

Measurement of Short-Chain Carbohydrates in Common Australian Vegetables and Fruits by High-Performance Liquid Chromatography (HPLC)

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Fermentable oligo-, di-, and monosaccharides and polyols (FODMAPs) are short-chain carbohydrates that can be poorly absorbed by the small intestine and may have a wide range of effects on gastrointestinal processes. FODMAPs include lactose, fructose in excess of glucose, fructans and fructooligosaccharides (FOS, nystose, kestose), galactooligosaccharides (GOS, raffinose, stachyose), and sugar polyols (sorbitol, mannitol). This paper describes an analytical approach based on HPLC with ELSD that quantifies the major FODMAPs in 45 vegetables and 41 fruits. Sorbitol and/or mannitol were measured in 18 vegetables (range = 0.09–2.96 g/100 g of fw), raffinose and/or stachyose in 7 vegetables (0.08–0.68 g/100 g of fw), and nystose and/or kestose in 19 vegetables (0.02–0.71 g/100 g of fw). Apple, pear, mango, clingstone peach, and watermelon all contained fructose in excess of glucose. Sorbitol was measured in 15 fruits (0.53–5.99 g/100 g of fw), mannitol was found in 2 fruits, and nystose or kestose was measured in 8 fruits. Understanding the importance of dietary FODMAPs will be greatly assisted by comprehensive food composition data.

KEYWORDS: Carbohydrates; high-performance liquid chromatography; FODMAPs; sugar polyols; fructose; galactooligosaccharides; fructooligosaccharides

INTRODUCTION

Carbohydrates are a major source of energy in the human diet with intakes ranging from 40 to 80% of total energy requirements. In addition to the provision of energy, it is clear that carbohydrates have a wide range of effects on human physiology (1) including effects on satiety and gastric emptying, control of blood glucose, insulin metabolism, and serum cholesterol and influencing colonic microflora and gastrointestinal processes such as laxation and fermentation.

Although carbohydrates are a diverse and complex family of compounds, the major classes of importance to human nutrition are sugars (glucose, sucrose, fructose, lactose, and maltose) and sugar polyols (sorbitol and mannitol), oligosaccharides, especially galactooligosaccharides (GOS) and fructooligosaccharides (FOS), and the polysaccharides (starch and nonstarch polysaccharides) (2, 3). Clearly, a greater understanding of the physiological effects of carbohydrates will only be possible with the separation, identification, and quantification of the different classes of carbohydrate present in food.

We have recently described a large group of short-chain carbohydrates that can be poorly absorbed by the small intestine and collectively termed these FODMAPs (fermentable oligo-

di- and monosaccharides and polyols) (4, 5). FODMAPs are found in a wide variety of foods and include lactose (in milk), free fructose (in pears, apples), fructans and FOS (in artichoke, garlic, onions), GOS (in legumes), and sugar polyols (in stone fruits, artificial sweeteners) (4). FODMAPs may have wide-ranging effects on gastrointestinal health, and this area clearly requires more research attention.

Fructans are an example of a FODMAP that has attracted a great deal of research interest. Fructans include FOS, with degrees of polymerization (DP) of 2–9 units, and inulin (DP \geq 10). The beneficial effects of fructans have been attributed to their malabsorption in the small intestine and delivery of carbohydrate to the large bowel, where they undergo rapid fermentation by bacteria with the subsequent expansion of bacterial populations, especially of bifidobacteria and lactobacilli (6, 7). These bacteria are believed to mediate a wide range of responses including suppressing the growth of potential pathogens in the colon (7), alleviating diarrhea (8), increasing the absorption of calcium (9), and stimulating the gastrointestinal immune system (10). Other byproducts of colonic fermentation include short-chain fatty acids (acetate, propionate, butyrate) and gases (H_2 , CO_2 , CH_4) (11).

The physiological and postulated health effects of fructans may also be mimicked by other FODMAPs. For example, there is evidence that GOS can also have prebiotic effects and favor the growth of bifidobacteria in the gastrointestinal tract (12).

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The osmotic effect of FODMAPs can increase fluid delivery to the large bowel and promote laxation (12), and their fermentation can result in the production of short-chain fatty acids (SCFA) (11) and lowering of the luminal pH, all of which may have several beneficial effects.

The monosaccharide, fructose, is one of the major FODMAPs present in the Western diet (13). It is present in high levels in fruits, fruit juices, honey, and high-fructose corn syrup (14). The absorption of fructose across the villous epithelium is via a low-capacity, carrier-mediated facilitated diffusion GLUT5 (15, 16). This low capacity to absorb fructose can result in its malabsorption. Indeed, oral loads of fructose are often incompletely absorbed as a normal physiological process, because a load of 50 g of fructose results in fructose malabsorption in 80% of healthy subjects (15). Interestingly, the absorption of free fructose is markedly enhanced in the presence of luminal glucose (15), and this effect is probably mediated via the low-affinity, facultative transporter—GLUT2 (16). Thus, it is important to know the quantity of fructose present in excess of glucose in a food to predict the potential for malabsorption.

For some individuals, however, the delivery of FODMAPs to the distal small and proximal large bowel and their subsequent rapid fermentation may lead to an exacerbation of symptoms (such as bloating, abdominal discomfort or pain, and altered bowel habit) associated with irritable bowel syndrome (IBS) and other functional gut disorders (4, 5, 17, 18). IBS affects one in seven Australians and is the most common reason for referral to a gastroenterologist. We have designed a dietary strategy that reduces the quantities of these fermentable FODMAPs in the diet. This approach has been highly successful in relieving functional gut symptoms of patients with IBS or inflammatory bowel disease (5, 17).

A considerable limitation to improving our understanding of the physiological importance of the FODMAP group of short-chain carbohydrates, however, is the lack of comprehensive food composition tables listing the quantities of these carbohydrates in a wide range of foods. Although information about the fructose, glucose, and lactose composition of foods is widely available (19–23), food composition data on the food content of fructans (including FOS and inulin) (24, 25), GOS (stachyose, raffinose) (20, 21), and sugar alcohols (sorbitol, mannitol) (21, 26) in a wide range of foods are limited in the international literature. Knowledge about the FODMAP composition of foods may have a number of uses in, for example, individuals attempting to increase their levels to gain the putative health benefits or in patients with gastrointestinal disorders who may want to limit their intake of FODMAPs due to undesirable gastrointestinal symptoms.

We have recently published tables listing the total fructan content of a wide range of common Australian vegetables and fruits (27). Total fructan levels were measured using the enzymic hydrolysis method described (28) and now commercially available in kit form (Megazyme Fructan HK Assay kit). The major objective of the present study was to develop an analytical technique based on high-performance liquid chromatography (HPLC) that could be used to quantify the other major FODMAP carbohydrates in foods. There are three common HPLC detectors for the analysis of sugars including refractive index (RI) detectors, evaporative light scattering detectors (ELSD), and electrochemical detectors (ECD). All detectors differ in sensitivity and their compatibility with the use of gradients. HPLC with ELSD is gaining in popularity in the area of carbohydrate analysis in foods as it is sensitive and compatible with gradients. In the present study HPLC with ELSD was

used to accurately separate and quantify the other major FODMAP carbohydrates of interest in foods including fructose (in excess of glucose), lactose, sorbitol, mannitol, stachyose, and raffinose as well as the fructooligosaccharides nystose and kestose. This method was then used to quantify levels of FODMAPs in common Australian vegetables and fruits.

MATERIALS AND METHODS

Food Sample Processing and Extraction. The foods chosen for analysis were 45 vegetables and 41 fruits commonly consumed in Australia. The same foods were analyzed recently for the total fructan content using the Megazyme Fructan HK kit (27). The food sampling and processing procedures of these foods have been described previously (27). All analyses were undertaken on raw food samples with the exception of chickpeas, which were soaked and then boiled before analysis.

Food samples were extracted and analyzed in triplicate. The extraction process was a slight modification of the method used in the previous study (27). Briefly, 1 g of freeze-dried food (0.5 mm particle size) was accurately weighed into a dry Pyrex beaker (100 mL capacity), and 80 mL of hot distilled water at 80 °C was added. The beaker was placed on a hot-magnetic stirrer and stirred with heat (around 80 °C) for 15 min until the sample was completely dispersed. Preliminary testing revealed that this heating does not result in hydrolysis of short-chain sugars. The solution was then cooled to room temperature and then quantitatively transferred to a 100 mL volumetric flask; the volume was adjusted to 100 mL. Samples were stirred, and two 10 mL samples were taken for centrifugation at 2600 rpm for 5 min at room temperature. The clear supernatant was then taken and further filtered through 0.22 μ m sterile Millex GP syringe driven filter units (Millipore, Carrigtwohill, Co. Cork, Ireland) to remove particles including bacteria and then filtered through an OASIS HLB Cartridge (Waters, Milford, MA) to remove ionized analytes, followed by immediate HPLC analysis. This filtering procedure was carried out to minimize the risk of coelution of noncarbohydrate substances. If analysis could not be undertaken immediately, then filtered samples were stored frozen at –20 °C and then reheated to 80 °C and allowed to cool to room temperature before HPLC analysis.

Chromatographic Procedure for Measuring FODMAPs. *Reagents and Standards.* Sugar standards included D-sorbitol (>99% pure, Fluka, BioChemika, Sigma-Aldrich Chemical), D-(+)-raffinose pentahydrate, D-mannitol, D-(–)-fructose, stachyose, nystose, and kestose (>99% pure, Fluka, BioChemika, Sigma-Aldrich Chemical), D-(–)-glucose (>99% pure Merck, Darmstadt, Germany), and lactose (>99% pure, Merck). All sugar standards were kept dry in a desiccator containing silica gel. Hydroscopic sugar standards were kept under nitrogen. Standard solutions were made up in water purified by a Millipore Milli-Q water purification system (Millipore, Milford, MA). High grade acetonitrile (Merck) was used. Standard solutions were kept at –40 °C for no longer than 4 months. Nitrogen gas used was ultrahigh grade.

HPLC Apparatus. The HPLC apparatus consisted of an ELSD Waters 2424, HPLC pump Waters 515, Waters autosampler 717 plus, and Waters column heater (Waters Temperature Control Module II). The data analysis system was Empower (Waters). For separation of FODMAP carbohydrates of interest, two separate columns were required. These were (i) a Waters Sugar-Pak column, 5 μ m, 6.5 \times 300 mm, and (ii) a Waters High-Performance Carbohydrate column, 4 μ m, 4.6 \times 250 mm. The guard column used with both columns was the High-Performance Carbohydrate Sentry Guard column, 3.9 \times 20 mm, 4 μ m particle size.

Samples were extracted as described above, except when running on the High-Performance Carbohydrate column when samples were extracted into the acetonitrile/water 75:25 (v/v) mobile phase. The water (purified by a Millipore Milli-Q water purification system) used for the mobile phase was filtered using a vacuum filter funnel with nylon membranes (0.22 μ m) (Alltech, Deerfield, IL).

Chromatographic Procedure. The chromatographic procedures employing the ELSD were as follows:

(i) For the Waters Sugar-Pak column, the pump flow rate was 0.5 mL/min, the column temperature was 90 °C, the gas flow was set at

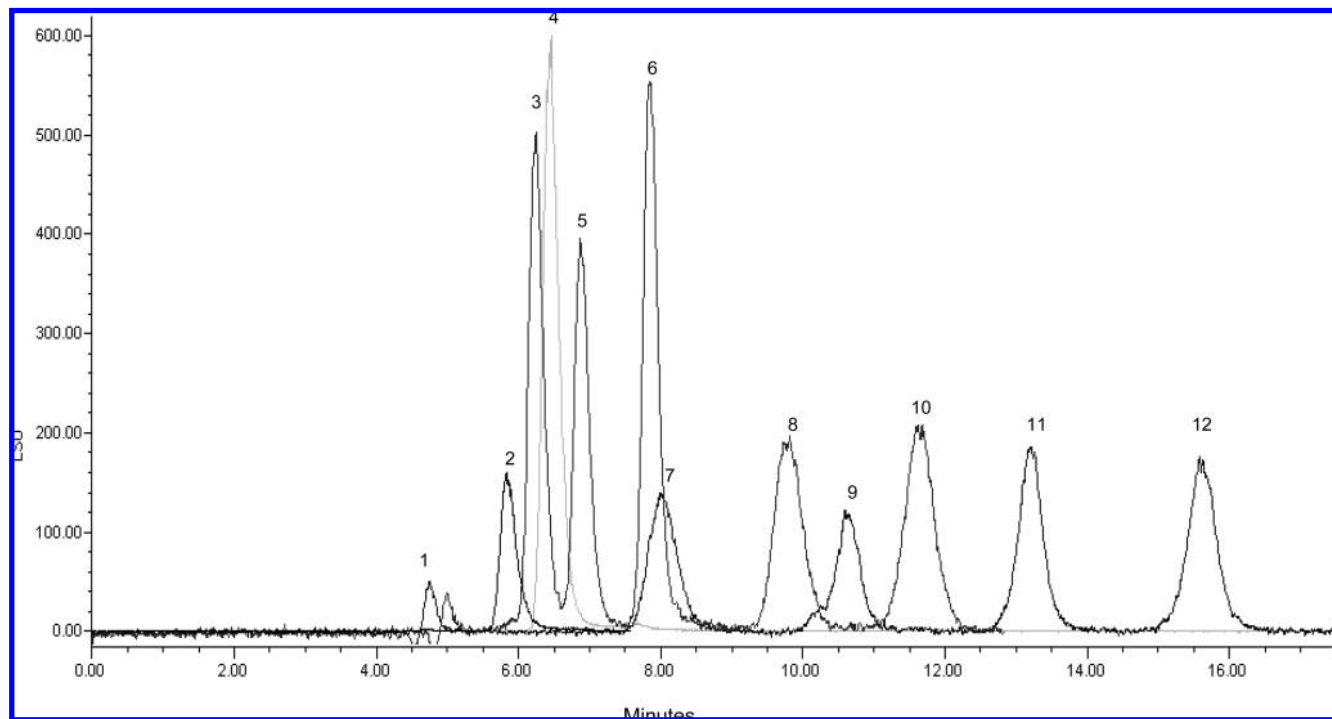


Figure 1. HPLC with ELSD chromatogram profile illustrating the location of standard sugars using the Sugar-Pak column with water as the mobile phase. Peaks: 1, unretained compounds; 2, nystose; 3, stachyose; 4, raffinose; 5, kestose; 6, sucrose and maltose; 7, lactose; 8, glucose; 9, galactose; 10, fructose; 11, mannitol; 12, sorbitol.

50 psi, the gain was 10, and the injection volume was 20 μL . The mobile phase consisted of HPLC-grade filtered water (Millipore) with added EDTA (50 mg/L).

(ii) For the High-Performance Carbohydrate column, the pump flow rate was 1.0 mL/min, the column temperature was 40 $^{\circ}\text{C}$, the gas flow was at 50 psi, the gain was 300, and the injection volume was 20 μL . The mobile phase consisted of 75:25 (v/v) acetonitrile/water.

Nitrogen was used to nebulize the effluent from both columns, and the evaporation temperature of the chromatographic eluent (i.e., the drift tube) was 55 $^{\circ}\text{C}$.

HPLC Data Evaluation. The linearity on a five-point calibration curve for each sugar was determined. The curves were not forced through the origin, and the intersection of the x -axis was not significantly different from zero. Standards for each sugar were made up in the range of 0.25–3 g/L, and a correlation coefficient of >0.998 was accepted. At least five standards were used to make up the standard curve to ensure that the unknown sample fell in the middle of the standard curve. The precision of analysis was checked after 10 repeated injections of each sugar. The relative standard deviation (RSD) was calculated [$\% \text{RSD} = (\text{standard deviation}/\text{mean value}) \times 100$] to assess repeatability. All samples were analyzed in triplicate and were reanalyzed if any one value differed by $>5\%$ from the mean for any one sugar. The set of standards was run at the start and the end of the sample running session, allowing correction of any drift in the elution profile. To ensure correct identification of the carbohydrates, several strategies were used, including overlaying the standards profile on the test food profile and routinely spiking the samples with standards and reanalyzing.

RESULTS

Elution Profile of Standards. A typical chromatographic profile of the standard FODMAP carbohydrates of interest using the Sugar Pak column is shown in **Figure 1**. The elution profile of standard sugars of interest include FOS (nystose GF₃ and kestose GF₂), GOS (stachyose, raffinose), monosaccharides (glucose, fructose), disaccharides (sucrose, lactose), and sugar polyols (mannitol and sorbitol). The Sugar Pak column with water as the mobile phase gave good and clear separation for glucose, galactose, fructose, mannitol, and sorbitol (**Figure 1**). Whereas nystose and

kestose were clearly separated, they eluted closely with the stachyose and raffinose. Longer chain FOS ($\text{DP} > 3$) were not separated in this system. The GOS verbascose was not identified in this system. Sugars that coeluted in this chromatographic system were maltose, sucrose, and lactose. To separate these sugars a second column (High-Performance Carbohydrate column) using acetonitrile and water as the mobile phase was used. This second column gave excellent separation of lactose and sucrose (see **Figure 2**) and also had the additional advantage of clearly separating the GOS, raffinose and stachyose, thereby allowing the confirmation of the results obtained from the Sugar Pak column. Fructose, sorbitol, mannitol, and glucose were not as well separated using the High-Performance Carbohydrate column system (**Figure 2**). Examples of chromatogram profiles are shown for the following: white onion via both columns (**Figure 3**); chickpea via both columns (**Figure 4**), pear via Sugar-Pak column (**Figure 5**), and mushroom via Sugar-Pak column (**Figure 6**).

Relative Standard Deviation and Detection and Quantification Limits. The %RSD from 10 injections of standard sugars were as follows for the Sugar Pak column (% RSD): stachyose (2.90); raffinose (4.04); sucrose (1.85); glucose (3.53); fructose (2.32); mannitol (2.85); and sorbitol (3.76). Those from the High-Performance Carbohydrate column were fructose (1.31), mannitol (2.28), sucrose (1.82), lactose (1.82), kestose (3.44), maltose (0.92), raffinose (1.33), and stachyose (0.55). The linearity on a five-point calibration curve was checked ($r^2 > 0.99$). The calibration curves were not forced through the origin, and the intersections of the x -axis were not significantly different from zero. The linear range for most sugars was 0.125–3 g/L except for nystose, which was 0.3–6 g/L. The detection limit was 0.05 g/L for all sugars except for nystose, for which it was 0.1 g/L. Quantification limits (5–10 times the detection limit) were 0.25–0.5 g/L for most sugars, and for nystose it was 0.50–1 g/L.

Content of FODMAPs in Vegetables and Fruits. The total amounts of FODMAP carbohydrates of interest (fructose,

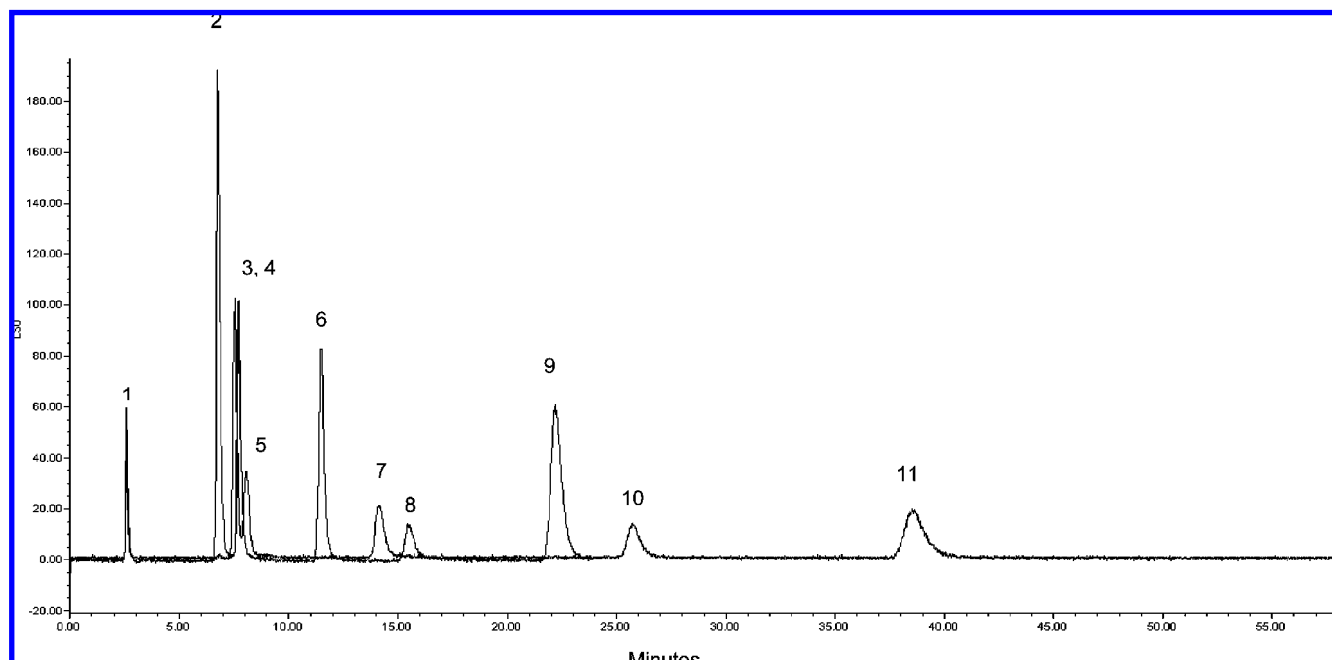


Figure 2. HPLC with ELSD chromatogram profile illustrating the location of standard sugars using the High-Performance Carbohydrate column with acetonitrile/water (75:25, v/v) as the mobile phase. Peaks: 1, unretained compounds; 2, fructose; 3, sorbitol and glucose; 4, mannitol; 5, maltose; 6, sucrose; 7, nystose; 8, lactose; 9, kestose; 10, raffinose; 11, stachyose.

sorbitol, mannitol, raffinose, stachyose, nystose, and kestose) as well as glucose and levels of fructose in excess of glucose are given in **Tables 1** and **2**. Results shown are expressed as grams per 100 g of “as eaten” fresh weight (fw).

Most vegetables contained various amounts of fructose and glucose (**Table 1**). No lactose was detected in these food samples. The sugar polyols (sorbitol and mannitol) were measured in 18 vegetables, with the levels ranging from the lowest of 0.09 g of mannitol in asparagus and 0.11 g of sorbitol in eggplant to the highest of 2.96 g of mannitol in cauliflower and 0.45 g of sorbitol in sweet corn. Raffinose was present in two vegetables only, chickpeas (0.68 g) and white onion (0.19 g) (**Table 1**). Stachyose was detected in six vegetables, with the highest levels in chickpeas at 0.57 g (**Table 1**). The short-chain FOS, nystose and kestose, were measured in 19 vegetables. The levels ranged from 0.02 g of nystose (GF₃) in bean sprouts to 0.71 g in garlic and broccoli, and for kestose (GF₂) levels ranged from 0.05 g in chicory leaves to 0.44 g in raddiccio lettuce.

Quantities of short-chain carbohydrates contained in common Australian fruits are shown in **Table 2**. All fruits contained fructose and glucose (**Table 2**). Fruits generally contained more monosaccharides than vegetables. Sorbitol was detected in 15 fruits, ranging from 0.53 g in lychee to 5.99 g in firm, peeled Packham pear. Mannitol was found in only two fruits (clingstone peach, 0.52 g; and seedless watermelon, 0.24 g). No GOS were detected in any fruit samples analyzed here (**Table 2**). FOS (nystose and kestose) were detected in 13 fruits but quantified in 8 fruits. Nystose was present in low but detectable levels ranging from 0.20 g in watermelon to 0.51 g in nectarine (**Table 2**). Kestose was present in low but detectable levels ranging from 0.02 g in custard apple to 0.11 g in longon and lemon juice.

Fructose occurred in excess of glucose in one vegetable, asparagus (0.41 g excess free fructose), and seven fruits including Granny Smith apples (0.14 g), Pink Lady apples (0.49 g), mango (0.49 g), Nashi pear (1.49), Packham pear (4.97 g), clingstone peach (4.16 g), and watermelon (0.65 g) (**Tables 1** and **2**).

Ripeness of food affected the levels of FODMAPs; for example, firm Packham pear contained higher levels of fructose

(9.32 g/100 g of fw), glucose (4.35 g), and sorbitol (5.99 g) compared to ripe Packham pear (fructose, 3.40 g; glucose, 1.1 g; and sorbitol, 2.29 g) (**Table 2**).

In **Table 3** a comparison has been made between the results obtained in the current study and results published elsewhere (*19, 21–23, 26*) for 11 common foods (6 vegetables and 5 fruits). The foods chosen were broccoli, carrots, sweet corn, chickpeas, onion, potato, apple, grapes, peach, pear, and watermelon. Although a variety of methods were used to measure sugars (*19, 21–23, 26*), there was generally quite good agreement between these different databases. No other database measured the number of short-chain carbohydrates (including sugar polyols, GOS, and FOS) as measured in the current study.

DISCUSSION

The major aim of this study was to develop an analytical technique, based on HPLC with ELSD, which could be used to quantify the major poorly absorbed short-chain carbohydrates (FODMAPs) in foods. Although there is some published information in this area (*19–25*), it is scattered throughout the literature, and no previous studies have attempted to quantify all of these carbohydrates in the same food samples.

Separation and identification of carbohydrates is a complex and challenging area of research. One of the major problems is the risk of coelution of the different carbohydrate sugars. HPLC with ELSD utilizing the Sugar-Pak column with water as the mobile phase provided good separation of most monosaccharides and sugar alcohols of interest (see **Figure 1**). There was, however, close coelution of sucrose with maltose and lactose and a close elution pattern of the GOS sugars, raffinose and stachyose, and the FOS sugars, nystose and kestose. For this reason a second column together with a different mobile phase was used. The High-Performance Carbohydrate column with acetonitrile and water (75:25, v/v) as the mobile phase clearly separated sucrose and lactose as well as separating the GOS sugars (see **Figure 2**). The coelution of the FODMAP lactose

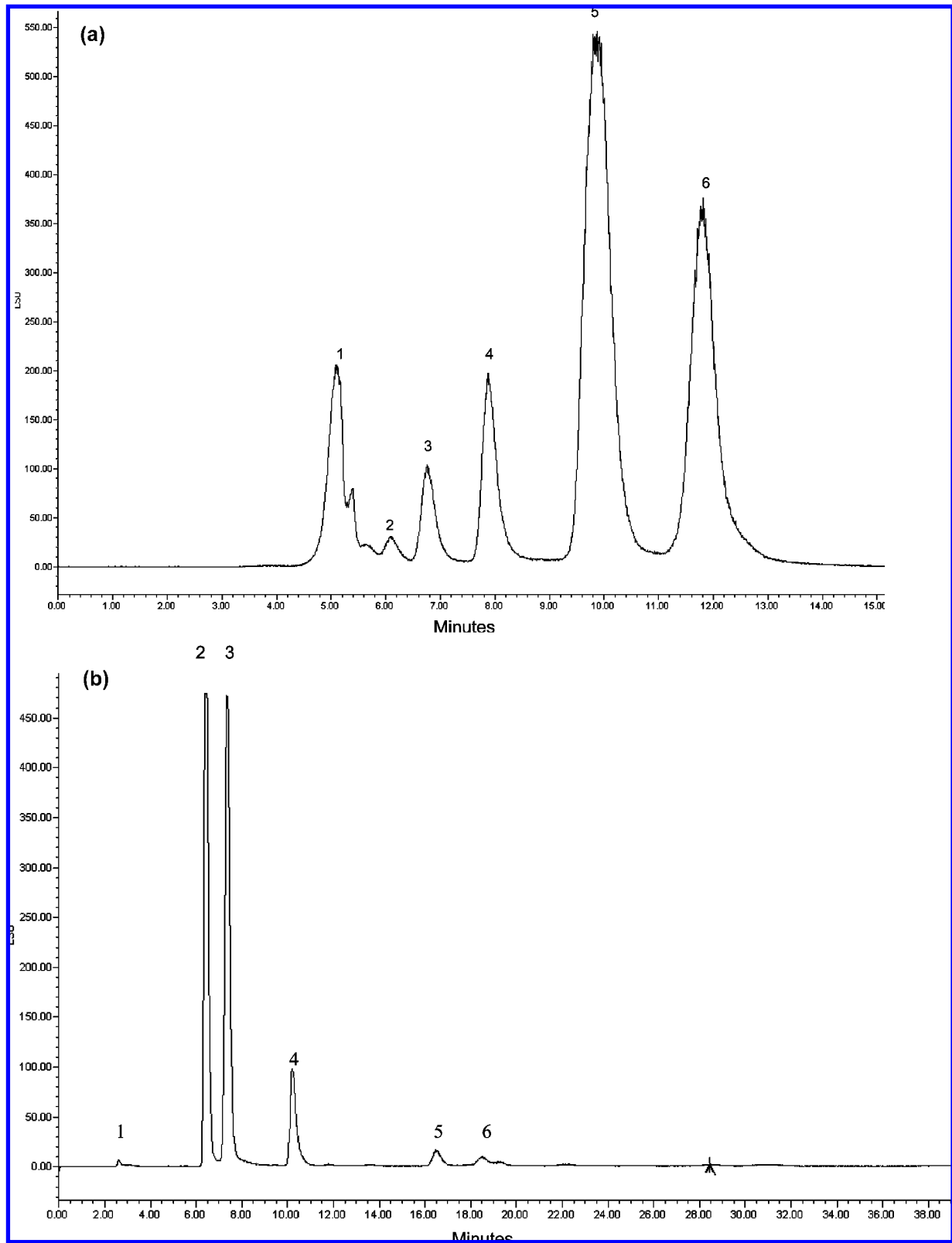


Figure 3. HPLC with ELSD chromatogram profile of white onion using (a) Sugar-Pak column (peaks: 1, unretained compound; 2, unknown sugar; 3, unknown sugar; 4, sucrose and maltose; 5, glucose; 6, fructose) and (b) High-Performance Carbohydrate Column (peaks: 1, unretained compounds; 2, fructose; 3, glucose; 4, sucrose; 5, kestose; 6, nystose).

with sucrose and maltose was not a major concern in the current study as lactose is not naturally present in vegetables and fruits (21–23) and only low levels of maltose are present in fruits and vegetables (21–23). However, the separation of these sugars will be important in future work that involves the analysis of processed grains, cereals, and milk-containing products.

Therefore, to achieve accurate separation and quantification of the major FODMAPs present in food, all samples should be run through two separate columns (see **Figures 1** and **2**).

The results show clearly that the major FODMAPs present in vegetables analyzed during this study were the sugar polyols, sorbitol and mannitol, and FOS in the form of nystose and

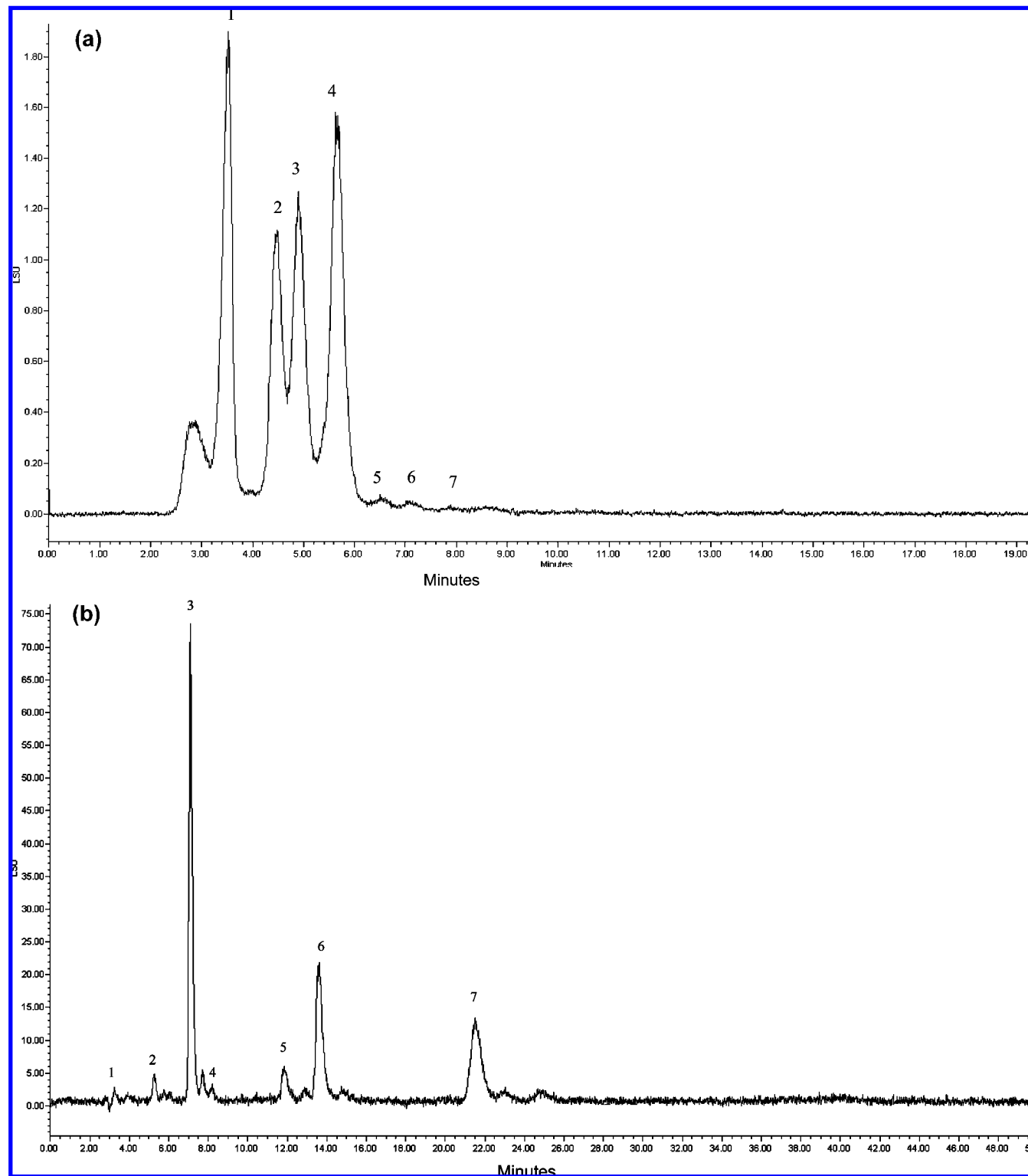


Figure 4. HPLC with ELSD chromatogram profile of chickpeas using (a) Sugar-Pak column (peaks: 1, unretained compound; 2, unknown sugar; 3, unknown sugar; 4, unknown sugar; 5, sucrose and maltose; 6, glucose; 7, fructose) and (b) High-Performance Carbohydrate column (peaks: 1, unretained compound; 2, fructose; 3, glucose/mannitol/sorbitol; 4, sucrose; 5, kestose; 6, raffinose; 7, stachyose).

kestose. High levels of mannitol were found in mushrooms and cauliflower. Excess fructose and sorbitol were the major FODMAPs in fruit. There are few published food composition tables that list levels of polyols in a wide range of foods. The current study concentrated on sorbitol and mannitol. However, xylitol together with a number of other sugar polyols (including lactitol, isomaltitol, and maltitol) are increasingly being added to foods during food processing as sweetening agents (29). Although these other sugar polyols were not investigated during

the present study, a method that does separate these polyols using HPAEC-PAD has been described previously (29).

In the present study chickpeas were the only legume analyzed. The presence of raffinose and stachyose confirmed previous work, in which different methodologies were applied (20, 21). This earlier work (20, 21) found the levels of GOS to be highest in legumes.

The chromatographic system (HPLC with ELSD) used in this study separated only the shortest chain fructans, kestose (GF₂)

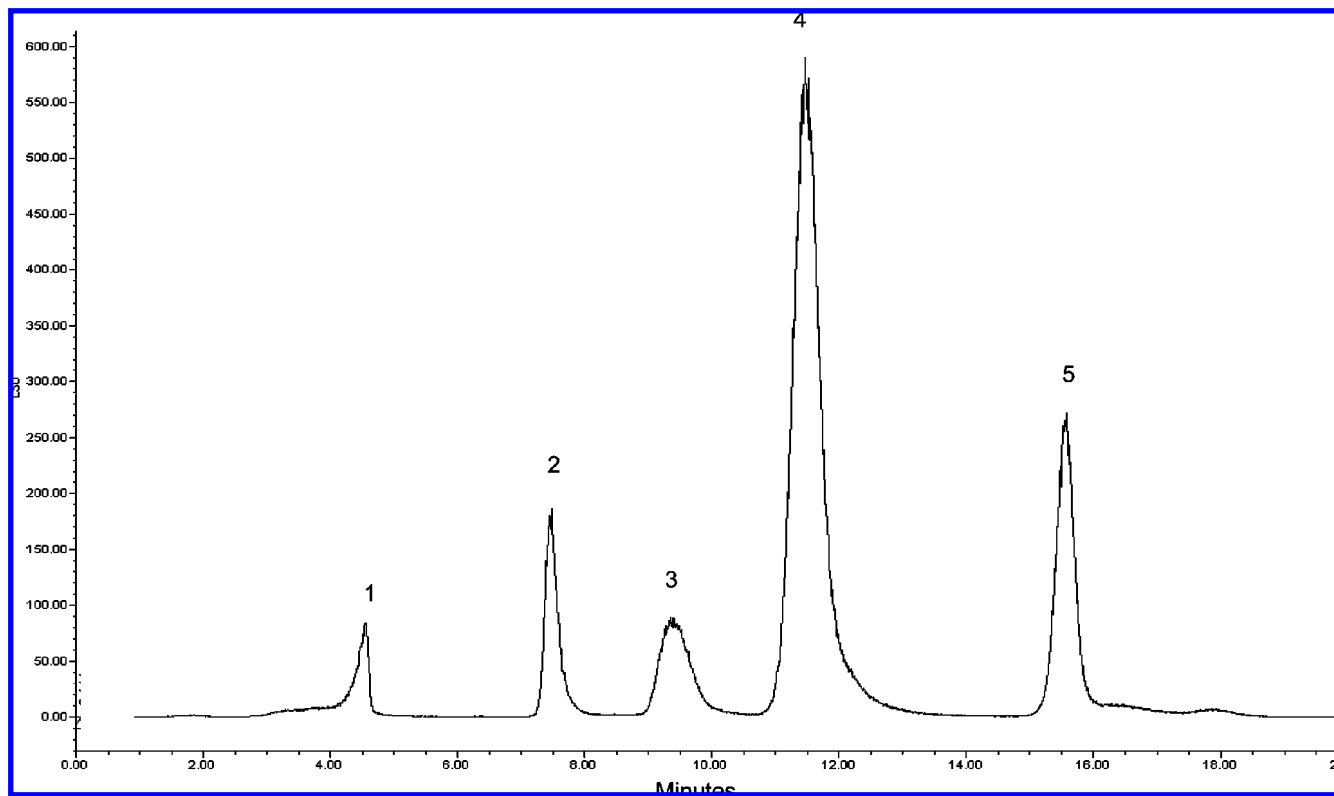


Figure 5. HPLC with ELSD chromatogram profile of Packham pear using the Sugar-Pak column. Peaks: 1, unretained compounds; 2, sucrose and maltose; 3, glucose; 4, fructose; 5, sorbitol.

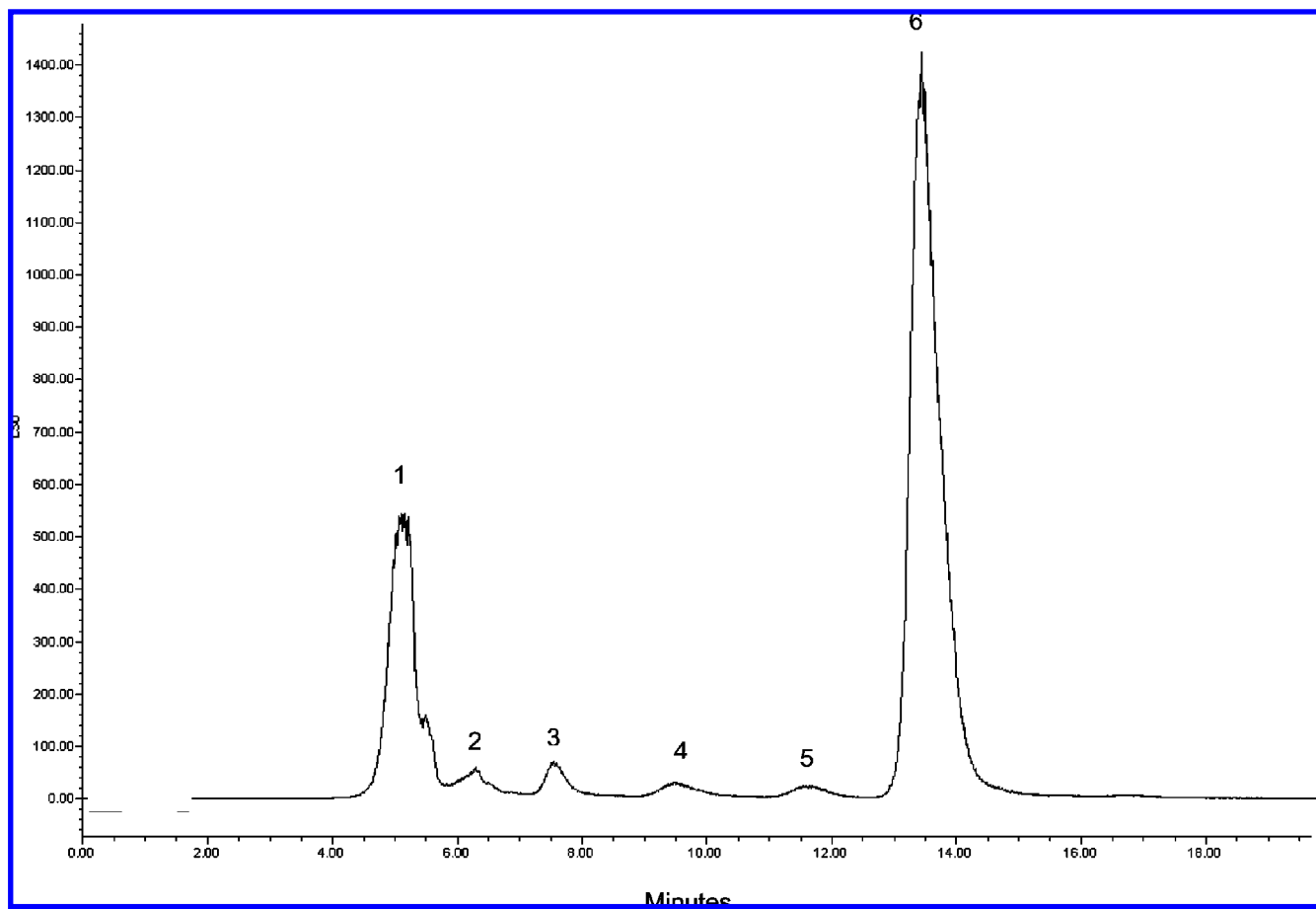


Figure 6. HPLC with ELSD chromatogram profile of mushrooms using the Sugar-Pak column. Peaks: 1, unretained compounds; 2, nystose; 3, sucrose and maltose; 4, glucose; 5, fructose; 6, mannitol.

Table 1. Short-Chain Carbohydrates Separated via HPLC with ELSD in Common Australian Vegetables (Grams per 100 g of Fresh Weight of Sample)

food	% moisture	monosaccharides ^b			sugar polyols ^b		GOS ^c		FOS ^c	
		fructose	glucose	excess fructose ^a	sorbitol	mannitol	raffinose	stachyose	nystose	kestose
asparagus	84	3.16	2.75	0.41	nd	0.09	nd	nd	0.34	0.09
artichoke, globe	83	0.18	2.19	–	nd	nd	nd	nd	tr	tr
artichoke, Jerusalem	75	nd	2.08	–	nd	nd	nd	nd	nd	tr
beans, green	87	1.17	1.62	–	0.17	0.11	nd	nd	nd	nd
bean sprouts	93	0.55	1.11	–	nd	nd	nd	nd	0.02	nd
beetroot	84	0.15	0.32	–	nd	nd	nd	0.14	0.22	0.11
bok choy	90	0.29	0.50	–	0.20	nd	nd	nd	nd	nd
broccoli	85	0.38	0.94	–	0.40	nd	nd	0.13	0.71	0.08
Brussels sprouts	81	0.06	0.41	–	0.19	nd	nd	nd	0.55	nd
cabbage, common	86	1.33	2.96	–	0.14	nd	nd	nd	0.46	nd
cabbage, savoy	86	0.97	1.80	–	0.13	nd	nd	nd	0.39	nd
<i>Capsicum</i> , green	87	2.37	4.00	–	0.37	nd	nd	nd	nd	nd
<i>Capsicum</i> , red	86	3.23	4.66	–	nd	nd	nd	nd	nd	nd
carrot	80	0.28	0.79	–	nd	nd	nd	nd	nd	nd
cauliflower	84	0.25	0.51	–	nd	2.96	nd	nd	nd	nd
chickpeas ^d	57	tr	0.99	–	nd	nd	0.68	0.57	nd	tr
chicory leaves	91	0.15	0.32	–	nd	0.10	nd	0.08	0.12	0.05
chilli, red	82	0.72	1.26	–	nd	nd	nd	nd	0.34	0.09
chives	83	0.48	0.88	–	nd	nd	nd	nd	nd	nd
choy sum	90	0.61	0.90	–	nd	nd	nd	nd	nd	nd
corn, sweetcorn	76	0.18	0.79	–	0.45	nd	nd	nd	nd	nd
cucumber, common										
peeled	89	1.49	2.61	–	nd	nd	nd	nd	nd	nd
unpeeled	89	1.16	2.05	–	nd	nd	nd	nd	nd	nd
eggplant	89	1.11	2.59	–	0.11					
endive, leaves	90	0.23	0.45	–						
fennel										
bulb	88	0.67	2.25	–	0.14	0.32	nd	0.10	0.16	0.15
leaves	92	0.26	1.97	–	0.28	0.21	nd	nd	0.17	nd
garlic	61	0.35	1.16	–	nd	nd	nd	nd	0.71	0.21
ginger root	82	0.08	0.45	–	nd	nd	nd	nd	nd	nd
lettuce, butter	85	0.80	0.82	–	nd	tr	nd	nd	tr	nd
lettuce, Red Coral	93	0.20	0.22	–	nd	nd	nd	nd	nd	nd
lettuce, raddiccio	88	0.64	0.77	–	nd	nd	nd	0.11	0.23	0.44
mushroom, button	89	0.01	0.20	–	0.11	2.63	nd	nd	0.19	0.08
okra	86	0.88	1.47	–	nd	nd	nd	nd	0.20	0.07
onion, white	84	1.38	3.36	–	nd	nd	0.19	nd	0.26	0.13
peas, snow	82	0.58	2.45	–	nd	1.16	nd	nd	0.51	0.13
potato, unpeeled	80	0.36	1.09	–	nd	nd	nd	nd	nd	nd
potato, sweet	76	0.15	1.07	–	nd	0.27	nd	nd	nd	nd
tomato, common	91	1.06	1.66	–	nd	nd	nd	nd	nd	0.09
tomato, cherry	89	0.84	0.92	–	nd	nd	nd	nd	nd	nd
tomato, roma	91	1.09	1.37	–	nd	nd	nd	nd	0.01	0.07
turnip	86	1.39	3.74	–	0.22	nd	nd	nd	nd	nd
spinach, baby	86	0.02	0.21	–	nd	nd	nd	nd	nd	nd
squash	88	1.57	2.47	–	nd	nd	nd	nd	nd	nd
zucchini	88	0.66	1.02	–	nd	nd	nd	nd	nd	nd

^a Excess fructose = fructose – glucose; tr, trace amounts detected only. ^b Fructose, glucose, sorbitol, and mannitol data were obtained from the Sugar Pak column (column 1). ^c Data for GOS (raffinose and stachyose) and FOS (nystose and kestose) were obtained using the High-Performance column (column 2). ^d Chickpeas were soaked and boiled. fw, fresh weight, nd, analyzed but not detected.

and nystose (GF₃). It is important to note, however, that foods contain fructans of different chain lengths (24), and the current method does not provide accurate information about the total fructan levels in vegetables and fruits. For accurate quantification of total fructan levels other methodologies need to be employed, for example, the methods as described previously (28, 30).

Our earlier study measured the total fructan levels in the same vegetable and fruit samples using the Megazyme Fructan kit (27). This assay involved the enzymatic hydrolysis of the fructans to release the free monosaccharides, which are then measured separately, spectrophotometrically (28). Using this approach we found that the foods containing the highest levels of total fructans included garlic (17.4 g of fructan/100 g of fw), Jerusalem artichokes (12.2 g of fructan/100 g of fw), and onions (1.8 g of total fructan/100 g of fw) (27). In the present study, however, these foods showed only traces or low levels of FOS (kestose and nystose) (Table 1), demonstrating the importance of also measuring total fructans using these other methods (28, 30).

There is increasing interest in modulating dietary intake of various short-chain carbohydrates (3, 6, 7, 12). The dietary information produced here may be used as a guide for people wanting to increase their dietary sources of FODMAPs (including fructans and GOS) that occur naturally in foods in order to gain the putative health benefits. However, the data will probably be more applied to the design of dietary approaches that reduce FODMAP intake. FODMAPs are important triggers of functional gut symptoms (5, 17), which are common in the population in general. A dietary strategy that reduces all FODMAPs in the diet has been associated with marked reductions in a range of functional gut symptoms in patients with IBS with or without fructose malabsorption (17) and in about three of four patients with IBS. FODMAP-food composition information may, therefore, be used by health professionals as part of a therapeutic approach to guide patients in choosing a diet that will produce relief from undesirable gastrointestinal symptoms.

Table 2. Short-Chain Carbohydrates Separated via HPLC with ELSD in Common Australian Fruits (Grams per 100 g of Fresh Weight of Sample)

food	% moisture	monosaccharides ^b			sugar polyols ^b		GOS ^c		FOS ^c	
		fructose	glucose	excess fructose ^a	sorbitol	mannitol	raffinose	stachyose	nystose	kestose
apple, Granny Smith										
unpeeled	82	1.63	1.49	0.14	0.70	nd	nd	nd	nd	nd
peeled	82	1.71	1.5	0.21	0.76	nd	nd	nd	nd	nd
apple, Pink Lady										
unpeeled	79	1.32	0.83	0.49	0.83	nd	nd	nd	nd	nd
peeled	81	1.89	1.16	0.73	0.75	nd	nd	nd	nd	nd
avocado	66	0.15	0.69	–	0.65	nd	nd	nd	nd	nd
banana, sugar										
firm	65	1.23	2.97	–	nd	nd	nd	nd	nd	nd
medium ripeness	64	2.18	7.34	–	nd	nd	nd	nd	nd	nd
banana, common										
firm	71	1.09	3.42	–	nd	nd	nd	nd	nd	nd
medium ripeness	72	2.45	5.38	–	nd	nd	nd	nd	nd	tr
blackberry	81	1.76	3.42	–	4.76	nd	nd	nd	nd	tr
blueberry	78	6.38	11.47	–	nd	nd	nd	nd	0.30	0.14
cantaloupe	86	0.71	1.52	–	nd	nd	nd	nd	nd	nd
custard apple	65	0.74	2.33	–	nd	nd	nd	nd	nd	0.02
dragon fruit	80	2.64	9.94	–	nd	nd	nd	nd	nd	nd
durian	62	0.67	1.85	–	nd	nd	nd	nd	nd	nd
grapes, black muscatel	78	5.43	10.32	–	nd	nd	nd	nd	nd	nd
grapes, Ralli seedless	78	7.91	11.52	–	nd	nd	nd	nd	nd	nd
grapes, Thompson	76	6.33	12.04	–	nd	nd	nd	nd	nd	nd
grapes, Red Globe	80	3.56	5.54	–	nd	nd	nd	nd	nd	nd
grapes, Red	64	9.97	17.62	–	nd	nd	nd	nd	nd	nd
grapefruit	83	1.29	2.73	–	nd	nd	nd	nd	nd	nd
kiwi fruit	72	4.05	6.85	–	nd	nd	nd	nd	nd	nd
lemon juice	84	0.65	1.39	–	nd	nd	nd	nd	nd	0.11
longon	77	2.02	6.74	–	0.68	nd	nd	nd	0.33	0.11
lychee	85	3.30	5.99	–	0.53	nd	nd	nd	nd	nd
mango	81	1.93	1.44	0.49	nd	nd	nd	nd	nd	nd
melon, honeydew	88	2.12	2.64	–	nd	nd	nd	nd	nd	nd
Nashi pear	80	4.35	2.58	1.77	1.01	nd	nd	nd	nd	nd
nectarine	83	0.62	1.49	–	1.01	nd	nd	nd	0.51	0.08
orange, navel	84	2.09	3.28	–	nd	nd	nd	nd	nd	nd
paw paw	87	0.69	0.85	–	nd	nd	nd	nd	nd	nd
pear, Packham										
firm, peeled	81	9.32	4.35	4.97	5.99	nd	nd	nd	nd	nd
Pear, Packham										
ripe, peeled	83	3.40	1.11	2.29	2.30	nd	nd	nd	nd	nd
peach, clingstone	79	5.64	1.48	4.16	0.90	0.52	nd	nd	nd	tr
peach, white	81	0.46	1.45	–	0.99	nd	nd	nd	nd	tr
peach, yellow	84	0.54	1.23	–	0.68	nd	nd	nd	nd	nd
prickly pear	82	1.74	6.89	–	nd	nd	nd	nd	nd	nd
pineapple	81	0.90	2.37	–	nd	nd	nd	nd	nd	0.10
rambutan	77	1.54	3.56	–	nd	nd	nd	nd	nd	tr
raspberry	84	2.09	3.09	–	nd	nd	nd	nd	0.22	0.08
watermelon, seedless	90	2.39	1.74	0.65	nd	0.24	nd	nd	0.20	nd

^a Excess fructose = fructose – glucose; tr, trace amounts detected only. ^b Fructose, glucose, sorbitol, and mannitol data were obtained from the Sugar Pak column (column 1). ^c Data for GOS (raffinose and stachyose) and FOS (nystose and kestose) were obtained using the High-Performance column (column 2). fw, fresh weight. nd, analyzed but not detected.

It is the osmotic effects and microbial fermentation of dietary short-chain carbohydrates that have escaped digestion and/or absorption in the small intestine that are believed to lead to the genesis of functional gut symptoms. Some of these carbohydrates will always be malabsorbed, and these include fructans and GOS and some ingested polyols.

Fructose is different in that there are absorptive mechanisms that include its own transporter and mechanisms by which glucose and amino acids can enhance its absorption. As noted in the Introduction, the absorption of free fructose is markedly enhanced in the presence of luminal free glucose (15), and this effect is probably mediated via the low-affinity, facultative transporter—GLUT2 (16). Thus, the degree by which fructose is incompletely absorbed is, therefore, dependent not only on the total fructose load but also on what is co-ingested. The amount of “free fructose”, that is, fructose in excess of glucose (i.e., fructose in excess of glucose =

free fructose – free glucose), in a specific food is therefore important information in defining what foods might be associated with incomplete fructose absorption. Fruits and vegetables in which the fructose and glucose are present in a 1:1 glucose to fructose ratio or greater are tolerated well because the fructose is completely absorbed. Indeed, sucrose (which has a glucose/fructose ratio of 1:1) is well absorbed when compared with fructose alone (15). On the other hand, foods that are high in excess free fructose, such as apples, pears, mango, and watermelon, are problematic in terms of triggering abdominal symptoms. The only vegetable analyzed in this study to have fructose in excess of glucose was asparagus.

More comprehensive food composition tables that list values for fructose and glucose in a wide range of foods have been published (19, 21–23, 26). No other food composition tables have provided data for this range of short-chain

Table 3. Comparison of Current Data with Other Published Results (Grams per 100 g of Fresh Weight of Sample)

food	monosaccharides			sugar polyols ^b		GOS ^c		FOS	
	% water content	fructose	glucose	sorbitol	mannitol	raffinose	stachyose	nystose	kestose
broccoli, raw									
current ^a	85	0.38	0.94	0.40	nd	nd	0.13	0.71	0.08
previous ^b	85	0.20	na	na	na	na	na	na	na
others ^c	87	0.89	0.73	na	na	na	na	na	na
others ^d	91	0.70	0.60	na	na	0.20	0.10	na	na
others ^e	89	1.30	0.80	na	na	na	na	na	na
others ^f	nr	na	na	na	na	na	na	nd	nd
carrots, raw									
current ^a	80	0.28	0.79	nd	nd	nd	nd	nd	nd
previous ^b	80	1.10	na	na	na	na	na	na	na
others ^c	88	0.39	0.28	na	na	na	na	na	na
others ^d	88	1.00	1.00	na	na	na	na	na	na
others ^e	na	0.80	0.80	na	na	na	na	na	na
others ^f	nr	na	na	na	na	na	na	nd	nd
corn, sweet									
current ^a	76	0.18	0.79	0.45	nd	nd	nd	nd	nd
previous ^b	76	0.20	na	na	na	na	na	na	na
others ^c	79	1.56	0.64	na	na	na	na	na	na
others ^d	76	0.60	0.80	na	na	na	na	na	na
others ^e	65	0.20	0.60	na	na	na	na	na	na
others ^f	nr	na	na	na	na	na	na	nd	nd
chickpea, cooked									
present ^a , boiled	57	tr	0.99	nd	nd	0.68	0.57	nd	tr
previous ^b	na	na	na	na	na	na	na	na	na
others ^c , canned	67	nd	nd	na	na	na	na	na	na
others ^d , boiled	60	0.10	0.10	na	na	0.40	0.50	na	na
others ^e	na	na	na	na	na	na	na	na	na
others ^f	na	na	na	na	na	na	na	na	na
others ^g	na	na	na	na	na	na	na	na	na
onion, raw									
current, ^a white	84	1.38	3.36	nd	nd	0.19	nd	0.26	0.13
previous, ^b white	84	3.20	na	na	na	na	na	na	na
others, ^c mature	86	1.76	2.21	na	na	na	na	na	na
others, ^d mature	91	0.90	2.40	na	na	1.40	0.70	na	na
others ^e	88	1.10	2.10	na	na	na	na	na	na
others ^f	nr	na	na	na	na	na	na	0.09	0.17
potato									
current, raw/unpeeled ^a	80	0.36	1.09	nd	nd	nd	nd	nd	nd
previous ^b	80	0.40	na	na	na	na	na	na	na
others, ^c cooked/unpeeled	72	0.17	0.15	na	na	na	na	na	na
others ^d	71	0.40	0.40	na	na	na	na	na	na
others ^e	84	0.04	0.20	na	na	na	na	na	na
others ^f	nr	na	na	na	na	na	na	0	0
apple fruits, raw									
current, ^a Pink Lady	79	1.32	0.83	0.83	nd	nd	nd	nd	nd
previous ^b	79	6.40	na	na	na	na	na	na	na
others, ^c Red Delicious	84	5.60	1.83	na	na	na	na	na	na
others, ^d not spec	84	7.60	2.30	0.30	na	na	na	na	na
others, ^e not spec	nr	10.50	3.20	na	na	na	na	na	na
others, ^f Red Delicious	nr	na	na	na	na	na	na	nd	0.01
others, ^g not spec	nr	6.00	2.35	0.51	na	na	na	na	na
grapes									
current, ^a Thompson	76	6.30	12.0	nd	nd	nd	nd	nd	nd
previous ^b	76	8.10	na	na	na	na	na	na	na
others, ^c Thompson	82	6.80	6.10	na	na	na	na	na	na
others ^d	81	6.90	6.60	0.10	nd	na	na	na	na
others ^e	na	na	na	na	na	na	na	na	na
others ^f	nr	na	na	na	na	na	na	na	na
peach									
current, yellow ^a	84	0.54	1.23	0.68	nd	nd	nd	nd	nd
previous ^b	84	1.8	na	na	na	na	na	na	na
others, unspec ^c	83	4.01	4.52	na	na	na	na	na	na
others ^d	88	1.3	1.1	0.2	nd	na	na	na	na
others ^e	na	na	na	na	na	na	na	na	na
others, ^f unspec	nr	na	na	na	na	na	na	nd	0.04
pear fruit									
current, ripe, peeled ^a	83	3.40	1.11	2.30	nd	nd	nd	nd	nd
current, firm, peeled ^a	81	9.32	4.35	5.99	nd	nd	nd	nd	nd
previous, firm peel ^b	83	9.70	na	na	na	na	na	na	na
others, ^c ripe, unpeeled	83	5.30	4.20	na	na	na	na	na	na
others, ^d raw, unspec	84	6.40	1.90	2.30	nd	na	na	na	na
others, ^e unspec	nr	6.50	1.70	na	na	na	na	na	na
others, ^f Bosc	nr	na	na	na	na	na	na	nd	0.01
watermelon									
current ^a	90	2.39	1.74	nd	0.24	nd	nd	0.20	nd
previous ^b	90	1.20	na	na	na	na	na	na	na
others ^c	91	2.72	0.67	na	na	na	na	na	na
others ^d	nr	3.30	1.60	na	na	na	na	na	na
others ^e	nr	3.50	1.80	na	na	na	na	na	na
others ^f	nr	na	na	na	na	na	na	nd	0.02

^a Current study using HPLC with ELSD. ^b Previous study (27) in which either fructose only was measured by an enzymic method or data were obtained from NUTAB database (19) [NUTAB use gas chromatography (GC) and HPLC for sugar analysis]. ^c Results (22) obtained using HPLC according to AOAC method 982.14. ^d Data (21) based on analysis via HPLC or GC. ^e Data (23) were obtained using enzymic, GC, HPLC. ^f Results (25) obtained using HPLC. ^g From a compilation of results (26) based on a range of methods from HPLC, GC, and paper chromatography. nd, analyzed but not detected; na, not analysed; nr, not reported; fw, fresh weight.

carbohydrates, including sugar polyols, GOS, and FOS, present in the same food. In **Table 3** a comparison has been made between the results obtained in the current study and results published elsewhere (19, 21–23, 26) for 11 common foods (**Table 3**). Although the values obtained in the present study are similar to other published food composition tables, there were some differences. This variation is to be expected given that the levels of these sugars (including glucose, fructose, sorbitol, GOS, and FOS) will vary greatly depending on the food variety, season, and climate as well as storage time and temperature (26). This is well illustrated by the results obtained here for ripe and unripe Packham pear, which showed large variation in the levels of glucose, fructose, and sorbitol (**Table 2**). Also, a wide range of methods (19, 21–23, 26) was used to quantify short-chain carbohydrates in foods, ranging from paper chromatography to enzymic methods, GC, and HPLC. This may also produce some variability in the results.

Another important aspect to the FODMAP hypothesis (4, 5, 17) is the concept that the total content of FODMAPs is important in the genesis of symptoms, not just the presence of specific types. One of the interesting observations from this study is that some foods contain a number of FODMAPs. These foods may be particularly problematic for people with IBS. For example, asparagus contained excess fructose, mannitol, nystose, and kestose (**Table 1**). Other vegetables that contained more than one type of FODMAP included beetroot, green beans, broccoli, Brussels sprout, cabbage, chicory leaves, chickpeas, radicchio, onion, fennel bulb, fennel leaves, mushrooms, and snow peas. Fruits containing more than one FODMAP included apples, longon, Nashi pear, Packham pear, nectarine, clingstone peach, and watermelon. It is important to note, however, that the major FODMAP in terms of total content to occur in vegetables are fructans, whereas for fruit, the major clinical problem is likely to generate from free fructose and, to a lesser extent, sorbitol.

In conclusion, determination of the short-chain carbohydrate content of foods requires the use of more than one analytical method. The HPLC method described in the current study permits separation and quantification of shorter chain fructans and galactans, in addition to individual hexoses and sugar polyols. Application of this new method to a series of fruits and vegetables widely available in Australia has considerably expanded our understanding of food composition and will assist in the refinement of design of dietary strategies to improve health outcomes.

S.J.S. has published cookbooks directed toward issues of dietary fructan restrictions, fructose malabsorption, and celiac disease. She has also published shopping guides for low fructose and fructan foods.

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