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Factors influencing quantities of sugars and organic acids in blackcurrant concentrates

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Abstract Use of endogenous non-volatile flavour components, i.e. sugars and organic acids, in fruit juice products is desirable. A study of 133 blackcurrant concentrates from three seasons examined variation in sugars and acids arising from storage of fruit at freezing or sub-ambient temperature, seasonal differences, geographical origin and choice of conventional thermal-evaporative or freeze concentration technology. Compared with freeze concentrates, conventional concentrates had significantly higher contents of total sugars and acids, notably malic acid, and higher fructose/glucose, lower malic/citric acid and similar sugar/acid ratios. Concentrates from frozen fruit generally had smaller amounts of fructose, total sugars and fructose/glucose ratios than those from fresh fruit, as well as less citric, ascorbic and total acids and lower sugar/acid ratios. Principal component analysis of 40 randomly chosen concentrates showed that variance is dominated by differences in fructose, total sugars and ascorbic acid contents and sugar/acid ratios. Geographical origin and concentration technology were major sources of variance but changes in post-production sub-ambient storage could not be excluded.

Key words Fruit · HPLC · Flavour quality · Sugar/acid ratios · Principal component analysis

Introduction

Sugars and organic acids, and their ratios, play important roles in the character and quality of the flavour of fruit juice products [1]. Commercially, endogenous sugars and acids have been supplemented with sugars and starch hydrolysates, as well as organic and mineral acids [1]. However, with changes in consumer demand and labelling it has become desirable to add only non-nutritive sweeteners to achieve acceptability.

Most blackcurrant drinks are produced from thermal-evaporative juice concentrates. During concentrate manufacture, major changes in fruit volatiles and Maillard browning reactions are favoured by increases in reactant concentrations and the elevated temperatures. Reactions between sugars and other components will continue in concentrates, albeit at reduced rates during subsequent storage at sub-ambient temperatures [2].

Alternative freeze concentration processes are now available, offering enhanced flavour quality [3]. Therefore, it is important to understand those factors that determine concentrate composition, particularly in terms of sugars and organic acids, in order to produce juice products that are perceived to have a natural character. Differences in post-harvest storage, the season of the fruit and processing technology all contribute to variation in juice products [4].

This work was aimed at establishing the significance of variation in the quantities of sugars and organic acids in thermal-evaporative blackcurrant concentrates from three seasons, prepared from either fresh or frozen fruit of various geographical origins, and comparing data with values for three freeze concentrates. This paper presents univariate and multivariate statistical analyses of data obtained by high-pressure liquid chromatography (HPLC) after sorbent extraction of the above-mentioned solutes from the concentrates.

Materials and methods

Samples. Blackcurrant concentrates ($n = 133$), processed from fruits of three seasons, were supplied by a UK manufacturer. A breakdown of samples from each season is provided in Table 1. All concentrates were stored at 4 °C in the dark.

Sorbent extraction. The method of van Horne [5] was adapted. Concentrate (1 ml) was diluted to 100 ml with deionized water, and acetic acid (AnalaR) and sucrose (AnalaR) were added, to 1 mg ml^{-1} final concentration, as internal standards. Solutions were neutralized with a few drops of 2 M NaOH (AnalaR). Cyclohexyl [CH; 1000 mg

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Table 1 Sugar contents of blackcurrant concentrates in 1989, 1990 and 1992 seasons. (*n* Total number of concentrates)

Season	Post-harvest storage/ Geographical origin (*n*)	Fructose (mg g^{-1})	Glucose (mg g^{-1})	Total sugars (mg g^{-1})	Fructose/glucose ratio
1989	UK Fresh fruit (mean values) (13)	131.69	78.0	209.69	1.69
	SD	4.79	2.59	7.38	0.01
	SE	1.33	0.72	2.05	0.00
	UK Frozen fruit (32)	122.18	75.76	197.94	1.61
	SD	5.69	4.28	9.78	0.04
	SE	1.01	0.76	1.73	0.01
	New Zealand fruit (2)	169.86	112.48	282.34	1.51
	SD	1.17	0.77	1.94	0.00
	SE	0.83	0.55	1.37	0.00
1990	UK Fresh fruit (mean values) (34)	191.93	131.00	322.93	1.47
	SD	15.13	8.83	22.76	0.07
	SE	2.59	1.51	3.90	0.01
	UK Frozen fruit (10)	174.65	131.06	305.71	1.33
	SD	0.68	4.33	4.32	0.05
	SE	0.22	1.37	1.37	0.01
	Polish fruit (7)	163.20	122.40	285.60	1.33
	SD	6.76	4.34	11.10	0.01
	SE	2.55	1.64	4.19	0.00
	Imported fruit (7)	174.53	129.35	303.88	1.35
	SD	6.18	3.80	9.96	0.01
	SE	2.34	1.44	3.77	0.00
	New Zealand fruit (2)	151.11	108.43	259.54	1.39
	SD	1.57	0.45	2.01	0.01
	SE	1.11	0.32	1.42	0.01
	Freeze concentrates (3)	135.23	111.41	246.64	1.21
	SD	22.87	16.38	39.07	0.05
	SE	13.21	9.45	22.56	0.03
1992	UK Fresh fruit (mean values) (12)	169.88	137.53	307.41	1.24
	SD	25.17	13.53	29.31	0.20
	SE	7.27	3.91	8.46	0.58
	UK Frozen fruit (11)	153.56	135.54	289.10	1.13
	SD	13.35	11.43	20.40	0.10
	SE	4.03	3.45	6.15	0.03

(Varian)] and quaternary amine [SAX; 500 mg (Varian)] phase sorbent extraction columns were conditioned with column volumes of methanol and water and then mounted in tandem. Diluted concentrate (2 ml) was passed through at 2 ml min^{-1} at 20 kPa vacuum. Filtrates were collected for sugar analysis. Top columns (CH) were then removed and SAX phases eluted with 2 ml of 0.2 M phosphoric acid (AnalaR) at 1 ml min^{-1} at 20 kPa vacuum. Eluates were collected for analysis of organic acids.

For both groups of solutes identification was performed by preliminary HPLC analyses without internal standards, in which rentention times of resolved peaks were matched with those of a number of standard pure solute solutions.

HPLC analysis. Sugars were quantified on Spherisorb NH_2 (5 × 240 mm) (Phase Separations, Clwyd, Wales) using acetonitrile/water (80/20) at ambient temperature and an ACS 740/14 evaporative mass detector (HPLC Technology, Macclesfield, England). Organic acids were determined on Spherisorb C18 (5 × 240 mm), eluting with 0.2 M phosphoric acid and monitoring effluent at 210 nm. Dry matter contents were calculated [6] and regression lines obtained using standard solutions of each identified component. Quantifications were performed in duplicate, calculating values using the internal standards.

Statistical analyses. Data were subjected to analysis of variance (ANOVA) using Minitab v8.2 and significance was inferred if $P < 0.05$. Principal component analyses were effected using Unscrambler II v3.1 (Camo A/S, Trondheim, Norway). This multivariate statistical procedure proceeds by extracting linear combinations of the original variables in data, to obtain a smaller number of variables (principal components, or factors). The first few factors explain the maximum of variance, whilst minimizing loss of information. Relationships between samples are described in two-dimensional spaces (Figs. 1–6), in which values (scores) on the first two principal components form the *x* and *y* coordinates (product spaces), respectively. The relationships between samples and the original variables are represented by plotting the distribution of variables (loadings) on the product spaces, to yield bi-plots.

Results

Tables 1 and 2 summarize available data on blackcurrant concentrates, including: season, geographical origin, post-harvest storage of fruit and concentration technology. Quantities of sugars and organic acids, ratios between individual sugars and between organic acids as well as total sugars/acid ratios were calculated. The significance of variation due to post-harvest storage and the geographical origin of the fruit was assessed. Differences were observed both between and within 1989 and 1990 concentrates for contents of fructose and total sugars and fructose/glucose ratios but not for glucose contents. Variation within 1989 and 1990 concentrates associated with the different geographical origins of the fruit were significant for all sugar

Table 2 Non-volatile acid contents of blackcurrent concentrates in 1989, 1990 and 1992 seasons

Season	Post harvest storage/ Geographical origin	Citric acid (mg g^{-1})	Ascorbic acid (mg g^{-1})	Malic acid (mg g^{-1})	Total acid (mg g^{-1})	Citric/malic acid ratio	Sugar/acid ratio
1989	UK Fresh fruit (mean values)	156.61	8.60	9.33	174.54	16.80	1.20
	SD	9.59	0.53	0.69	10.81	0.40	0.78
	SE	2.66	0.15	0.19	3.00	0.11	0.02
	UK Frozen fruit	148.58	6.14	8.89	163.61	16.71	1.21
	SD	11.66	0.70	0.86	13.22	0.41	0.11
	SE	2.06	0.12	0.15	2.34	0.07	0.02
	New Zealand fruit	171.81	7.50	10.66	189.97	16.12	1.49
	SD	0.44	0.04	0.06	0.54	0.05	0.01
	SE	0.31	0.02	0.05	0.38	0.04	0.01
1990	UK Fresh fruit (mean values)	142.37	12.44	12.96	167.77	10.98	1.92
	SD	12.50	2.34	3.77	17.64	1.57	0.21
	SE	2.14	0.40	0.65	3.03	0.27	0.04
	UK Frozen fruit	142.36	10.46	13.30	166.12	10.70	1.84
	SD	5.23	0.99	1.30	5.73	0.86	0.06
	SE	1.65	0.31	0.41	1.81	0.27	0.02
	Polish fruit	162.24	16.75	20.87	199.87	7.77	1.43
	SD	14.94	1.41	1.69	17.12	0.46	0.11
	SE	5.65	0.53	0.64	6.47	0.17	0.04
	Imported fruit	154.06	10.92	15.32	180.30	10.06	1.69
	SD	15.77	2.51	2.70	19.92	0.85	0.22
	SE	5.96	0.95	1.02	7.53	0.32	0.08
	New Zealand fruit	148.27	9.75	14.36	172.38	10.33	1.51
	SD	1.21	7.07 e^{-3}	0.22	1.42	0.07	0.00
	SE	0.85	5.00 e^{-3}	0.16	1.00	0.06	0.00
	Freeze concentrates	128.13	3.36	0.88	132.37	145.60	1.86
	SD	24.97	2.01	0.47	26.97	53.98	0.31
	SE	0.86	1.16	0.27	15.57	31.16	0.18
1992	UK Fresh fruit (mean values)	234.36	10.08	2.47	246.91	94.88	1.25
	SD	31.26	2.35	0.76	31.10	34.34	0.18
	SE	9.02	0.68	0.22	8.98	9.91	0.05
	UK Frozen fruit	163.97	6.40	1.81	172.18	90.59	1.68
	SD	18.36	2.06	0.95	19.68	68.69	0.28
	SE	5.54	0.62	0.29	5.93	20.71	0.08

parameters. In 1989 concentrates, variations in the contents of individual (citric, ascorbic and malic) and total acids in relation to post-harvest fruit storage were significant except for citric/malic acid and sugar/acid ratios. Differences due to geographical origin were significant for all parameters except ascorbic acid content. In 1990 concentrates, post-harvest fruit storage differences were significant in terms of all organic acid parameters whereas geographical origin was correlated with significant variation only for quantities of citric and total acids and not other acid parameters.

ANOVA was performed to study the effect of concentration technology. Significant differences in the amounts of fructose, glucose and total sugars as well as all acid contents and sugar/acid ratios but not the citric/malic acid ratio were observed. Two-way ANOVA indicated that the combination of post-harvest storage of fruit and concentration technology contributed significantly to the variation in the amounts of glucose and total sugars as well as the fructose/glucose ratio but not fructose contents and all acid values except malic acid content.

For 1992 concentrates, ANOVA indicated that the amounts of fructose and citric, ascorbic and total acids varied significantly in relation to post-harvest storage as did fructose/glucose and sugar/acid ratios.

Principal component analysis was also performed on the combined sugar and acid data sets from concentrates for each season. For the 1989 concentrates, samples scores on the first two components accounted for 97% variance but showed no clear differentiation within UK concentrates on the basis of post-harvest storage of fruit (Fig. 1). There was, however, clear separation between New Zealand and UK concentrates. Loadings indicated the former group of concentrates to have high levels of fructose, glucose and total sugars. Separation on the basis of fruit storage was apparent on the third component (Fig. 2). This factor accounted for only 3% of total variance, and was highly correlated with high fructose/glucose ratios in UK concentrates obtained from frozen fruit.

Figure 3 shows a principal component analysis bi-plot for 1990 concentrates: PC1 accounts for 66% of the total variance. Discrimination was based upon differences in the amounts of fructose and total sugars as well as the fructose/glucose ratios. Among UK concentrates, those made from frozen fruit had slightly lower (but significantly different) levels of these parameters. Factor 2, accounting for 18% of the variance, did not show clear separation of samples according to their known source of variation. Loadings, however, revealed that concentrates were separated on the basis of total acids, with citric and malic acids having large

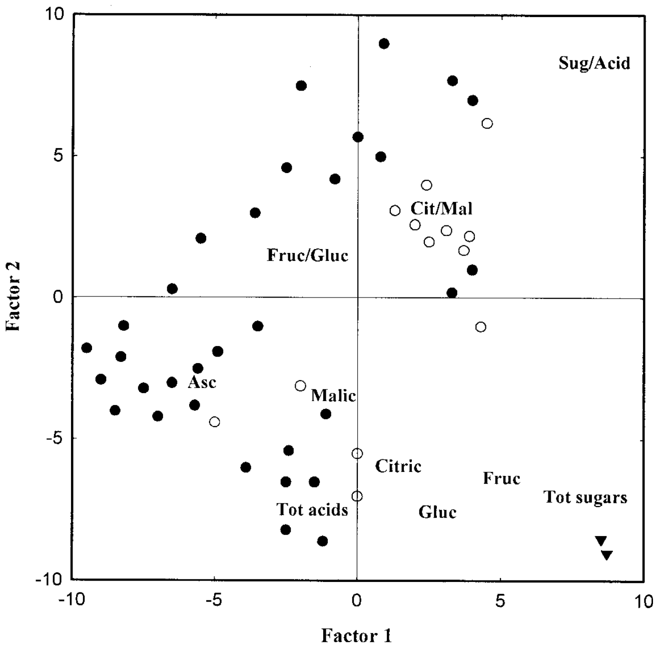

Fig. 1 Principal component analysis (*PCA*) bi-plot on the first and second factors, showing relationships between fresh (○), frozen (●) UK and New Zealand (▼) concentrates of the 1989 season, based on sugar and acid contents

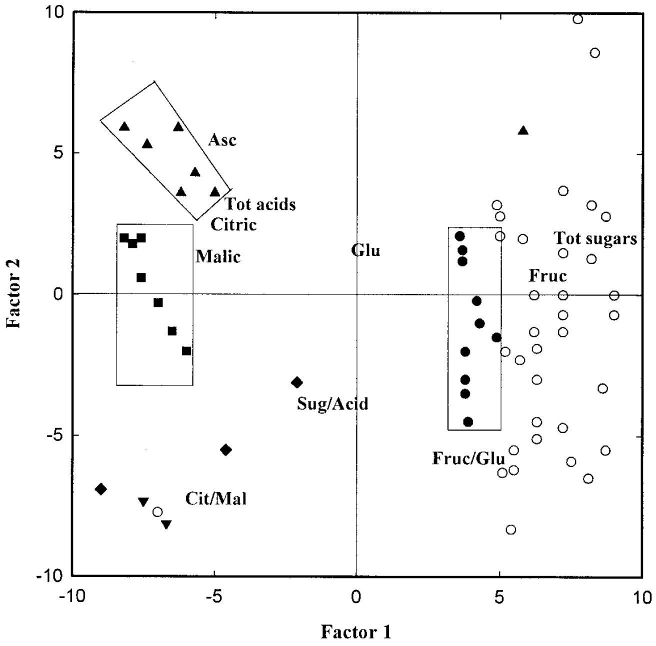

Fig. 3 PCA bi-plot on the first and second factors, showing relationships between fresh (○), frozen (●) fruit UK concentrates, imported (■), Polish (▲) and New Zealand (▼) and freeze (◆) concentrates of the 1990 season, based on sugar and acid contents

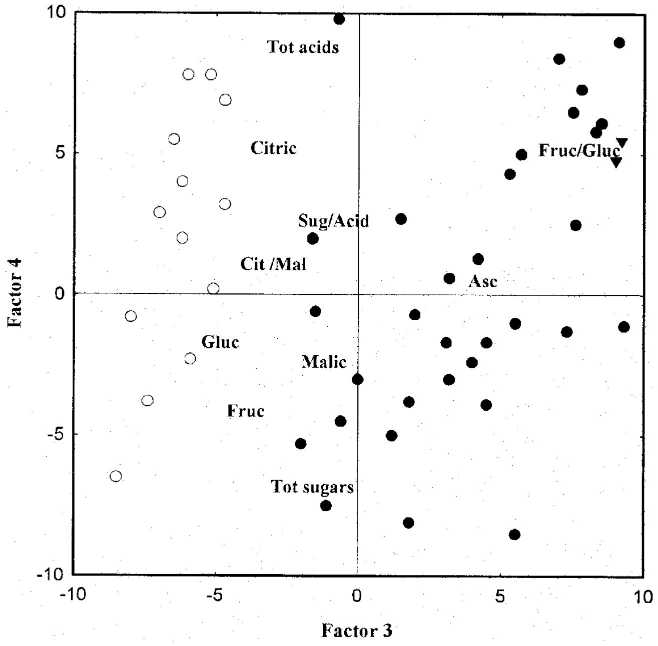

Fig. 2 PCA bi-plot on the third and fourth factors, showing relationships between fresh (○), frozen (●) UK and New Zealand (▼) concentrates of the 1989 season, based on sugar and acid contents

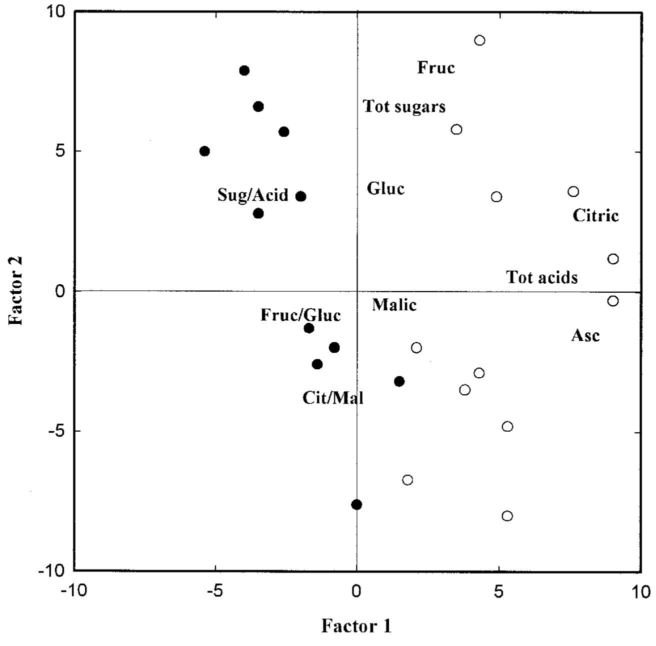

Fig. 4 PCA bi-plot on the first and second factors, showing relationships between fresh (○) and frozen (●) concentrates of the 1992 season, based on sugar and acid contents

influences. Polish concentrates were discriminated on the basis of containing relatively large amounts of total, citric and malic acids, whereas those from New Zealand contained relatively little. UK fresh-fruit concentrates were widely distributed on the basis of this component.

For 1992 concentrates (Fig. 4) PC1 explained 77% of the variance, clearly separating concentrates manufactured from fresh and frozen fruit on the basis of amount of total acids, mainly citric and ascorbic acids. In contrast, PC2 (14% of the variance), separated concentrates on the basis

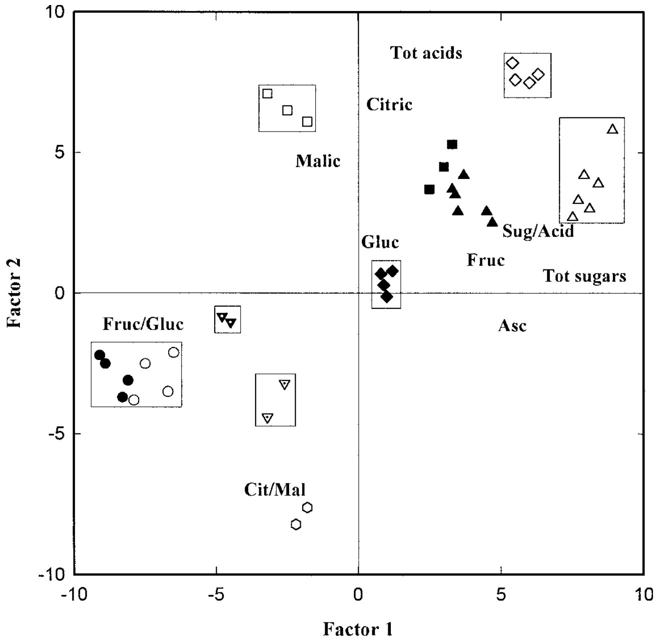

Fig. 5 PCA bi-plot on the first and second factors, showing relationships between season, post-harvest storage, geographical origin and concentration technology on sugar and acids contents of blackcurrant concentrates. (○ 1989 UK fresh fruit, ● 1989 UK frozen fruit; ▽ 1989 New Zealand, △ 1990 UK fresh fruit, ▲ 1990 UK frozen fruit, ■ 1990 imported fruit, □ 1990 Polish, ▼ 1990 New Zealand, ⬡ 1990 freeze samples, ◇ 1992 UK fresh fruit, ◆ UK frozen fruit)

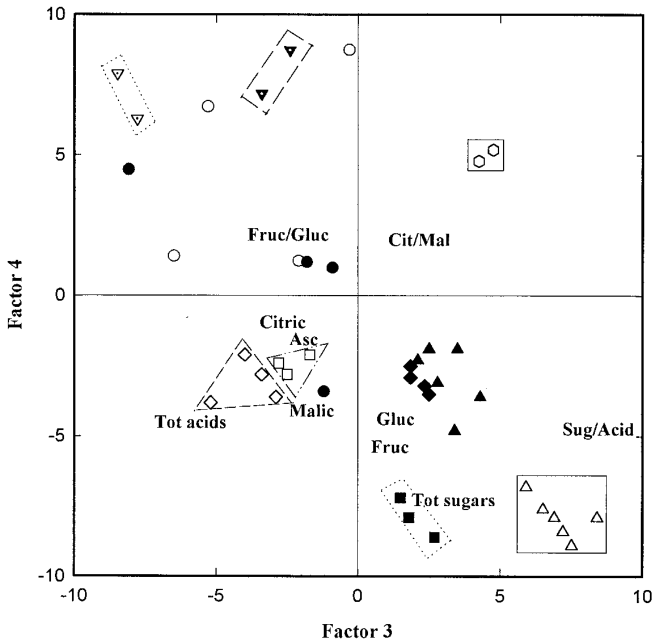

Fig. 6 PCA bi-plot on the third and fourth factors, showing relationships between season, post-harvest storage, geographical origin and concentration technology on sugar and acids contents of blackcurrant concentrates. See legend to Fig. 5 for explanation of symbols

of their fructose and total sugar contents. This was not correlated with any known source of difference.

The results of principal component analysis on the data of 40 randomly selected concentrates from all three seasons, varying in post-harvest storage, geographical origin and concentration method, are indicated in Fig. 5. Factor 1, 37% variance, grouped UK 1990 and 1992 concentrates as well as imported concentrates and could be related to differences in fructose, total sugars and ascorbic acid contents and sugar/acid ratios. Factor 2 (19% variance) discriminated UK 1990 and 1992, Polish and imported concentrates on the basis of citric and total acid contents. Inspection of scores on PC3, 8% variance (Fig. 6), separated concentrates primarily on the basis of differences in sugar/acid ratios.

Discussion

From this study it is apparent that freeze concentrates have significantly lower contents of individual and total acids compared with conventional thermal-evaporative concentrates made from fresh berries, but similar values for citric/malic acid ratios. The effect of post-harvest storage of berries is also clear: thermal concentrates from fresh berries have significantly higher ($P < 0.050$) ascorbic acid contents (11.1–12.4 mg g^{-1}) than those from frozen fruit (9.8–10.0 mg g^{-1}). Fresh-fruit thermal concentrates also have significantly higher ($P < 0.050$) citric/malic acid ratios, at 11.6–12.5, than those from frozen fruit, at 10.0–10.5, which is a reflection of increases in malic acid content from 10.4–12.7 to 13.5–14.5 mg g^{-1}. Such differences associated with post-harvest fruit storage were significant for total acids and sugar/acid ratios.

Although there were significant variations in fructose and total sugars contents associated with use of frozen fruit, glucose contents were similar. When the combination of season and post-harvest storage was considered, neither variation in fructose nor total sugars content was significant. In all three seasons the fructose/glucose ratio was significantly related with post-harvest fruit storage. When the geographical origin of the fruit was considered, only variations in the quantities of citric and total acids were significant.

Of the individual seasons, it was apparent that 1989 thermal concentrates could be discriminated into two clusters on the basis of post-harvest fruit storage, with those from fresh berries containing more fructose and, consequently, having higher fructose/glucose ratios. Concentrates from New Zealand frozen fruit contained more of all sugars, but had lower fructose/glucose ratios compared with those made from UK berries. Results for 1990 concentrates were similar; however, 1992 concentrates had lower fructose/glucose ratios compared with those from preceding years.

Variation in the sugar contents of thermal concentrates can have two origins: differences in fruit and its post-harvest storage prior to processing; and variation in the rates or extent of post-processing reactions during storage at sub-ambient temperatures. Differences in blackcurrant composition can arise from factors related to genotype, soils and agronomic practice. During post-harvest storage of fruit, concentrate manufacture and the subsequent storage at sub-ambient temperatures, glucose and fructose will participate in the initial stages of Maillard reactions, forming Amadori and Heyns intermediates. In such reactions, glucose, the preferred substrate [7], is slowly converted to 3-substituted furans and furaneols [8–10]. Although 3-(2*H*)-furanones may theoretically be formed, the acidity of the concentrates does not favour such reactions [11].

Prolonged storage of concentrates, such as those of the 1989 season, was predicted to reduce ascorbic acid contents. Thomson and Fennema [12] suggested losses of up to 30% in ascorbic acid content during freezing, storage and subsequent thawing with many fruits, a view not shared by Loeffler [13] or Sulc [14]. Thomson and Fennema [12] did, however, concede that a low pH and low oxygen content may restrict losses to $<5\%$. In the present study, 1992 concentrates had particularly high contents of all acids and relatively little variation ($<3\%$), which could be ascribed to the use of fresh or frozen fruit. Differences between fruits are important in determining ascorbic acid losses [15], but cultivar differences could not be concluded from this study since single-variety blackcurrant concentrates were not available.

The reduced contents both of sugars and acids in freeze concentrates compared with those conventionally prepared can be ascribed to losses in ice formed during the freezing process.

Although the composition of fruit juice products may originate in differences in the original fruit, blending both of fruit, prior to processing, and concentrates is commonly done by manufacturers. The processing plant itself is a major factor that contributes to differences in fruit concentrate composition [16], but differences in post-harvest fruit storage influence respiratory activity in berries [17] and nutrient loss [18]. The absence of sucrose in concentrates may be the result of inversion, recorded to occur in blackberry [2] and apple [19, 20] concentrates. In this study glucose contents may have decreased during post-processing storage at sub-ambient temperatures, whereas the major effect in blackberry concentrates was on acid content [2]. The highest sugar/acid ratios in concentrates was observed in those made from fresh and frozen fruit in 1989; the lowest were observed to occur in 1990 concentrates, most likely a reflection of the prevailing weather at the time of harvest.

In summary, any conclusion that fresh fruit yields the best quality juice cannot be extrapolated to considerations of acids and sugars in blackcurrant concentrates [21]. Differences in the amounts of individual and total acids will influence perceptions of the flavour, character and quality of juice products. Differences in sugar contents also influence product mouthfeel, as exogenous sucrose contributes more compared with endogenous glucose or fructose [1]. The natural non-volatiles in juice products have not been extensively studied. They may, however, play important roles in determining sensory factors such as mouthfeel and temporal characteristics of flavour [22], suggesting that more studies of the influences of differences is warranted in the search for juice products perceived to be of premium quality by consumers.

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