¹). Most of the berries contained also sucrose (0.2–5.1 g l⁻¹). The data enable equivalent comparison of Nordic berries and underline the wide variation in their acid and sugar content and so the possibilities for production of numerous organoleptic profiles.

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Introduction

The composition of berries is of interest for a variety of reasons. Organic acids and sugars, and their ratios, together with different aromatic compounds, play important roles in the character and quality of the flavour and organoleptic properties of berries. Berries of the northern regions are known to be rich in organic acids. Consequently, berry juices have a very low pH (pH 2.7–3.5) and, owing to the low sugar content, the flavour is also very acidic. Interest in the composition of berries has also intensified because of increased awareness of their possible health benefits. For example, the content of sugars will be of interest to nutritionists and processors of foods for con-

sumers with special dietary requirements (Wrolstad & Shallenberger, 1981; Haila et al., 1992; Boccorh et al., 1998). From the industrial point of view, the characteristic acid and sugar composition of individual berries affects significantly the processing properties of the berry (Charley, 1977; Wrolstad & Shallenberger, 1981; Boccorh et al., 1998) and is essential when developing wine-making processes using berries as the raw material.

Despite the importance of the organic acids and soluble sugars in berries, only rare, fragmentary and incoherent data are available on their contents in Nordic berries. The methods of analysis vary from one study to another and often only total acids, titratable acids or total sugars have been determined. Moreover, the reports concern only a limited number of berries (Salo & Suomi, 1972; Kallio & Markela,

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1982; Varo et al., 1984; Haila, 1990; Haila et al., 1992; Huopalahti et al., 2000; Kallio et al., 2000). Thus, comparison of the data from different studies is difficult. Therefore, six wild and five cultivated berries, all grown in Finland, were processed similarly into juice and analysed identically. Systematic data were gathered which together with the existing data can help in the selection of berries for a variety of applications.

Material and methods

Samples

Six wild and five cultivated berries were chosen from those most commonly grown in Finland. The wild berries were bilberry [European blueberry (*Vaccinium myrtillus* L.)], lingonberry [cowberry (*Vaccinium vitis-idaea* L.)], cranberry [mooseberry (*Vaccinium oxycoccus* L.)], cloudberry [mulberry (*Rubus chamaemorus* L.)], red raspberry (*Rubus idaeus* L.) and black crowberry [*Empetrum nigrum* ssp. *hermaphroditum* (Hagerup) Böcher]. Bilberries and black crowsberries were obtained frozen from Pakkasmarja Oy (Suonenjoki, Finland). Lingonberries, cranberries and red raspberries were picked from the forests of southern Finland and cloudberry from Finnish Lapland. All of the berries were frozen immediately after harvest.

The cultivated berries were blackcurrant (*Ribes nigrum* L.), whitecurrant (*Ribes × pallidum* Otto & F. Dietr.), redcurrant (*Ribes rubrum* L.), gooseberry (red) (*Ribes uva-crispa* L.) and strawberry (*Fragaria × ananassa* Duch.). Blackcurrants, whitecurrants and strawberries were obtained frozen from Pakkasmarja Oy (Suonenjoki, Finland). Redcurrants and gooseberries (red) were from a garden in southern Finland and frozen immediately after harvest.

All berries were stored at -18°C or -25°C for 1 year at the most and allowed to thaw for 3 days at $+4^{\circ}\text{C}$ before use. Then, 750 g of thawed berries was minced and the juice was extracted in a hydraulic press (Hafico, Germany) with a maximum pressure of 300 kp cm^{-2} .

Sample preparation

Before high-pressure liquid chromatographic (HPLC) analyses of sugars, the juices were exposed to a Bond Elut strong anion-exchange column (Varian, USA) to remove the acidic compounds (Varian, 2000). The samples were diluted and centrifuged if necessary, and always filtered through a membrane filter (pore size $0.2\ \mu\text{m}$). Organic acids (citric and malic acids) and sugars (sucrose, fructose and glucose) were analysed using a HP Series 1100 HPLC (Hewlett Packard, USA) equipped with an Aminex HPX-87

H^{+} column ($300 \times 7.8\text{ mm}$, $9\ \mu\text{m}$) (Bio-Rad Laboratories, USA). Column temperature was 35°C and elution was carried out with $5\text{ mM H}_2\text{SO}_4$. The flow rate was 0.6 ml min^{-1} . For benzoic acid analyses a Hypercil BDS C8 reverse-phase column ($250 \times 4.6\text{ mm}$, $5\ \mu\text{m}$) (Hypercil, UK) was used. The column temperature was 30°C , the eluent was 0.05 M phosphate buffer and methanol (1:1) and the flow rate was 1.0 ml min^{-1} . Acids were detected with an ultraviolet detector at 214 nm and sugars with a refractive index (RI) detector (both from Hewlett Packard). Acid concentrations were calculated using formic acid and sugar concentrations with xylitol as the internal standard. Quantifications were performed in duplicate and were based on peak high or peak area measurements.

Results and discussion

The main acids of the berry juices were invariably citric and malic acid. In addition, lingonberry, cranberry, cloudberry and black crowberry contained benzoic acid. The main sugars of the investigated berry juices were fructose and glucose. Most of the berries also contained sucrose. The main acids and sugars in the wild and cultivated berry juices investigated are shown in Figs. 1 and 2 with respect to total acid or total sugar concentration. Tables 1–4 summarize available literature data on pH values, acid and sugar concentrations of the berry juices with comparison to the present results. For reference, the

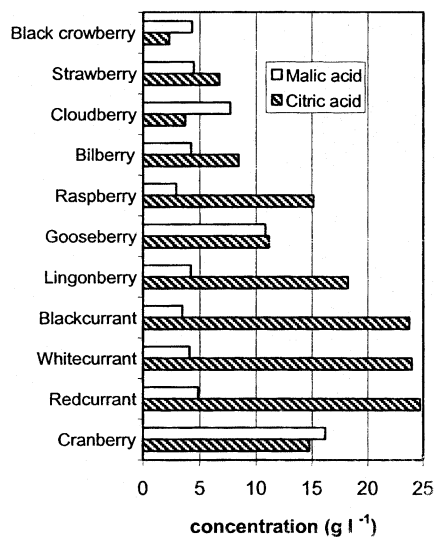


Fig. 1. The main organic acids in Finnish berry juices.

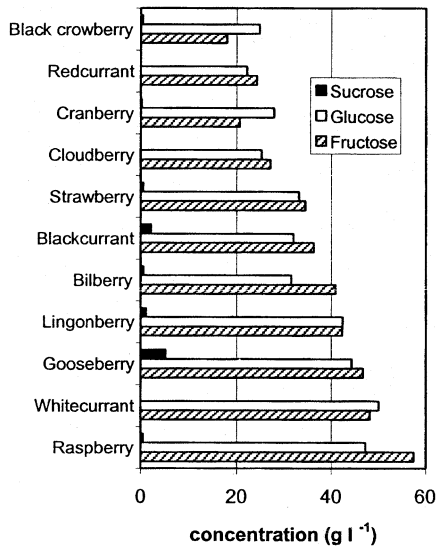


Fig. 2. The main sugars in Finnish berry juices.

tables contain literature data on some berries outside the northern region (Charley, 1977; Southgate et al., 1978; Marwan & Nagel, 1986; Spanos & Wrolstad, 1987; Plowman, 1991; Rodriguez et al., 1992; Souci et al., 1994; Herrmann, 1996).

Fig. 1 shows that the total acid content among the investigated berry juices was highest in cranberry and in different currant juices (27.2–30.9 g l⁻¹). In the currant juices citric acid comprised up to 87% (23.8–24.7 g l⁻¹) of total acids. In cranberry juice, in which the total acid content was highest, malic and citric acids were present in almost equal amounts. A similar ratio between the two acids was found, for example, in gooseberry juice. Juices of black crowberry, strawberry, cloudberry and bilberry had very low total acid content (6.6–12.6 g l⁻¹). In addition to citric acid, malic acid formed a significant proportion of total acids in these juices.

Raspberry, whitecurrant and gooseberry juices contained the highest amounts of sugars (96.1–105.2 g l⁻¹) (Fig. 2). The lowest sugar contents were in juices from black crowberry, redcurrant and cranberry (43.1–48.7 g l⁻¹). Thus, black crowberry was poor in both acids and sugars. The fructose:glucose ratio varied among the berry juices from 0.73 to 1.30 (data not shown), probably owing to the post-harvest storage. During this period, glucose and fructose in the berry participate in the initial stages of Maillard browning reactions. In such reactions glucose is the preferred substrate and they influence its content (Boccorh et al., 1998; Anon., 2002). Concentrations of sugars, especially sucrose, varied widely. This vari-

ation probably reflects the enzymatic or chemical hydrolysis of sucrose to glucose and fructose, which occurs as a result of disruption of the cellular structure during juice extraction (Plowman et al., 1989). Skrede (1983) reported that during thawing as much as 70% of sucrose is degraded by invertase. As a whole, the sugar concentration can be greatly influenced by the ripeness and post-harvest processes of the berry.

The acid concentrations of the juices of wild berries are shown in Table 1. The acid concentrations in the present results appeared mostly to be higher than has previously been reported for Nordic berries (Salo & Suomi, 1972; Solberg, 1980; Kallio & Markela, 1982; Fuchs & Wretling, 1991; Huopalahti et al., 2000). Correspondingly, the pH values were lower than reported (Kuusi, 1969; Solberg, 1980; Kallio & Markela, 1982). For example, Huopalahti et al. (2000) reported citric and malic acid concentrations for cranberry to be as low as 9.4 and 2.8 g l⁻¹, respectively. Only the total acid concentrations of bilberry and cloudberry reported by Salo & Suomi (1972) and titratable acidity of cranberry reported by Huopalahti et al. (2000) were higher than analysed in this study. As a whole, the results of the current study agree most closely with the results of Solberg (1980) and Fuchs & Wretling (1991).

In general, the organic acid concentrations and pH values of juices of cultivated berries (Table 2) agree well with the few available reports found in the literature (Kuusi, 1969; Skrede, 1982; Haila, 1990; Fuchs & Wretling, 1991; Haila et al., 1992; Kallio et al., 2000). This may be partly due to the wide variation range given in the literature or to more constant growing conditions compared with those of wild berries. Only Salo & Suomi (1972), Haila (1990) and Haila et al. (1992) reported lower acid concentrations for blackcurrant and redcurrant than measured in the present study. Salo & Suomi (1972) reported higher total acid concentrations for blackcurrant, gooseberry and strawberry than those found in the present study. Kallio et al. (2000) reported citric acid concentration as high as 7.3–15.8 g l⁻¹ for strawberry. In general, berries grown in southern Europe seem to contain less acid than those grown in the north (Tables 1 and 2). For example, in the studies of Rodriguez et al. (1992) on Spanish whitecurrant, citric acid and malic acid contents were only 19.9 and 0.4 g l⁻¹, respectively. The corresponding amounts according to the present study were 24.0 and 4.1 g l⁻¹. Differences in climate are likely to explain these distinctions.

The soluble sugar concentrations in wild berry juices are presented in Table 3. In general, the measured values were higher than reported in the literature (Salo & Suomi, 1972; Solberg, 1980; Varo et al.,

Table 1. Acid concentrations of the wild berry juices

Berry	Citric acid (g l ⁻¹)	Malic acid (g l ⁻¹)	Total organic acids (g l ⁻¹)	Titrateable acids (g l ⁻¹)	Other acids	pH	Origin	Reference
Bilberry (<i>Vaccinium myrtillus</i>)	8.42 ± 0.16	4.22 ± 0.10	14.181–14.329 10	8.4–14.1	Quinic acid Tartaric acid, chlorogenic acid, quinic acid	2.98 ± 0.03 3.11–3.25 3.0	Finland Finland Finland Norway Sweden Spain Germany	Present data Kuusi (1969) Salo & Suomi (1972) Solberg (1980) Fuchs & Wretling (1991) Rodriguez et al. (1992) Souci et al. (1994)
Lingonberry (<i>Vaccinium vitis-idaea</i>)	18.23 ± 0.67	4.20 ± 0.48	19.47 16	21.4–27.1	Benzoic acid Benzoic acid	2.67 ± 0.03 2.78–2.90 2.7	Finland Finland Finland Norway Sweden Germany	Present data Kuusi (1969) Salo & Suomi (1972) Solberg (1980) Fuchs & Wretling (1991) Souci et al. (1994)
Cranberry (<i>Vaccinium oxycoccus</i>)	14.76 ± 0.53	16.18 ± 0.41	27.6 31	38	Benzoic acid	2.37 ± 0.03 2.80–2.81 2.5	Finland Finland Finland Norway USA Finland	Present data Kuusi (1969) Salo & Suomi (1972) Solberg (1980) Marwan & Nagel (1986) Huopalahti et al. (2000)
Cloudberry (<i>Rubus chamaemorus</i>)	3.74 ± 0.02	7.70 ± 0.04	14.52		Benzoic acid (0.48 ± 0.05)	3.20 ± 0.03 3.25–3.41	Finland Finland Finland	Present data Kuusi (1969) Salo & Suomi (1972)
Red raspberry (<i>Rubus idaeus</i>)	15.15 ± 0.28	2.93 ± 0.05	13.5–31.1	12.3–27.8	Isocitric acid	3.28 ± 0.03 2.94–3.23	Finland USA, Canada, New Zealand, Germany, Romania, Hungary Sweden New Zealand Spain	Present data Spanos & Wroldstad (1987)
Black crowberry (<i>Empetrum nigrum</i>)	2.27 ± 0.30	4.30 ± 0.19	3 0.6	15.0–24.0	Benzoic acid Quinic acid, phosphoric acid, benzoic acid	3.52 ± 0.03 3.6 3.7	Finland Norway Finland	Present data Solberg (1980) Kallio & Markela (1982)

Table 2. Acid concentrations of the cultivated berry juices

Berry	Citric acid (g l ⁻¹)	Malic acid (g l ⁻¹)	Total organic acids (g l ⁻¹)	Titratable acids (g l ⁻¹)	Other acids	pH	Origin	Reference
Blackcurrant (<i>Ribes nigrum</i>)	23.75 ± 0.32	3.47 ± 0.11				3.04 ± 0.03 2.95–3.00	Finland Finland Finland Finland Sweden Finland Spain Germany	Present data Kuusi (1969) Salo & Suomi (1972) Haila (1990) Fuchs & Wretling (1991) Haila et al. (1992) Rodríguez et al. (1992) Souci et al. (1994)
	19.4–22.4 21.4–32.8 17.0–32.0 25.965–37.558 23.5–31.1	1.8–2.6 1.7–2.8 0.704–1.671 2.2–4.4	37.422 21.3–25.0 19.1–34.8	24.4–39.0	Quinic acid Quinic acid, caffeic acid, para-coumaric acid, protocatechuic acid			
Whitecurrant (<i>Ribes × pallidum</i>)	24.00 ± 0.47	4.09 ± 0.10				3.04 ± 0.03	Finland Spain	Present data Rodríguez et al. (1992)
	19.881	0.384						
Redcurrant (<i>Ribes rubrum</i>)	24.72 ± 0.80	4.85 ± 0.29				2.91 ± 0.03 3.10	Finland Finland Finland Spain Finland Germany	Present data Kuusi (1969) Salo & Suomi (1972) Rodríguez et al. (1992) Haila et al. (1992) Souci et al. (1994)
	20.591–28.159 13.8–22.9 16.9–23.0	0.507–2.133 1.9–9.8 2.4–6.4	27.216 21.2–25.1		Quinic acid, para- hydroxybenzoic acid			
Gooseberry (red) (<i>Ribes uva-crispa</i>)	11.13 ± 0.13	10.83 ± 0.15				2.96 ± 0.03 2.95	Finland Finland Finland	Present data Kuusi (1969) Salo & Suomi (1972)
			24.966–32.68					
Strawberry (<i>Fragaria × ananassa</i>)	6.75 ± 0.28	4.47 ± 0.20				3.50 ± 0.03 3.40–3.45 3.45–3.67	Finland Finland Finland Norway Finland Finland Sweden Germany, Italy, Finland Finland	Present data Kuusi (1969) Salo & Suomi (1972) Skrede (1982) Haila et al. (1992) Fuchs & Wretling (1991) Herrmann (1996) Kallio et al. (2000)
	3.6–10.6 5.0–9.4 6.8–12.2 5.0–10.3	2.4–5.2 2.4–4.9 0.7–4.9	13.158–15.51 6.0–14.8 8.1–13.5	9.4–11.7 8.4–15.1	Ascorbic acid			
	7.3–15.8	2.2–6.9		7.4–11.6		3.20–3.66		

Table 3. Sugar concentrations of the wild berry juices

Berry	Fructose (g l ⁻¹)	Glucose (g l ⁻¹)	Sucrose (g l ⁻¹)	Total sugars (g l ⁻¹)	Other sugars	Origin	Reference
Bilberry (<i>Vaccinium myrtillus</i>)	40.87 ± 0.22	31.45 ± 0.27	0.63 ± 0.04 0–5.957	72.94 ± 0.52 70.416–80.983 67	Galactose, arabinose, xylose	Finland Finland Norway Finland Sweden Not defined Germany	Present data Salo & Suomi (1972) Solberg (1980) Varo et al. (1984) Fuchs & Wretling (1991) Anon. (1993) Souci et al. (1994)
Lingonberry (<i>Vaccinium vitis-idaea</i>)	42.30 ± 0.27	42.38 ± 0.39	1.17 ± 0.01 3.465	85.85 ± 0.67 83.82 73	Galactose, arabinose, xylose	Finland Finland Norway Finland Sweden Not defined Germany	Present data Salo & Suomi (1972) Solberg (1980) Varo et al. (1984) Fuchs & Wretling (1991) Anon. (1993) Souci et al. (1994)
Cranberry (<i>Vaccinium oxycoccus</i>)	7.4–33.6	26.6–40	2 <1–3	57–98 87			
	20.69 ± 0.01	27.75 ± 0.11	0.22 ± 0.04 0	48.66 ± 0.34 49.68	Galactose, arabinose, xylose Xylose	Finland Finland Finland	Present data Salo & Suomi (1972) Mäkinen & Söderling (1980) Solberg (1980) Varo et al. (1984) Anon. (1993)
Cloudberry (<i>Rubus chamaemorus</i>)	12	22	0	40 34		Norway Finland Not defined	
	27.01 ± 0.03	25.21 ± 0.03	0 0 0	52.21 ± 0.05 37.95 77	Galactose, arabinose, xylose	Finland Finland Finland Not defined	Present data Salo & Suomi (1972) Varo et al. (1984) Anon. (1993)
Red raspberry (<i>Rubus idaeus</i>)	57.52 ± 0.05	47.21 ± 0.13	0.50 ± 0.02 20 3 0–9.1	105.22 ± 0.19 62 42.1–72.3		Finland England, USA, Switzerland Finland USA, Canada, New Zealand, Germany, Romania, Hungary Sweden New Zealand Not defined	Present data Southgate et al. (1978) Varo et al. (1984) Spanos & Wroistad (1987)
	22	16					
	24.1–37.9	18.0–35.1					
	10–66	8–40	<1–8	18–106			Fuchs & Wretling (1991)
	16.2–27.8	12.2–19.5	9–30	34–41			Plowman (1991) Anon. (1993)
Black crowberry (<i>Empetrum nigrum</i>)	17.99 ± 0.06	24.75 ± 0.09	0.41 ± 0.00	43.14 ± 0.16 45		Finland Norway	Present data Solberg (1980)

Table 4. Sugar concentrations of the cultivated berry juices

Berry	Fructose (g l ⁻¹)	Glucose (g l ⁻¹)	Sucrose (g l ⁻¹)	Total sugars (g l ⁻¹)	Other sugars	Origin	Reference
Blackcurrant (<i>Ribes nigrum</i>)	36.22 ± 0.11	31.89 ± 0.27	2.12 ± 0.04	70.22 ± 0.41	Galactose, mannose, arabinose, xylose Maltose	Finland Finland England, USA, Switzerland Finland Finland Sweden Finland Not defined Germany	Present data Salo & Suomi (1972) Charley (1977) Southgate et al. (1978) Varo et al. (1984) Haila (1990) Fuchs & Wretling (1991) Haila et al. (1992) Anon. (1993) Souci et al. (1994)
39	30	10	80.784				
40	35	3	45.5–129.0				
22.8–27.0	17.5–21.6	6.2–9.3	104				
29–64	22–47	<1–6	46.4–57.0				
20.9–31.7	16.0–27.2	4.5–11.8	52–118				
26.8–54.0	20.2–46.2	5.3–10	41.8–62.4				
			103				
Whitecurrant (<i>Ribes × pallidum</i>)	48.11 ± 0.27	50.02 ± 0.25	0	98.13 ± 0.52		Finland England, USA, Switzerland Germany	Present data Southgate et al. (1978) Souci et al. (1994)
30	31	11	72				
30	31	5.8					
Redcurrant (<i>Ribes rubrum</i>)	24.19 ± 0.20	22.15 ± 0.28	0	46.34 ± 0.47	Galactose, mannose, arabinose, xylose	Finland Finland England, USA, Switzerland Finland Finland Not defined Germany	Present data Salo & Suomi (1972) Southgate et al. (1978) Varo et al. (1984) Haila et al. (1992) Anon. (1993) Souci et al. (1994)
30	29	2.856	60.312				
44	29	5	64				
18.6–29.0	12.4–25.4	0–1.7	32.7–54.4				
18.7–30.0	7.3–29.0	0.9–5	75				
Gooseberry (red) (<i>Ribes uva-crispa</i>)	46.70 ± 0.26	44.32 ± 0.33	5.11 ± 0.04	96.12 ± 0.62	Galactose, mannose, arabinose, xylose	Finland England, USA, Switzerland Finland Not defined	Present data Salo & Suomi (1972) Southgate et al. (1978) Varo et al. (1984) Anon. (1993)
37	36	11	84				
24	28	2	54				
Strawberry (<i>Fragaria × ananassa</i>)	34.43 ± 1.68	33.07 ± 1.72	0.58 ± 0.01	68.08 ± 3.41	Galactose, arabinose, xylose	Finland England, USA, Switzerland Norway Norway Finland Finland Sweden Finland Not defined Germany, Italy, England, Finland, Switzerland Finland	Present data Salo & Suomi (1972) Southgate et al. (1978) Skrede (1982) Skrede (1983) Varo et al. (1984) Haila (1990) Fuchs & Wretling (1991) Haila et al. (1992) Anon. (1993) Herrmann (1996) Kallio et al. (2000)
26	22	11	57.86–61.472				
18.1–27.2	15.2–25.6	10.961–20.502	59				
22.6–30.0	4.8–20.3	17.3–24.7					
24	29	20.6–30.2					
20.2–26.4	19.1–25.2	21	46.2–74.9				
28–51	23–43	4.4–30.5	54–96				
19.8–25.8	17.1–24.8	<1–11	45.1–68.7				
16.0–38.6	13.8–36.5	4.3–29.3	47–74				
21.4–41.4	18.9–45.2	0–29.3	36.8–85.4				
		9.0–38.7	53.5–109.6				

1984; Fuchs & Wretling, 1991; Anon., 1993). For example, according to Varo et al. (1984) the fructose content of lingonberry is only 29 g l⁻¹, glucose content 36 g l⁻¹ and sucrose content 2 g l⁻¹, and for cranberry 12, 22 and 0 g l⁻¹, respectively. The low sugar concentration of raspberry reported in the literature (Varo et al., 1984; Fuchs & Wretling, 1991) may be explained if the analysed berry was cultivated, not wild-grown as in the present study. Amongst the sugar concentrations only Salo & Suomi (1972), Solberg (1980) and Anon. (1993) reported higher total concentrations for cranberry, black crowberry and lingonberry than shown in this study. Cloudberry juice contained notably lower glucose and fructose concentrations than reported by Varo et al. (1984) as well as less total sugar than reported by Anon. (1993). However, Salo & Suomi (1972) measured only 37.95 g l⁻¹ total sugars, whereas the present study showed a concentration of over 52.21 g l⁻¹ for the same berry. Only Fuchs & Wretling (1991) reported similar results to those obtained here.

The present results on the sugar concentrations in juices from the representative cultivated berries (Table 4), particularly strawberry, were in fairly good agreement with available literature reports (Haila, 1990; Fuchs & Wretling, 1991; Haila et al., 1992; Anon., 1993; Kallio et al., 2000). The similar results of Fuchs & Wretling (1991) were probably due to the wide range of variation for the concentrations. In addition, previous data have shown that when the concentration of sucrose is high, the concentrations of glucose and fructose are correspondingly low (Skrede, 1982, 1983; Varo et al., 1984; Haila, 1990; Haila et al., 1992). Inversion of sucrose has been observed to occur during thawing of the berries and also as a result of the juicing process (Plowman, 1991). One exception was the sugar concentration of redcurrant as reported by Varo et al. (1984), where the values were much higher (fructose 44, glucose 29 and sucrose 2 g l⁻¹) than those obtained in the present study (24.2, 22.2 and 0 g l⁻¹, respectively). However, the berry variety used by Varo et al. was not reported, and totally different analytical methods were used.

The berries underwent exactly the same storage, juice preparation and analytical procedures, thus enabling their comparison. Nevertheless, differences between the present and existing data are still obvious. Seasonal and variety differences as well as geographical origin probably serve at least as a partial explanation for this. Support for geographical influences comes from the fact that scattering between the present and previous results is in general smaller amongst berries from northern regions. Variables such as post-harvest storage and degree of ripeness are difficult to eliminate in a comparative study in-

cluding several berries. However, in the present study storage conditions, sample treatment and methods of analysis were excluded as variables and thus useful data were obtained that may be used in a wide field of applications.

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