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Food Chemistry 53 (1995) 117–124
Elsevier Science Limited
Printed in Great Britain.

Alkaline stability of the oligosaccharides present in beet medium invert sugar

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(Received 8 June 1994; accepted 29 June 1994)

The stability of the components of beet medium invert sugar, D-glucose, D-fructose, sucrose, and oligosaccharides, in the eluent used for anion-exchange high-performance liquid chromatography (HPLC) analysis with pulsed amperometric detection has been determined. The monosaccharides undergo degradation when stored in 100 mM sodium hydroxide but the degradation products produced elute before the beet medium invert sugar fingerprint oligosaccharides. Sucrose and the fingerprint beet medium invert sugar oligosaccharides do not produce detectable degradation products when stored under similar conditions. The presence of sodium acetate, the eluting salt in the HPLC method, does not alter the degradation product spectrum. The fingerprint peaks in the oligosaccharide region, are components of beet medium invert sugar and not artefacts of the anion-exchange HPLC method; they are stable under the HPLC analysis conditions used.

INTRODUCTION

The size and value of the pure fruit juice market has encouraged the lucrative business of falsification or extension of real juice with a lower cost material. Juice adulteration seldom involves any health hazard, although there may be some deterioration in the sensory quality; it is a fraudulent practice and as such is illegal. The most simple form of extension would be the addition of water though this might be detected by a reduction in the soluble solids content. As sugar is the main component of juice its addition with water would provide a more sophisticated method of extension.

The ratio of the main carbohydrates in orange juice, D-glucose, D-fructose, and sucrose, is approximately 1 : 1 : 2 although the exact ratio will vary according to country of origin, climatic conditions, cultivar and storage during which time some sucrose inversion may occur (Echeverria & Valich, 1988). If the extension of orange juice by simple sugar addition is not to be readily detected then this ratio must be maintained in the extended juice. Commercial sugar (sucrose) is available from two sources: cane and beet, either of which can be used to extend orange juice. The ratio of D-glucose to D-fructose in inverted cane sugar and high fructose corn syrup is 1 : 1 and addition of either of these sugars and sucrose would provide the correct composition, 1 : 1 : 2, or alternatively beet medium invert sugar which has a D-glucose, D-fructose, sucrose ratio of 1 : 1 : 2 could be used. This would give the same major sugar composition as that found in natural orange juice and therefore the extension of juice with these sugars would not be detected by carbohydrate ratio analysis.

The carbon stable isotope ratio, $^{13}C/^{12}C$, in plant-derived material is dependent upon the way in which the plant fixes carbon dioxide (Bender, 1971). Those plants which have a C_4 photosynthetic pathway are enriched in ^{13}C compared with those which have a C_3 pathway. Doner (1988) has used this fact to develop a method of detecting C_4-derived cane or corn sugar in orange juice. Oranges have a C_3 photosynthetic pathway and therefore pure orange juice will have a lower

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$^{13}C/^{12}C$ ratio than juice with added cane or corn sugar. However, sugar beet like orange is a C_3 plant and therefore will have the same method of carbon dioxide fixation as oranges and therefore a similar $^{13}C/^{12}C$ ratio. Alternative methods for detecting the addition of partially inverted beet sugar are therefore required. A development of the stable iosotope method for the detection of beet-derived sugar involves the determination of the $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratio in sucrose extracted from orange juice or concentrate (Doner *et al.*, 1987). This method, however, has not gained widespread acceptance as the $^{18}O/^{16}O$ ratio is affected by climatic conditions and the $^{1}H/^{2}H$ ratio is experimentally difficult to obtain so making the method less robust. The method is also not applicable to orange juice which has been reconstituted from a commercial concentrate by the addition of water.

Beet medium invert sugar is commercially produced from beet sucrose by either controlled acid or enzyme hydrolysis to give a product which is approximately 50% hydrolysed, i.e. contains D-glucose, D-fructose, and sucrose in a 1 : 1 : 2 ratio. The naturally occurring oligosaccharides in the beet sucrose may remain intact during the hydrolysis. There is also a possibility of the formation of additional oligosaccharides during the production process, either by enzymic or chemical action. The fingerprint of the oligosaccharides in beet medium invert sugar, as determined by anion-exchange high-performance liquid chromatography (HPLC), has been suggested as a possible method for the determination of the presence of beet medium invert sugar in samples of orange juice (Swallow *et al.*, 1991).

Recently it has been suggested that the oligosaccharides used to determine beet medium invert sugar addition to orange juice are not components of the added sugar but are artefacts of the HPLC system (Tsang *et al.*, 1992). If this is indeed the case then it would invalidate the HPLC method. Thus, this work was undertaken in order to establish the stability and degradation products of D-glucose, D-fructose and sucrose in the HPLC system and to determine if the oligosaccharides previously identified as being components of beet medium invert sugar are indeed so or are artefacts of the HPLC method.

MATERIALS AND METHODS

Materials

The beet medium invert sugar (BMIS) used in this study was a gift from Mr Alan Brooks (RHM Research, High Wycombe, UK). D-Fructose (glucose-free) was obtained from BDH Laboratory Supplies (Lutterworth, UK), D-glucose from Sigma Chemical Company (Poole, UK) and beet sucrose from British Sugar Corporation (Norwich, UK). The aqueous sodium hydroxide (NaOH) used for the preparation of the HPLC eluents and in the carbohydrate stability tests was 50% w/w (FSA Laboratory Supplies, Loughborough, UK) and was diluted using 18·2 MΩ water. The sodium acetate (NaOAc) (FSA Laboratory Supplies, Loughborough, UK) was AR grade. Saccharinic acids were produced as reference materials, α-isosaccharino-1,4-lactone according to the method of Whistler and BeMiller (1963*a*), D-glucometasaccharinic acids according to the method of Corbett (1963), and α-D-glucosaccharino-1,4-lactone according to the method of Whistler and BeMiller (1963*b*).

Sample preparation

Solutions of D-glucose, D-fructose, beet sucrose and beet medium invert sugar were prepared in 18·2 MΩ grade water (10 ml), 100 mM NaOH (10 ml) or 100 mM NaOH containing 5, 10, 20, 50 or 100 mM NaOAc (10 ml) and stored as detailed in Table 1.

Anion-exchange HPLC analysis

Anion-exchange HPLC analysis of the carbohydrate solutions was performed using two CarboPac PA1 columns, 250 × 4·0 mm i.d., operated in series (Dionex (UK) Ltd, Camberley, UK). A guard column of the same packing material was also used. The HPLC instrument consisted of a 625 LC solvent delivery system with a non-metallic flow path, a 464 pulsed amperometric detector (PAD) fitted with a gold working electrode and base stable reference electrode and a whisp 712 injector (Waters Chromatography Division of Millipore (UK) Ltd, Watford, UK). A single piston reciprocating pump (Scientific Systems Inc., State College,

Table 1. Concentration (mg/10 ml) and storage times of the sample solutions, prepared in 18·2 MΩ water, 100 mM sodium hydroxide or 100 mM sodium hydroxide containing sodium acetate used to assess the alkali stability of D-glucose, D-fructose, beet sucrose and beet medium invert sugar

Sample	mg	Solvent	Storage (h)	
			4°C	Ambient
D-Glucose	133	Water	45·75	14·40
	140	NaOH	45·75	17·25
		NaOH	66·12	19·49
D-Fructose	142	Water	45·75	8·73
	133	NaOH	45·75	11·55
		NaOH	51·20	31·52
Beet sucrose	247	Water	64·67	1·12
	243	NaOH	64·67	4·05
Mixture		Water	70·15	1·20
D-Glucose	130			
D-Fructose	139			
Beet sucrose	237			
Mixture		NaOH	70·12	4·05
D-Glucose	127			
D-Fructose	134			
Beet sucrose	245			
BMIS	670	Water	45·75	3·00
	671	NaOH	45·75	5·85
BMIS	906	5 mM NaOAc	31·00	15·15
	853	10 mM NaOAc	31·00	18·02
	853	20 mM NaOAc	31·00	20·88
	815	50 mM NaOAc	31·00	23·70
	896	100 mM NaOAc	31·00	26·58

PA, USA) with 2 m of polymer tubing was used to add 300 mM NaOH, at a flow rate of 0·7 ml/min, to the eluent stream between the columns and detector. For the analysis of carbohydrates the PAD was operated in the cathodic mode with the following sequence of potentials: 50 mV for 200 mS; 800 mV for 200 mS and –600 mV for 500 mS. After equilibrating the system with 100 mM NaOH a sample aliquot (200 μl) was injected. The initial eluent composition was held constant for 7 min after which the NaOAc concentration was increased, linearly, to 3 mM over the next 16 min followed by a linear increase to 100 mM NaOAc over the next 30 min. The NaOAc concentration was held at this level for a further 10 min after which the columns were regenerated by flushing with 300 mM NaOH for 60 min followed by re-equilibrating in 100 mM NaOH for 40 min before injecting the next sample. The eluent flow rate throughout was 0·7 ml/min.

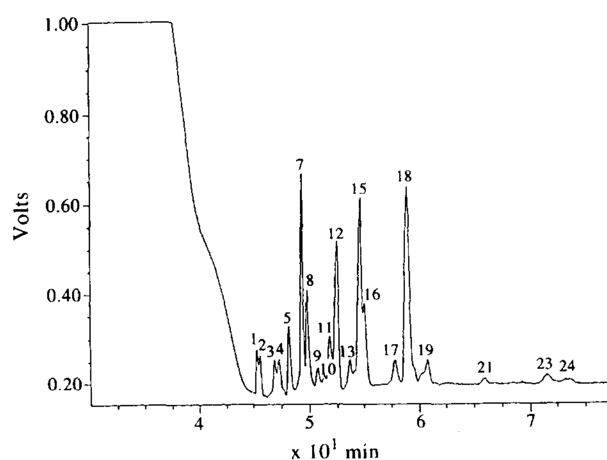

Fig. 1. Anion-exchange HPLC chromatogram of the oligosaccharide region of a fresh sample of beet medium invert sugar prepared in 18·2 MΩ water.

RESULTS AND DISCUSSION

Anion-exchange HPLC analysis of beet medium invert sugar from different suppliers is reported to give similar oligosaccharide profiles which are different from those of orange juice or orange concentrate analysed under the same conditions (Low *et al.*, 1991). This has also been observed with all samples of beet medium invert sugar and pure fresh orange juice analysed in the authors' laboratory. However, for this method to be an acceptable way of monitoring the addition of beet medium invert sugar to orange juice/concentrate it is necessary to establish that these oligosaccharides are indeed present in beet medium invert sugar and are not, as proposed by Tsang *et al.* (1992), alkali-catalysed degradation products of D-glucose, D-fructose or sucrose.

When the beet medium invert sugar is prepared for anion-exchange HPLC analysis it is dissolved in 18·2 MΩ water and it is only when injected into the HPLC system (100 mM NaOH eluent stream) that the carbohydrates, D-glucose, D-fructose, and sucrose, are exposed to alkali conditions. Until the carbohydrates elute from the column they will be exposed to 100 mM NaOH containing an increasing amount of NaOAc (0–100 mM). The oligosaccharide residence time in the HPLC system is less than 90 min. Any alkaline degradation would have to take place during this time for the products to be identified during the analysis.

Figure 1 shows the anion-exchange HPLC chromatogram of a sample of beet medium invert sugar prepared in water immediately prior to analysis. The four fingerprint oligosaccharide peaks used in the identification of added beet medium invert sugar are 7, 12, 15, and 18. Samples of the individual sugars, D-glucose, D-fructose, and beet sucrose, a mixture of the three prepared in the same ratio and concentration as in the beet medium invert sugar sample, and beet medium invert sugar were prepared in 18·2 MΩ water and 100 mM NaOH and stored as detailed in Table 1. Beet medium invert sugar was also prepared and stored in 100 mM NaOH containing 5, 10, 20, 50, or 100 mM NaOAc to assess the effect of storage on the oligosaccharide profile.

Figure 2 shows the oligosaccharide regions of the anion-exchange HPLC chromatograms of D-glucose, prepared in 18·2 MΩ water (stored at 4°C for 45·75 h followed by 14·4 h at ambient temperature), and in 100 mM NaOH (stored at 4°C for 45·74 h followed by 17·25 h at ambient temperature and at 4°C for 66·12 h followed by 19·49 h at ambient temperature), respectively. It can clearly be seen that when D-glucose is prepared in water there is only one very small peak, G, which elutes after approximately 30 min (Fig. 2A). This is before the beet medium invert sugar fingerprint oligosaccharides. The D-glucose on-column load is the same for both the D-glucose sample and the beet medium invert sugar prepared in 18·2 MΩ water prior to analysis (Fig. 1) and therefore if the fingerprint oligosaccharides were due to on-column degradation of D-glucose the same profile would be expected in the HPLC chromatograms of these samples. The fingerprint oligosaccharides in beet medium invert sugar are therefore not products of the alkaline degradation of D-glucose when it is the only sugar present in the sample. Additional peaks are observed when D-glucose is dissolved and stored in 100 mM NaOH before analysis (Fig. 2B), which are attributed to the alkaline degradation products of D-glucose. However, these degradation products all elute prior to the fingerprint oligosaccharides in beet medium invert sugar, and indeed, only peak 4 is common to both chromatograms. Increasing the storage time of the D-glucose in 100 mM NaOH solution to 66·12 h at 4°C followed by 19·49 h at ambient temperature does not increase the number of peaks (Fig. 2C).

The oligosaccharide region of the anion-exchange HPLC chromatogram of D-fructose, the second monosaccharide component in beet medium invert sugar, prepared in 18·2 MΩ water (stored at 4°C for 45·75 h followed by 8·73 h at ambient temperature), and in 100 mM NaOH (stored at 4°C for 45·75 h followed by

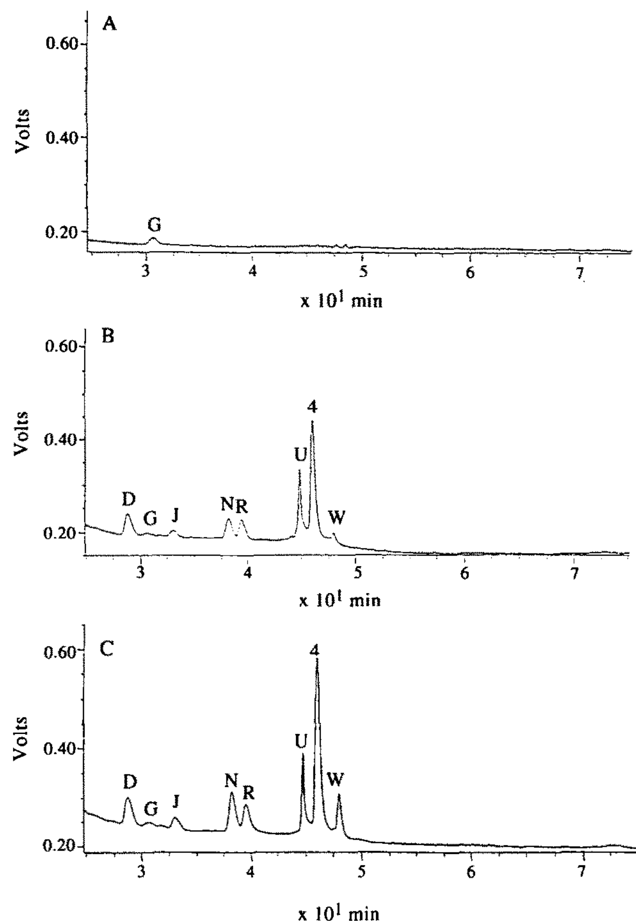

Fig. 2. Anion-exchange HPLC chromatograms of a sample of D-glucose prepared in 18·2 MΩ water and stored at 4°C for 45·75 h followed by 14·40 h at ambient temperature (A), and in 100 mM sodium hydroxide at 4°C for 45·74 h followed by 17·25 h at ambient temperature (B), and at 4°C for 66·12 h followed by 19·49 h at ambient temperature (C).

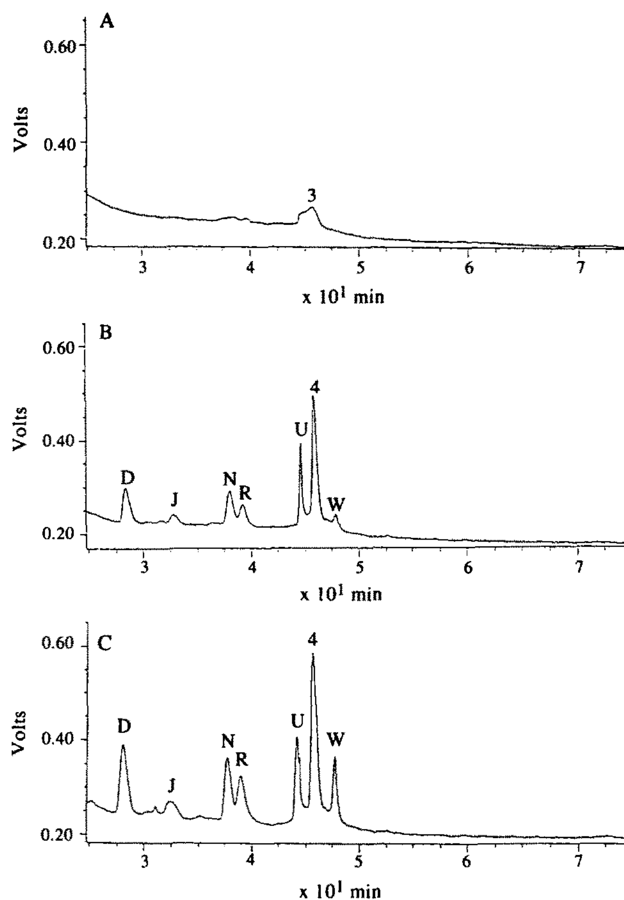

Fig. 3. Anion-exchange HPLC chromatograms of a sample of D-fructose prepared in 18·2 MΩ water and stored at 4°C for 45·75 h followed by 8·73 h at ambient temperature (A), and in 100 mM sodium hydroxide at 4°C for 45·75 h followed by 11·55 h at ambient temperature (B), and at 4°C for 51·20 h followed by 31·52 h at ambient temperature (C).

11·55 h at ambient temperature and at 4°C for 51·2 h followed by 31·52 h at ambient temperature) is shown in Fig. 3. When prepared and stored in 18·2 MΩ water only one small broad peak, 3, is visible which elutes after approximately 45 min (Fig. 3A). The profile obtained when D-fructose is dissolved and stored in 100 mM NaOH shows additional peaks (Fig. 3B) with the profile being similar to that observed when D-glucose is prepared and stored under the same conditions. No additional peaks were observed when the storage time was increased (Fig. 3C).

The bar chart showing the response/g of sugar for D-glucose and D-fructose prepared and stored in 100 mM NaOH is shown in Fig. 4. Increasing the length of time the monosaccharides were stored in alkali increases the degradation product peak heights but, as seen in the HPLC chromatograms, does not result in any additional degradation product peaks. This suggests that these peaks are due to the end-products in the degradation pathway and not to intermediate compounds. If the fingerprint beet medium invert sugar peaks were due to any intermediate compounds in the alkaline degradation pathway then it should be possible to see them in the HPLC chromatograms of the shorter storage time as degradation is still on going. From the bar chart it can also be seen that more degraded material is produced from D-fructose than D-glucose even though the D-fructose was stored for a shorter period of time at ambient temperature. The alkaline degradation products of both monosaccharides, D-glucose and D-fructose, elute before the beet medium invert sugar fingerprint oligomers.

In aqueous solutions an equilibrium is established between the pyranose, furanose and acyclic forms of

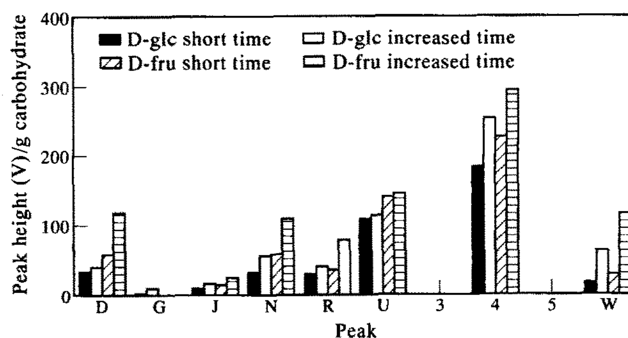

Fig. 4. Quantification of the degradation products of D-glucose and D-fructose produced when the monosaccharides were prepared and stored in 100 mM sodium hydroxide prior to analysis.

sugar molecules. When the aqueous solution is alkaline, enolisation of the acyclic forms of reducing monosaccharides will occur. Enolisation of D-glucose, an aldose, produces the 1,2-enediol and of D-fructose, a ketose, both the 1,2 and 2,3-enediols (Fig. 5). As the enolisation process is reversible, when starting with D-glucose or D-fructose (or D-mannose the C2 epimer of D-glucose), an aqueous solution will contain all three of the monosaccharides. The enediol intermediates in alkaline solution will subsequently undergo a non-reversible rearrangement to saccharinic acids (Nef, 1907, 1910, 1914). The Nef–Isbell (Isbell, 1944) mechanism for the alkaline degradation of D-glucose and D-fructose, the two monosaccharides in beet medium invert sugar, is shown in Fig. 6. The metasaccharinic acid isomers (3-deoxy-D-ribo-hexonic acid and 3-deoxy-D-arabino-hexonic acid) are formed from the 1,2-enediol, a ketoaldehyde, by an intramolecular Cannizzaro reaction. The branched saccharinic acids, the isosaccharinic acid

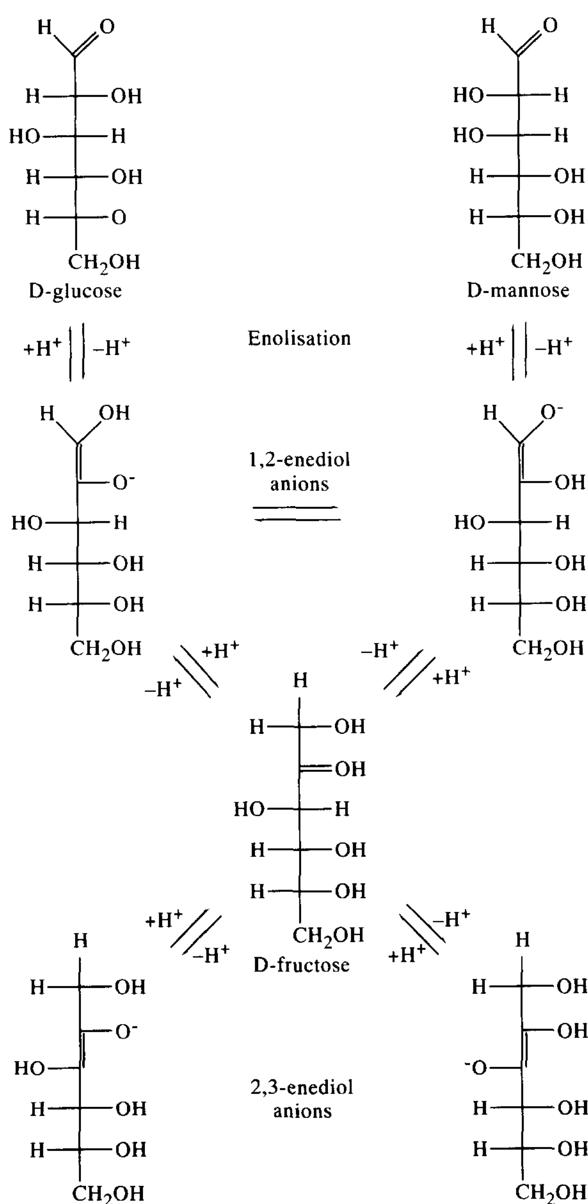

Fig. 5. The alkali-catalysed interconversion of D-glucose, D-fructose and D-mannose.

isomers (2-C-(hydroxymethyl)-3-deoxy-D-erythro-pentonic acid and 2-C-(hydroxymethyl)-3-deoxy-D-threo-pentonic acid) and the glucosaccharinic acid isomers (2-C-methyl-D-ribo-pentonic acid and 2-C-methyl-D-arabino-pentonic acid) are produced via the 2,3-enediols by the elimination of water to give an α-diketone followed by a benzilic acid rearrangement. Any degradation of the D-glucose or D-fructose components of beet medium invert sugar which occurs due to the alkaline conditions used for HPLC analysis would therefore produce some or all of these six saccharinic acids. Analysis of saccharinic acid standards using the same anion-exchange HPLC conditions has shown that they elute before the beet medium invert sugar fingerprint peaks.

The oligosaccharide region of the anion-exchange HPLC chromatogram of beet sucrose prepared in 18·2 MΩ water (stored at 4°C for 64·67 h followed by 1·12 h at ambient temperature) and 100 mM NaOH (stored at 4°C for 64·67 h followed by 4·05 h at ambient temperature) is shown in Fig. 7. In both chromatograms only one peak is visible which elutes after the large beet sucrose peak, peak 4. The elution position of this peak corresponds to that of raffinose, a trisaccharide (β-D-fructofuranosyl-*O*-α-D-galactopyranosyl-(1-6)-α-D-glucopyranoside). Raffinose has been used to identify sucrose of beet origin (Tsang *et al.*, 1990) and therefore its presence in this sample was expected. The normalised height of this peak, tentatively identified as raffinose, is the same when the beet sucrose is prepared and stored in 18·2 MΩ water or 100 mM NaOH. This is consistent with its identification as raffinose, being a non-reducing sugar, it will not undergo alkaline degradation.

Although the alkaline degradation products of D-glucose and D-fructose have been shown to elute before the fingerprint peaks in beet medium invert sugar and sucrose does not degrade, it was felt necessary to evaluate the stability of the three sugars as a mixture. Samples, containing the three sugars at the same concentration and in the same ratio as is found in beet medium invert sugar and orange juice, were prepared to investigate the possibility of the formation of the fingerprint components by a synergistic effect. Figure 8 shows the anion-exchange HPLC chromatograms of the oligosaccharide region of the sugar mixture prepared in 18·2 MΩ water (stored at 4°C for 70·12 h followed by 1·2 h at ambient temperature, or 100 mM NaOH stored at 4°C for 70·12 h followed by 4·05 h at ambient temperature). Raffinose, from the beet sucrose component, is visible in the HPLC chromatogram of the sugar mixture prepared and stored in water. In the chromatogram of the sugar mixture prepared and stored in 100 mM NaOH only those peaks previously identified in the chromatograms of D-glucose and D-fructose prepared and stored in 100 mM NaOH can be seen. It therefore appears that there is no synergistic effect between D-glucose, D-fructose and sucrose which produces alkaline degradation products eluting in the beet medium invert sugar oligomer fingerprint region of the anion-exchange HPLC chromatogram. It can therefore be concluded that the fingerprint peaks in the

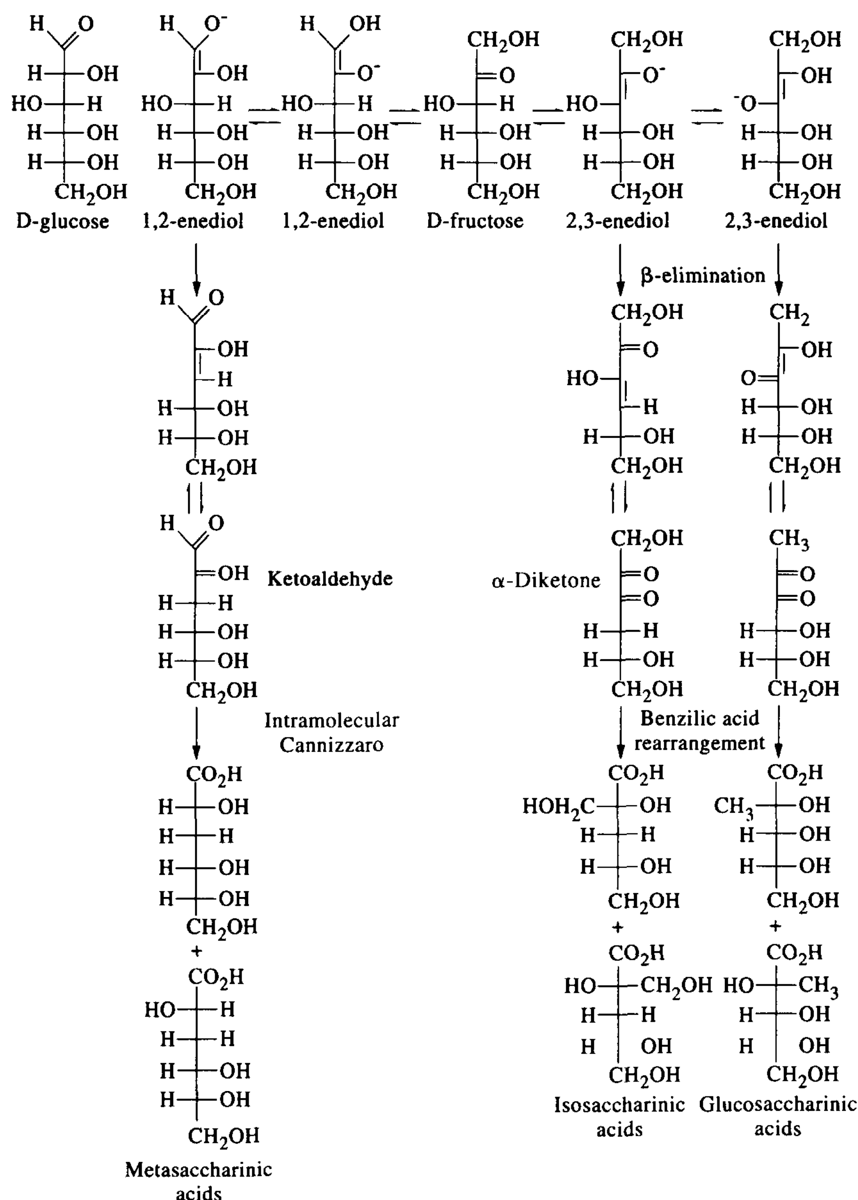

Fig. 6. The Nef–Isbell mechanism of the alkaline degradation of D-glucose, and D-fructose to the saccharinic acids.

anion exchange HPLC profile of beet medium invert sugar used for the identification of its addition to orange juice are not artefacts generated by the alkaline conditions used in the analysis.

Having established the elution positions of the alkaline degradation products of D-glucose and D-fructose in the anion-exchange HPLC system, the stability of the beet medium invert sugar fingerprint components in the HPLC eluent, 100 mM NaOH, was determined. Anion-exchange HPLC chromatograms were obtained for samples of beet medium invert sugar prepared in 18·2 MΩ water (stored at 4°C for 45·75 h followed by 3·00 h at ambient temperature) and 100 mM NaOH (stored at 4°C for 45·75 h followed by 5·85 h at ambient temperature) (Fig. 9). Comparing the oligosaccharide profile of beet medium invert sugar prepared and stored in 18·2 MΩ water (Fig. 9A) with that of a freshly prepared solution (Fig. 1), peak 15 after storage has split and peak 11 is no longer evident. In the anion-exchange HPLC chromatogram of the beet medium invert sugar prepared and stored in 100 mM NaOH, it is possible to see peaks, N, R, and U, which had been previously observed in the D-glucose and D-fructose samples which had also been prepared and stored in 100 mM NaOH. The monosaccharide alkaline degradation products, D, G, and J, are masked by the large beet medium invert sugar sucrose peak. It is not possible to identify any additional peaks in the chromatogram of the beet medium invert sugar chromatogram. The results of peak height normalisation, for the HPLC analysis of beet medium invert sugar prepared and stored in either 18·2 MΩ water or 100 mM NaOH, are shown in Fig. 10. There is a good correlation between the peak heights of the beet medium invert sugar key oligomers, peaks 7, 12, 15, and 18, when the sample is prepared and stored in either solvent. Where there is incomplete resolution or the appearance of a shoulder, as with oligomer peaks 7 and 15, there is more error in the peak height quantification which is reflected in there being a difference of 7% in the heights of these two

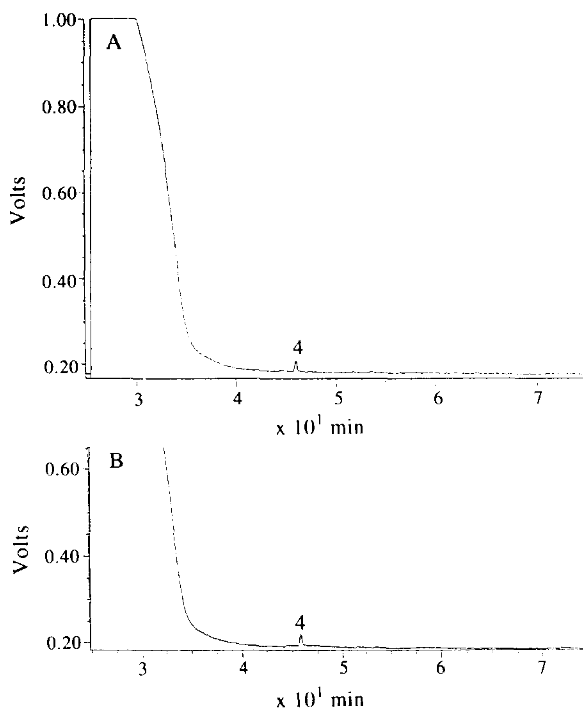

Fig. 7. Anion-exchange HPLC chromatograms of sucrose prepared and stored in 18·2 MΩ water at 4°C for 64·67 h followed by 1·12 h at ambient temperature (A), and in 100 mM sodium hydroxide at 4°C for 64·67 h followed by 4·05 h at ambient temperature (B).

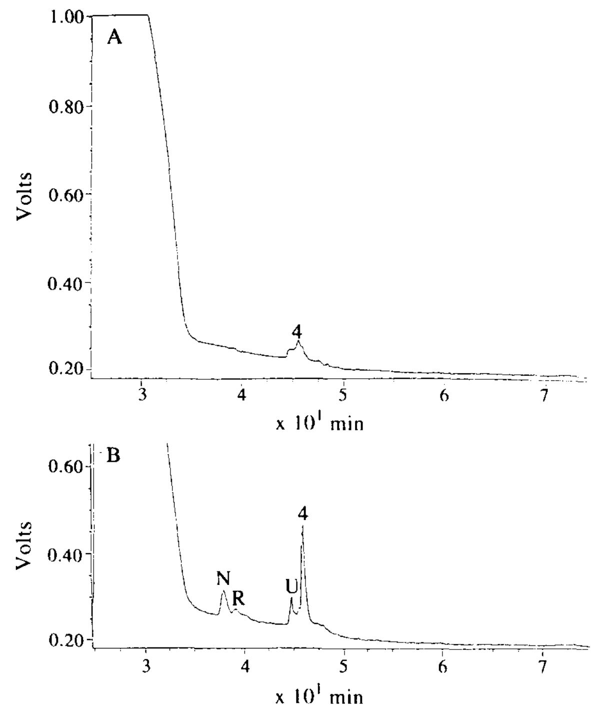

Fig. 8. Anion-exchange HPLC chromatograms of a D-glucose, D-fructose, and sucrose mixture prepared and stored in 18·2 MΩ water at 4°C for 70·12 h followed by 1·20 h at ambient temperature (A), and in 100 mM sodium hydroxide at 4°C for 71·12 h followed by 4·05 h at ambient temperature (B).

peaks but, where baseline resolution is achieved (peaks 12 and 18) the difference is smaller, approximately 4%. The phenomenon of peak splitting has been observed with peaks 15 and, to a lesser extent 18, many times during the course of our work and has been the subject of a separate piece of work which is currently being prepared for publication. However, it should be noted that it is dependent upon column/system operational efficiency and is independent of sample. It can therefore be concluded that the beet medium invert sugar does not contain any carbohydrate which undergoes degradation in the presence of 100 mM NaOH to produce compounds which are used as identifiers of beet medium invert sugar addition. The oligosaccharide components of beet medium invert sugar are stable in 100 mM NaOH and are therefore postulated to be non-reducing sugars.

The initial eluent in the anion-exchange HPLC analysis of beet medium invert sugar is 100 mM NaOH but, to elute the oligosaccharides, a gradient of increasing NaOAc is required. Therefore, during the course of the chromatography, the beet medium invert sugar oligosaccharides will be exposed to 100 mM NaOH containing NaOAc. The cationic environment has been reported to affect the rate of alkaline degradation of carbohydrates; for example, calcium ions are known to catalyse alkali-induced rearrangements leading to an increase in the rate of degradation (O'Meara & Richards, 1960). The influence of the NaOAc concentration on the alkaline degradation of the monosaccharides, D-glucose, and D-fructose, present in beet medium invert sugar and any additional degradation products, was determined by preparing and storing

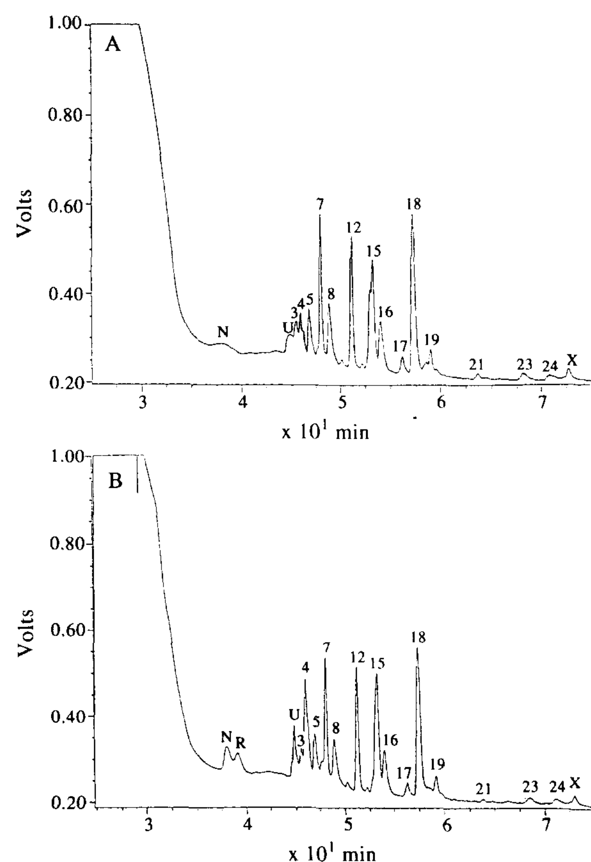

Fig. 9. Anion-exchange HPLC chromatograms of beet medium invert sugar prepared and stored in 18·2 MΩ water at 4°C for 45·75 h followed by 3·00 h at ambient temperature (A), and in 100 mM sodium hydroxide at 4°C for 45·75 h followed by 5·85 h at ambient temperature (B).

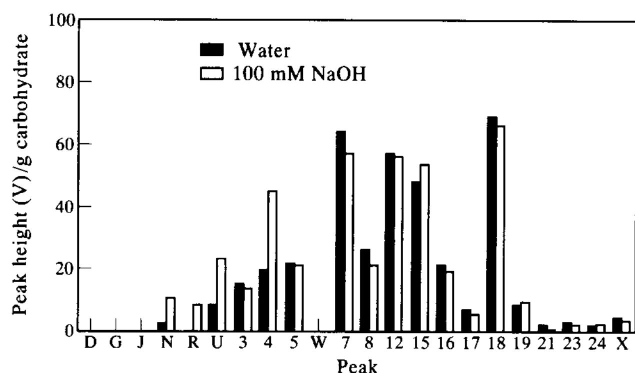

Fig. 10. Quantification of the oligosaccharide profile and degradation products of beet medium invert sugar prepared and stored in either 18·2 MΩ water or 100 mM sodium hydroxide.

beet medium invert sugar in 100 mM NaOH containing 5, 10, 20, 50, or 100 mM NaOAc The samples were stored at 4°C for 31·0 h followed by ambient temperature for 15·15, 18·02, 20.88, 23.70, and 26·58 h, respectively. Baseline stability and reproducibility of elution volumes was poor during the analysis of the samples containing NaOAc. This was attributed to the samples being injected in a solution with a higher solvent strength than the starting eluent — conditions not recommended for HPLC as it disrupts the sample/stationary phase equilibrium at the point of injection (Dolan & Snyder, 1989). The problem was made more apparent when the peak heights were normalised to the carbohydrate content. Figure 11 shows the normalised peak heights for the four fingerprint oligosaccharides, peaks 7, 12, 15, and 18. Throughout the range of NaOAc evaluated, the peaks attributed to the degradation products of D-glucose and D-fructose were evident but no additional peaks could be seen. The fingerprint oligomers were also evident throughout the range of concentrations with only peak 15, the peak which splits in some chromatographic runs, changing significantly in height up to 5 mM salt. The other peak heights were relatively constant up to a concentration of 100 mM when peak 7 increased in height.

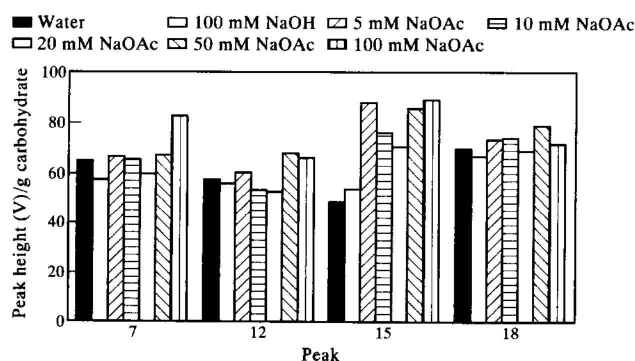

Fig. 11. Quantification of the fingerprint oligosaccharides present in beet medium invert sugar when prepared and stored in 18·2 MΩ water, 100 mM sodium hydroxide, or 100 mM sodium hydroxide containing 5, 20, 50, or 100 mM sodium acetate.

It can therefore be concluded that the beet medium invert sugar peaks used as identifiers for its addition to orange juice are not artefacts of the HPLC system produced by alkaline degradation of the carbohydrate and that they themselves are stable under the analysis conditions used. The anion-exchange HPLC method is suitable for the identification of fresh pure orange juice or concentrate to which beet medium invert sugar has been added.

ACKNOWLEDGEMENT

This work was supported by The Ministry of Agriculture Fisheries and Foods and is Crown Copyright.

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