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Effect of Extraction Procedure on Measured Sugar Concentrations in Onion (*Allium cepa* L.) Bulbs

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Bulb samples from a range of onion cultivars grown over three consecutive years were freeze-dried and the resulting powders extracted using three previously reported methods. The extracts were analyzed for fructose, glucose, and sucrose content using HPLC coupled with ELSD, and for fructans using MALDI-MS. The three methods gave differing results, indicating that the extraction procedure is crucial in the determination of the concentration and ratios of nonstructural carbohydrates in onion bulbs. O'Donoghue et al.'s method (O'Donoghue, E. M.; Somerfield, S. D.; Shaw, M.; Bendall, M.; Hedderly, D.; Eason, J.; Sims, I. *J. Agric. Food Chem.* **2004**, *52*, 5383−5390), which utilized a more polar solvent (62.5% (v/v) aqueous methanol) and also had the benefit of shorter extraction times and lower temperatures, was far superior to 80% (v/v) ethanol-based methods in extracting significantly greater amounts of fructose, glucose, and sucrose from all onion bulbs tested. Discrepancies between and within cultivars tested also demonstrated that the ratio of monosaccharides to sucrose was affected by extraction method, such that some caution should be given to interpreting some previous work on elucidating the nonstructural carbohydrate composition in onion.

KEYWORDS: Fructans; MALDI-MS; nonstructural carbohydrates

INTRODUCTION

Water-soluble carbohydrates in onion bulbs include glucose, fructose, and sucrose, and a series of oligosaccharides called fructans (*1*), and constitute 60−80% of the dry weight (*2*). Fructan content in onion bulbs tends to decrease during refrigerated, ambient atmosphere (*3*−*6*) and low-oxygen storage (*7*). A maximum soluble sugar concentration occurs between 5 and 8 weeks after harvest (*8*, *9*).

It has been postulated that carbohydrate content is correlated with storage life. Suzuki and Cutliffe (*4*) found a significant, but small, positive correlation between fructan content and percent marketable bulbs of eight cultivars stored at 6−10 °C for 4 months. Higher fructose content at harvest was correlated with extended storage life in onion cv. Robusta bulbs stored at 4 °C for 3 months (*2*).

There is increasing interest in the role that some nonstructural carbohydrates play in defining taste preference (*10*); therefore, it is desirable to examine the efficacy and suitability of methods used to quantify these compounds.

Much research has been undertaken to quantify soluble nonstructural carbohydrates (NSCs) in various onion cultivars. A variety of extraction methods have been utilized, and these are summarized in **Table 1**. Extraction methods vary according to duration, temperature, and solvent composition. In the present study three commonly employed methods were compared against one another to attest the efficacy of each in extracting NSCs. The concentrations of sucrose, glucose, and fructose in extracts from a series of low- and high-pungency onion cultivars grown over three consecutive years were determined using standard HPLC. In addition, the effect of two of the extraction methods on subsequent quantification of fructans was also evaluated using matrix-assisted laser desorption ionization−mass spectrometry (MALDI-MS).

MATERIALS AND METHODS

Plant Material. Onion cultivars were grown over three consecutive years in the United Kingdom using conventional methods and were supplied by either F. B. Parrish and Son Ltd. (Beds., U.K.), Moulton Bulb Co. Ltd. (Lincs., U.K.), Rustler Produce Ltd. (Cambs., U.K.), or G's Marketing Ltd. (Cambs., U.K.). The cultivars harvested in 2003 consisted of Supasweet (SS1), Radar, and Shakespeare; in 2004 cvs. SS1, Buffalo, and Shakespeare were grown; and the 2005 harvest consisted of cvs. SS1, Buffalo, Extra-Supasweet (ESS1), Domenica, Element, and a commercially bought cv. SS1. The onions fall into two categories: cvs. SS1, ESS1, and Buffalo are low dry matter, low-pungency onions and are monosaccharide-dominated, whereas cvs. Domenica, Element, Radar, and Shakespeare are relatively high dry matter, of medium to high pungency, and are disaccharide-dominated.

Individual 0.5 cm thick equatorial slices (dry scales removed) were taken from 10 randomly selected onion bulbs for each cultivar and immediately snap-frozen in liquid nitrogen. Samples were stored briefly at −40 °C before being freeze-dried in an Edwards Modulyo freeze-drier (W. Sussex, U.K.) and then milled to a fine powder, before being returned to the freezer until use. For the 2003 onions, freeze-dried

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Table 1. Summary of the Extraction and Quantification Methods of Nonstructural Carbohydrates Measured in Postharvest Onion Bulbs

NSC[1] analytes	extraction method	bulb section	cultivar	storage conditions	quantification method	range measured	ref
total soluble sugars	no details	bulb tissue	Ebenezer[H]	high, medium or low temperature 10 weeks	no details	549.9-653.8 mg g^{-1} of DW	*15*
total CHOs[2]	none; fresh diluted juice	bulb tissue	not specified	not stored	anthrone colorimetric method	107.5 mg g^{-1} of FW	*16*
fructan	fresh tissue, 80% (v/v) EtOH at 90 °C for 5 min	equatorial slice divided into leaf bases (no dry skin)	PLK[3]	not stored	gel permeation chromatography; column packed with porous polyacrylamide beads (Bio-Gel P-2)	0.5-18 mg g^{-1} of FW	*1*
fructose glucose sucrose	fresh tissue 80% (v/v) EtOH, reflux	bulb tissue	cultivars with a range of DM[4] (*n*=10)	not stored	free glucose, glucose oxidase peroxidase technique free and combined fructose, resorcinol method	3-23 mg g^{-1} of FW 1-28 mg g^{-1} of FW 8-22 mg g^{-1} of FW	*17*
fructose glucose sucrose	fresh tissue with 80% (v/v) EtOH, reflux	bulb tissue	Robusta[H]	4 °C, 70% RH	descending paper chromatography, solvent butan-1-ol/EtOH/water (4:1.1:1.9 v/v) 50 h, 20 °C	3.6-25 mg g^{-1} of FW 16-28 mg g^{-1} of FW 8-20 mg g^{-1} of FW	*2*
total CHOs	fresh tissue (v/v) with water, then 0.5 mL of slurry extracted in 7.5 mL of 100% (v/v) EtOH	latitudinal wedge of bulb tissue	Granex-Grano[L]	1, 4, or 21 °C 65-70% RH 24 weeks	colorimetricallly by the phenol-sulfuric acid method	29-37 mg g^{-1} of FW	*18*
fructan	200 mg of oven-dried (70 °C) tissue extracted in 50 mL of room temperature water with shaking for 60 min	bulb tissue	cultivars with a range of DM (*n*=8)	6-10 °C 40-60% RH 24 weeks	fructan (including fructose and sucrose), automatic Roe's method	271-415 mg g^{-1} of DW	*4*
fructose glucose sucrose	0.5 g of FD[5] tissue in 20 mL of 80% (v/v) EtOH at 50 °C	inner scales outer scales	Sentinel[L]	0, 15 or 30 °C	GC-FID[6]; column packed with 3% OV-101 on 80/100 mesh Supelcoport; column temp 190 °C for 2 min, then increased at 8 °C min^{-1} to 270 °C; injector temp 300 °C; detector temp 350 °C	60-180 mg g^{-1} of DW 160-260 mg g^{-1} of DW 120-210 mg g^{-1} of DW	*8*
glucose	homogenized with water	quarter wedge, no sprout	Valenciana sintética[H]	3-32 °C 40-50% RH 42 weeks	anthrone colorimetric method	450-800 mg g^{-1} of DW	*19*
fructose glucose sucrose fructan	FD tissue in 80% (v/v) EtOH at 75 °C	bulb base, inner scale, outer scale	Hystar[H] Hysam[H] Centurian[H]	16 °C 17 weeks	total CHOs, anthrone-H_2SO_4 method F[7],G[8],S[9], HPLC-PED[10]; CarboPak PA1 column; isocratic elution in 150 mM NaOH for 30 min; flow rate = 1 ml min^{-1}; fructan = total CHOs (F+G+S)	20-160 mg g^{-1} of DW 40-270 mg g^{-1} of DW 90-150 mg g^{-1} of DW 10-260 mg g^{-1} of DW	*5*
fructose glucose sucrose fructan	50 g fresh tissue in 75 mL 96% (v/v) EtOH at 80 °C	outer, middle and inner scales	Hyton[H] Hyduro[H]	1 °C 75-80% RH 40 weeks	HPLC-RID[11]; ion exchange column; 60 °C; mobile phase 10^{-4} N NaOH in water; flow rate = 1 mL min^{-1} fructan, hydrolyzed sugars (2N TFA[12] at 40 °C for 60 min) minus non-hydrolyzed sugars	26-192 mg g^{-1} of DW 85-144 mg g^{-1} of DW 85-188 mg g^{-1} of DW 89-419 mg g^{-1} of DW	*20*
F+G+S glucose	5 g of FD tissue in 50 mL of water for 30 min at 100 °C	inner bud tissue	Rouge Amposta[H]	18 °C 70% RH 24 weeks	HPLC-DRD[13]; Polyspher CH-CA column, 80 °C, mobile phase water, flow rate = 0.5 mL min^{-1}	400-650 mg g^{-1} of DW 110-220 mg g^{-1} of DW	*21*
fructose glucose sucrose fructan	1 g of FD tissue in 70% (v/v) EtOH, reflux for 10 min fructan, aliquot treated with inulinase to release F and G	dry skin, outer leaves, top, bottom, inner part	Hysam[H]	not stored	HPLC-RID; Aminex cation exchange column, 85 °C, mobile phase water, flow rate = 0.5 mL min^{-1}	2.5-80 mg g^{-1} of DW 30.3-163.3 mg g^{-1} of DW 5.4-103.1 mg g^{-1} of DW 0.8-316.3 mg g^{-1} of DW	*22*
fructose glucose sucrose fructan total CHOs	as above	inner fleshy leaves	cultivars with a range of DM (*n*=5)	0 °C 60-65% RH 24 weeks	as above	43.1-241.8 mg g^{-1} of DW 34.9-263.8 mg g^{-1} of DW 31.7-130.6 mg g^{-1} of DW 40.2-458.1 mg g^{-1} of DW 535-665 mg g^{-1} of DW	*23*
F+G+S fructan	F+G+S. 5 g of FD tissue in 50 mL of water for 30 min at 100 °C fructan, 1 g of FD tissue in 80 mL of 70% (v/v) EtOH, reflux for 10 min	bulb tissue	Rouge Amposta[H]	4 °C, 85% RH 10 °C, 80% RH 20 °C, 65% RH24 weeks	F+G+S, HPLC-DRD as in ref *21* fructan, HPLC-PAD; carbohydrate column PA1 (CarboPak); gradient elution with NaOH and sodium acetate, flow rate = 1 mL min^{-1}	30-70 mg g^{-1} of FW 25.59-68.13 mg g^{-1} of FW	*9*
fructose glucose sucrose	1 g of fresh tissue in 10 mL of 80% (v/v) CH_3CN in H_2O for 3 min	outer fleshy part, inner part	Tropea[H]	5 °C, 30% RH 25 °C, 66% RH 30 °C, 50% RH 6 weeks	HPLC-RID; column Hypersil 5 APS 2, mobile phase 80% (v/v) CH_3CN in H_2O, flow rate = 0.5 mL min^{-1}	175.2-177.5 mg g^{-1} of DW 252.4-276.2 mg g^{-1} of DW 53.7-148.7 mg g^{-1} of DW	*24*
fructose glucose sucrose	5 g of tissue homogenized in 50 mL water for 30 min at 100 °C	bulb tissue	Rouge Amposta[H]	18 ± 1 °C 65 ± 1% RH 2 weeks	as above	50-55 mg g^{-1} of FW	*25*

Table 1. (Continued)

NSC[1] analytes	extraction method	bulb section	cultivar	storage conditions	quantification method	range measured	ref
fructose glucose sucrose fructan	60 mg of FD tissue in 7 mL of water saturated with CaOH at 100 °C for 15 min	bulb tissue	Sherpa[H]	0.5, 1.0 or 21% O_2, <0.3% CO_2 36 weeks	HPAEC-PAD[14]; flow rate = 1 mL min^{-1}; total G and S measured after hydrolysis of extract with HCl	40-200 mg g^{-1} of DW 100-180 mg g^{-1} of DW 80-140 mg g^{-1} of DW 20-320 mg g^{-1} of DW	*7*
total soluble sugars	5 g of FD tissue homogenized in 50 mL of water, 30 min boiling water bath	inner bud tissue	Rouge Amposta[H]	20 °C, 65 % RH 8 weeks	as in ref *9*	5-23 mg g^{-1} of FW	*26*
fructose glucose sucrose fructan	F,G,S, 5 g of FD tissue in 50 mL water for 30 min in a boiling water bath fructan, 10 g of FD tissue in 80% (v/v) EtOH, reflux for 10 min	bulb tissue	Jaune d'Espagne[H]	4 °C, 85% RH 10 °C, 80% RH 20 °C, 65% RH 24 weeks	F,G,S, HPLC-DRD as in ref *21* fructan, HPLC-PAD as in ref *9*	156-190 mg g^{-1} of DW 176-215 mg g^{-1} of DW 66-93 mg g^{-1} of DW 255-295 mg g^{-1} of DW	*27*
fructose glucose sucrose	10 g of fresh tissue in 80 mL of 70% (v/v) EtOH, reflux for 10 min	bulb tissue	Tenshin[H]	10 ± 1 °C, 70 ± 1% RH 20 °C, 55% RH 25 weeks	HPLC-PAD as in ref *9*	2-22 mg g^{-1} of FW 3-17 mg g^{-1} of FW 3-18 mg g^{-1} of FW	*28*
fructose glucose sucrose fructan	G,F,S, 10 mg of FD tissue in 1 mL of 62.5% (v/v) MeOH at 55 °C fructan, 10 mg fresh tissue in 1 mL water at 80 °C for 15 min	quadrant	PLK[H] Grano[L]	not stored	F,G,S, HPLC-ELSD[15]; Rezex monosaccharide column, 85 °C; mobile phase water, flow rate = 0.6 mL min^{-1} fructan, enzyme assay kit	18.1-20.3 mg g^{-1} of FW 4.7-12.0 mg g^{-1} of FW 4.6-50.8 mg g^{-1} of FW	*13*
sucrose fructan	10 g of fresh tissue in 80 mL of 70% (v/v) EtOH, reflux for 10 min	bulb tissue	Tenshin[H]	10 ± 1 °C, 70 ± 1% RH 20 °C, 55% RH 24 weeks	HPLC-PAD as in ref *9*	3-18 mg g^{-1} of FW 2-25 mg g^{-1} of FW	*29*
fructose fructan	as above	fleshy bulb tissue	Tenshin[H]	15 ± 1 °C, 45 ± 1% RH 20 °C, 55% RH 24 weeks	HPLC-PAD as in ref *20*	11.5-13.62 mg g^{-1} of FW 8-26 mg g^{-1} of FW	*30*
fructose glucose sucrose	50 mg of FD tissue in 50 mL of 80% (v/v) EtOH, reflux for 1 h	equatorial slice	SS1[L] Buffalo[L] Shakespeare[H]	not stored	HPLC-ELSD, Novapak-NH_2 reverse phase column, mobile phase acetonitrile/water (80:20, v/v), flow rate = 2 mL min^{-1}	46-260 mg g^{-1} of DW 65-241 mg g^{-1} of DW 57-126 mg g^{-1} of DW	*10*
fructan	1 g of FD tissue in 100 mL of water, 80 °C for 15 min	bulb tissue	Renate[H] Ailsa Craig[H] SS1[L]	5 % O_2, 3% CO_2, 2 °C 12-32 weeks	enzyme assay kit	25-290 mg g^{-1} of DW	*31*
fructose glucose sucrose fructan	10–100 mg of FD tissue refluxed in 80% (v/v) EtOH for 1 h, concentrated and redissolved in 1 mL of water	whole bulb	cultivars with a range of DM (n=11)	not stored	HPLC-ELSD	No absolute values stated	*11*
fructose glucose sucrose fructan	G, F, S, 150 mg of FD tissue in 3 mL of 62.5 % MeOH at 50 °C for 15 min fructan, 10 mg of FD tissue in 1 mL of water at 80 °C for 15 min	bulb quadrant	Renate[H] Carlos[H] SS1[L]	5 % O_2, 3% CO_2 or air, 2 °C 6 weeks	F,G,S, HPLC-ELSD as in ref *13* fructan, enzyme assay kit	20-350 mg g^{-1} of DW 100-360 mg g^{-1} of DW 30-130 mg g^{-1} of DW 25-360 mg g^{-1} of DW	*32*
fructose glucose sucrose fructan	as above	bulb quadrant	SS1[L]	4, 12 or 20 °C 6-13 weeks	as above	125-310 mg g^{-1} of DW 130-320 mg g^{-1} of DW 40-100 mg g^{-1} of DW 25-65 mg g^{-1} of DW	*33*

[H] High dry matter. [L] Low dry matter. [1] NSC, nonstructural carbohydrates. [2] CHOs, carbohydrates, [3] PLK, Pukekohe Longkeeper. [4] DM, dry matter. [5] FD, freeze-dried. [6] FID, flame ionization detector. [7] F, fructose. [8] G, glucose. [9] S, sucrose. [10] PED, pulsed electrochemical detector. [11] RID, refractive index detector. [12] TFA, trifluoroacetic acid. [13] DRD, differential refractometer detector. [14] PAD, pulsed amperometric detector. [15] ELSD, evaporative light scattering detector.

powders for each cultivar (10 bulbs) were pooled, giving 12 samples for cv. SS1 and 3 samples each for cvs. Radar and Shakespeare. However, for 2004 and 2005 individual bulbs were kept separate. For 2004 onions, there were 6 samples (of 10 bulbs each) for cv. SS1, 4 for cv. Buffalo, and 2 for cv. Shakespeare. For 2005 there were 4 samples (of 10 bulbs each) in total for each cultivar. Nonstructural carbohydrates were extracted from the dried powders using three previously published methods for onions and/or potatoes with slight modifications (viz. refs *11*−*13*).

Sample Extraction Methods. The Kahane method was based on that previously published (*11*) with slight modifications, in that rather than oven-drying samples prior to extraction, homogenized freeze-dried samples (50 mg) were used. These powders were mixed with 80% (v/v) ethanol (50 ml) and refluxed for 1 h. The samples were filtered through syringe filters (0.45 μm pore diameter; Millipore Corp., Bedford, MA) and readjusted to 50 mL with 70% (v/v) ethanol. Samples were then concentrated in a rotary evaporator under reduced pressure at <50 °C. These concentrated extracts were stored at 4 °C before dilution in 1 mL of water (HPLC grade) and filtration through 0.2 μm syringe filters. The remaining 1 mL samples were then stored at −40 °C until needed. Extracts were diluted 1:10 with water (HPLC grade) immediately before analysis.

The Viola and Davies method was based on a previously published method originally designed for extraction of NSCs from potato (*12*). Freeze-dried onion powder (150 mg) was combined with 3 mL of 80% (v/v) ethanol and mixed well. Vials (7 mL polystyrene bijou vials; Sterilin, Staffs., U.K.) of the slurry were placed in a shaking water bath at 70 °C for 2 h, removed briefly, and vortexed (Vortex Genie 2, Scientific Industries, Bohemia, NY) for 20 s every 30 min. The cooled samples were centrifuged using a Heraeus Biofuge Pico table-top centrifuge (Thermo Electron Corp., Waltham, MA) at 16000*g* for 10 min, filtered through a 0.2 μm filter, and stored at −40 °C until required. Extracts were diluted 1:10 with water (HPLC grade) immediately before analysis.

The O'Donoghue method was based on that previously published (*13*). Freeze-dried onion powder (150 mg) was combined with 3 mL of 62.5% methanol (v/v) and mixed well. Vials of the slurry were placed in a shaking water bath at 55 °C for 15 min, removed briefly, and

Table 2. Effect of Different Extraction Methods on Measured Sugar Concentrations in 2003-Harvested Onions[a]

cultivar	method	fructose (mg g^{-1} of DW)	glucose (mg g^{-1} of DW)	sucrose (mg g^{-1} of DW)	total sugar (mg g^{-1} of DW)	ratio (F + G)/S
SS1	O'Donoghue	315.2 (3.2)[b]	316.1 (3.4)	82.0 (3.5)	713.2 (6.4)	8.2 (0.3)
	Viola and Davies	241.3 (3.8)	247.4 (5.3)	52.2 (2.4)	541.0 (9.4)	10.0 (0.5)
	Kahane	200.8 (8.1)	210.7 (7.7)	45.9 (1.4)	457.4 (16.6)	9.0 (0.3)
Radar	O'Donoghue	218.8 (11.9)	293.5 (8.5)	100.8 (7.3)	613.1 (21.2)	5.3 (0.4)
	Viola and Davies	159.5 (12.8)	226.5 (7.7)	54.7 (6.6)	440.7 (26.9)	7.4 (0.5)
	Kahane	114.0 (7.9)	164.0 (13.7)	52.8 (5.3)	330.8 (25.5)	5.4 (0.2)
Shakespeare	O'Donoghue	237.3 (9.6)	281.2 (12.3)	94.3 (4.8)	612.7 (16.6)	5.6 (0.3)
	Viola and Davies	163.7 (10.2)	213.1 (2.9)	46.9 (3.2)	423.8 (15.8)	8.2 (0.4)
	Kahane	161.2 (7.4)	199.1 (10.9)	71.9 (2.2)	432.1 (14.8)	5.0 (0.1)

[a] Freeze-dried powders were pooled, giving 12 samples for cv. SS1 and 3 samples each for cvs. Radar and Shakespeare. [b] Standard error is given in parentheses.

Table 3. Effect of Different Extraction Methods on Measured Sugar Concentrations in 2004-Harvested Onions[a]

cultivar	method	fructose (mg g^{-1} of DW)	glucose (mg g^{-1} of DW)	sucrose (mg g^{-1} of DW)	total sugar (mg g^{-1} of DW)	ratio (F + G)/S
SS1	O'Donoghue	293.3 (2.1)[b]	298.5 (2.1)	60.7 (2.3)	652.5 (4.1)	12.7 (0.6)
	Kahane	254.1 (2.6)	225.7 (4.0)	57.9 (2.2)	537.7 (5.4)	10.4 (0.4)
Buffalo	O'Donoghue	333.6 (5.3)	280.3 (4.7)	64.1 (3.0)	678.0 (6.8)	12.6 (0.7)
	Kahane	261.4 (6.6)	170.8 (5.6)	57.8 (2.2)	489.0 (9.6)	9.5 (0.6)
Shakespeare	O'Donoghue	51.3 (3.8)	82.8 (5.6)	145.3 (4.7)	279.4 (9.7)	1.0 (0.1)
	Kahane	46.3 (3.0)	65.2 (5.1)	126.9 (5.7)	238.3 (9.7)	0.9 (0.1)

[a] There were 6 samples (of 10 bulbs each) for cv. SS1, 4 for cv. Buffalo, and 2 for cv. Shakespeare. [b] Standard error is given in parentheses.

Table 4. Effect of Different Extraction Methods on Measured Sugar Concentrations in 2005-Harvested Onions[a]

cultivar	method	fructose (mg g^{-1} of DW)	glucose (mg g^{-1} of DW)	sucrose (mg g^{-1} of DW)	total sugar (mg g^{-1} of DW)	ratio (F + G)/S
SS1	O'Donoghue	290.1 (4.6)[b]	309.7 (4.4)	84.2 (3.8)	684.0 (9.0)	10.1 (0.8)
	Viola and Davies	213.2 (4.2)	224.9 (4.6)	45.5 (2.5)	483.6 (9.7)	14.5 (1.0)
Buffalo	O'Donoghue	308.3 (4.1)	311.8 (4.5)	71.4 (3.5)	691.5 (9.4)	11.1 (0.5)
	Viola and Davies	239.4 (2.8)	241.0 (3.8)	40.5 (1.7)	520.9 (5.9)	14.6 (0.7)
ESS1	O'Donoghue	286.2 (2.0)	300.9 (3.4)	77.9 (2.9)	665.0(3.7)	9.6 (0.6)
	Viola and Davies	230.2 (2.0)	240.3 (3.8)	49. 1 (2.1)	519.5 (4.9)	13.1 (0.9)
Domenica	O'Donoghue	262.9 (4.8)	311.0 (3.2)	72.1 (3.7)	646.0 (6.1)	11.7 (0.8)
	Viola and Davies	197.1 (3.8)	231.2 (3.3)	42.4 (2.8)	470.7 (6.6)	19.5 (1.8)
Element	O'Donoghue	136.3 (5.9)	244.6 (4.2)	181.2 (4.1)	562.1 (10.3)	2.4 (0.1)
	Viola and Davies	97.8 (4.2)	176.3 (4.2)	106.7 (3.7)	380.9 (9.1)	2.9 (0.1)
SS1 (commercial)	O'Donoghue	283.0 (5.7)	309.5 (4.6)	96.0 (5.6)	688.5 (9.5)	10.6 (1.0)
	Viola and Davies	198.9 (3.5)	206.2 (4.4)	43.2 (2.1)	448.3 (6.5)	13.5 (0.9)

[a] There were 4 samples (of 10 bulbs each) in total for each cultivar. [b] Standard error is given in parentheses.

Table 5. Pearson's Rank Correlation Matrix Comparing Fructose, Glucose, and Sucrose Using Three Extraction Methods ($P < 0.001$ unless Otherwise Stated)

	2003			2004			2005		
method	fructose	glucose	sucrose	fructose	glucose	sucrose	fructose	glucose	sucrose
O'Donoghue versus Viola and Davies	0.96	0.84	0.87				0.76 (0.76)	0.51 (0.57)	0.77 (0.84)
O'Donoghue versus Kahane	0.68	0.35	0.10						
Viola and Davies versus Kahane	0.70	0.20	–0.20	0.93	0.84	0.91			

vortexed for 20 s every 5 min. The cooled samples were centrifuged, filtered, and diluted as described above for the Viola and Davies method.

Soluble Sugar Analysis. Fructose, glucose, and sucrose concentrations in onion bulb tissue were determined using a HPLC system comprising a P580 pump, a Dionex STH column thermostat, and a GINA 50 autosampler (Dionex, Sunnyvale, CA). The diluted crude onion extract (20 μL) or standard sugar solution was injected into one of two columns. System 1 included a Rezex RCM monosaccharide Ca^{+} size exclusion column of 300 mm × 7.8 mm diameter, 8 μm particle size (Phenomenex, Torrance, CA; part no. 00H-0130-K0) with a Carbo-Ca^{2+} security guard cartridge of 4 mm × 3 mm diameter (Phenomenex; part no. AJ0-4493). The mobile phase was HPLC grade water at a flow rate of 0.6 mL min^{-1}. Column temperature was held at 75 °C using a Dionex STH column thermostat. System 2 included a Waters Carbohydrate Analysis column of 300 mm × 3.9 mm diameter, 5 μm particle size, and 80 Å pore size (Waters, Herts., U.K.) with μBondapak NH_2 guard columns of 20 mm × 3.9 mm diameter, 10 μm particle size (Waters, Milford, MA) with the column temperature set

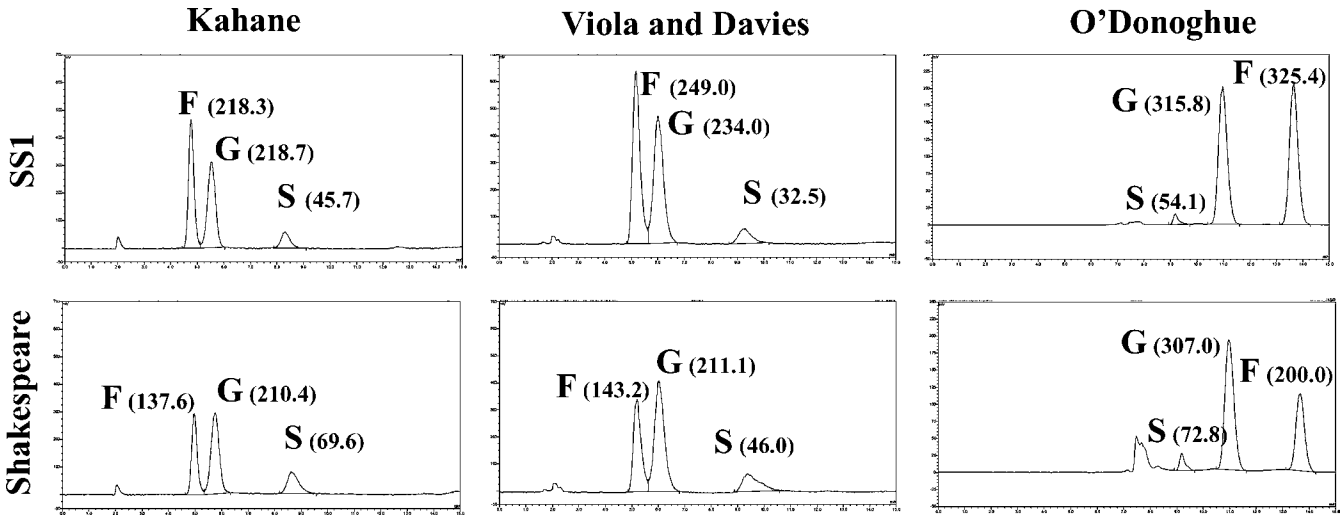

Figure 1. Examples of typical HPLC profiles of an onion cvs. SS1 and Shakespeare bulb sample ($n = 10$) from the 2003 series of samples extracted by the O'Donoghue, Viola and Davies, and Kahane methods.

at 30 °C. Mobile phase was 80:20 acetonitrile/water (v/v) at a flow rate of 2 mL min^{-1}.

Eluted carbohydrates from all extractions were monitored by an evaporative light scattering detector (ELSD 2420, Waters) connected to the Dionex system using a UCI-50 universal chromatography interface. ELSD was chosen as the preferred method of detection due to greater baseline stability and sensitivity as compared with conventional detection by refractive index (*10*). The presence and abundance of fructose, glucose, and sucrose were automatically calculated by comparison of peak area with peak area of external standards using Chromeleon version 4.6 software (Dionex). Assays ($n = 370$) were performed in triplicate.

Fructan Analysis. Fructans were determined by MALDI-MS utilizing an Applied Biosystems 4700 Proteomics analyzer (Applied Biosystems, Foster City, CA) and a 2,4,6-trihydroxyacetophenone matrix as previously described (*14*). Three cultivars (namely, SS1, Radar, and Shakespeare) harvested in 2003 were analyzed for fructans using samples extracted using the O'Donoghue or Viola and Davies method. Samples taken immediately after curing and after 2 months of storage were utilized.

Statistical Analyses. Data were subjected to analysis of variance using Statistica version 7 (StatSoft Inc., Tulsa, OK) or Genstat version 8 (VSN International Ltd., Herts., U.K.). Tests for correlations between mean values for NSC concentrations were made using Pearson's rank correlation. Correlations are presented with the Pearson's correlation coefficient (r) and P value based on a two-tailed test.

RESULTS

The O'Donoghue-based aqueous methanol extraction consistently resulted in significantly ($P < 0.001$) higher concentrations of fructose, glucose, and sucrose being extracted from various low and high dry matter onion cultivars grown over 3 years as compared to both the Viola and Davies- and Kahane-based ethanolic methods (**Tables 2**−**4**). Specifically, the concentrations of fructose, glucose, and sucrose extracted from 2003-harvested onion cv. SS1 bulbs were ca. 1.3-, 1.3-, and 1.6-fold higher, respectively, using the O'Donoghue-based method as compared to the Viola and Davies-based extraction (**Table 2**). Moreover, the O'Donoghue-based extraction was ca. 1.6-, 1.5-, and 1.8-fold better at extracting fructose, glucose, and sucrose, respectively, from cv. SS1 samples than the Kahane-based method. Typical chromatograms for a single onion cv. SS1 sample extracted by the three methods are shown (**Figure 1**); in this particular case, the O'Donoghue-based extraction method was ca. 1.3-fold more efficacious in extracting fructose and glucose and ca. 1.1-fold better for sucrose than both of the ethanol-based extraction methods. A similar trend was also observed for onion cv. Radar and Shakespeare samples harvested in 2003 (**Table 2**), whereby, for example, the O'Donoghue method was 1.9-, 1.8-, and 1.9-fold more effective in extracting fructose, glucose, and sucrose, respectively, from cv. Radar than the Kahane method. The Kahane-based extraction was the least efficacious at extracting fructose and glucose than either the O'Donoghue- or Viola and Davies-based methods, but extracted similar concentrations of sucrose compared to the O'Donoghue method for some onion cultivars (**Figure 1**; **Tables 3** and **4**).

Pearson's rank correlations were drawn between the three extraction methods (**Table 5**). There was generally good correlation between the O'Donoghue and Viola and Davies methods with $P < 0.05$ for all correlations. In contrast, correlation was much poorer between the O'Donoghue and Kahane methods for 2003 onions ($P > 0.05$), especially for glucose (0.35) and sucrose (0.10). There was also poor correlation between Kahane and Viola and Davies extractions for 2003 onions ($P > 0.05$) for glucose (0.20) and sucrose (−0.20), despite both methods being 80% (v/v) ethanol-based extractions. The Kahane-based extraction procedure generally underestimated NSC concentrations. However, for the 2004 data better correlations were obtained between the two methods.

Correlations were also obtained for different cultivars using each extraction procedure. Results confirmed that NSC concentrations in low dry matter onion cultivars showed greatest variability and were affected more by extraction efficiency than high dry matter onion bulbs. For example, when fructose extractions by either O'Donoghue or Viola and Davies methods were compared, high dry matter onions such as cv. Radar (0.99) gave much better correlation than cvs. ESS1 (0.37) and Buffalo (0.11). Glucose correlations were excellent for cvs. Radar (0.93) and ESS1 (0.84) but less so for cv. Buffalo (0.16). Sucrose gave similar results, with cvs. Radar (0.96) and ESS1 (0.84) having better correlation than cv. Buffalo (0.34). When O'Donoghue and Kahane methods were compared, the high dry matter onions again gave better correlation for NSCs. Similar trends could be seen when Kahane and Viola and Davies methods were compared.

Extracts from onion cv. SS1, Radar, and Shakespeare bulb samples using the O'Donoghue and Viola and Davies methods

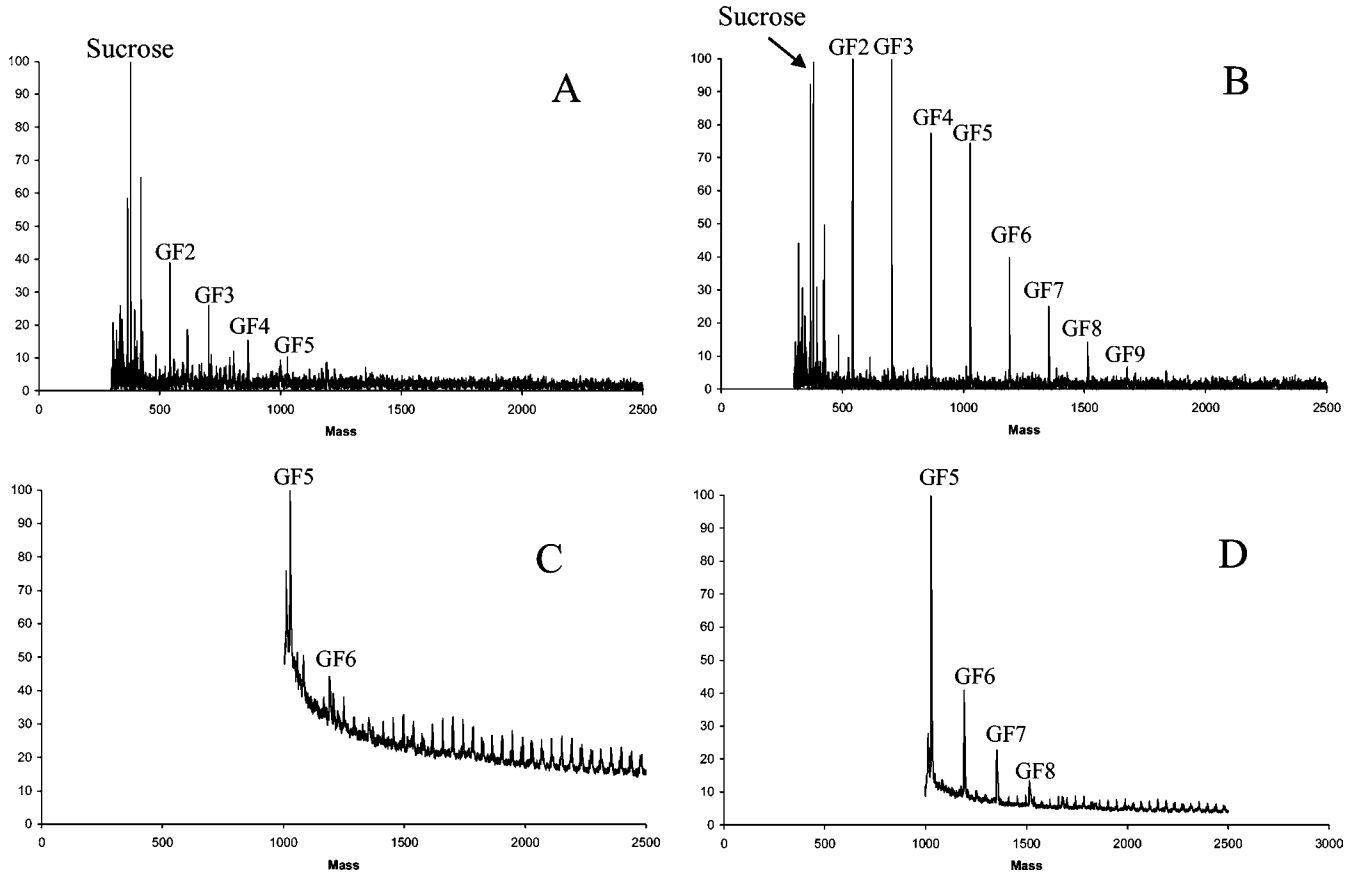

Figure 2. Examples of typical MALDI profiles of two cultivars selected from the 2003 onions, which were extracted by two different methods: (**A**) cv. SS1, O'Donoghue extraction; (**B**) cv. Shakespeare, O'Donoghue extraction; (**C**) cv. SS1, Viola and Davies extraction; (**D**) cv. Shakespeare, Viola and Davies extraction. GF*x* refers to the degree of polymerization of the fructans, where G refers to the glucoside unit and *x* the number of attached fructose units. Note that the profile is scaled such that the highest peak in the profile has a height of 100.

were analyzed for fructan content using MALDI-MS. Spectra for low fructan content (cv. SS1) and high fructan content (cv. Shakespeare) onions extracted using both methods are shown in **Figure 2**. The O'Donoghue-based method gave better resolution of fructans within extracts when studied with MALDI-MS. As reported previously, the addition of potassium salts to the extracts to promote ionization (*14*) led to clear detection of the (M + K) ions for a range of fructans with degrees of polymerization from 2 (sucrose) to 10. A clear loss of fructans over the 2 month storage period was observed (**Figure 3**) as previously shown by other authors (*3*−*6*).

Comparison of the spectra of onions extracted by the two techniques clearly showed that the Viola and Davies-based method is less suitable than the O'Donoghue method for subsequent fructan analysis. However, it is thought that plasticizer and/or polymer material may have leached from the plastic vials used, which may have occurred more for the Viola and Davies method and led to a saturation of the process at molecular weights of <1000 and also caused interference for cv. SS1 (**Figure 2C**). Better spectra were obtained for the high-fructan Shakespeare cultivar, probably because there were more fructans to extract and, therefore, the peaks for the individual fructan species could still be visualized despite interference.

DISCUSSION

The analysis of onions for nonstructural carbohydrates has been widely studied, and a range of extraction techniques have been used (**Table 1**). The results from the present study clearly demonstrate that different extraction methods have differing efficacies for the extraction of sugars. The O'Donoghue-based extraction was the most efficacious method at extracting nonstructural carbohydrates from freeze-dried onion powder for a range of onion cultivars, yet it uses the lowest temperature and has the shortest extraction time of the three methods tested. The brevity of this method and its relative simplicity make it especially suitable for extractions of large numbers of onion samples.

Reasons for the discrepancies between extraction methods are most probably based on the solvents used. The solvent mixture used in the O'Donoghue method was methanol based [62.5% (v/v)] and thus more polar than the 80% ethanol (v/v) used in the other two methods tested. Fructose and glucose are approximately twice as soluble in a water/methanol mixture than in a water/ethanol mixture, and sucrose is nearly 3 times more soluble (*34*, *35*). In our experiments the final concentrations of sugars in the extract were far below saturation concentrations (*34*, *35*) for these solvent mixtures; however, it is indicative that sugars will dissolve more readily in the water/methanol mixture.

It was noted that the freeze-dried powder obtained from bulbs of low dry matter cultivars was less dense than that from high dry matter onions. The difference in the physical nature of these powders may have caused variation in solvent extraction efficiencies. Furthermore, the more polar solvent [62.5% (v/v) methanol] used within the O'Donoghue procedure could simply have penetrated and wetted the powder more effectively than

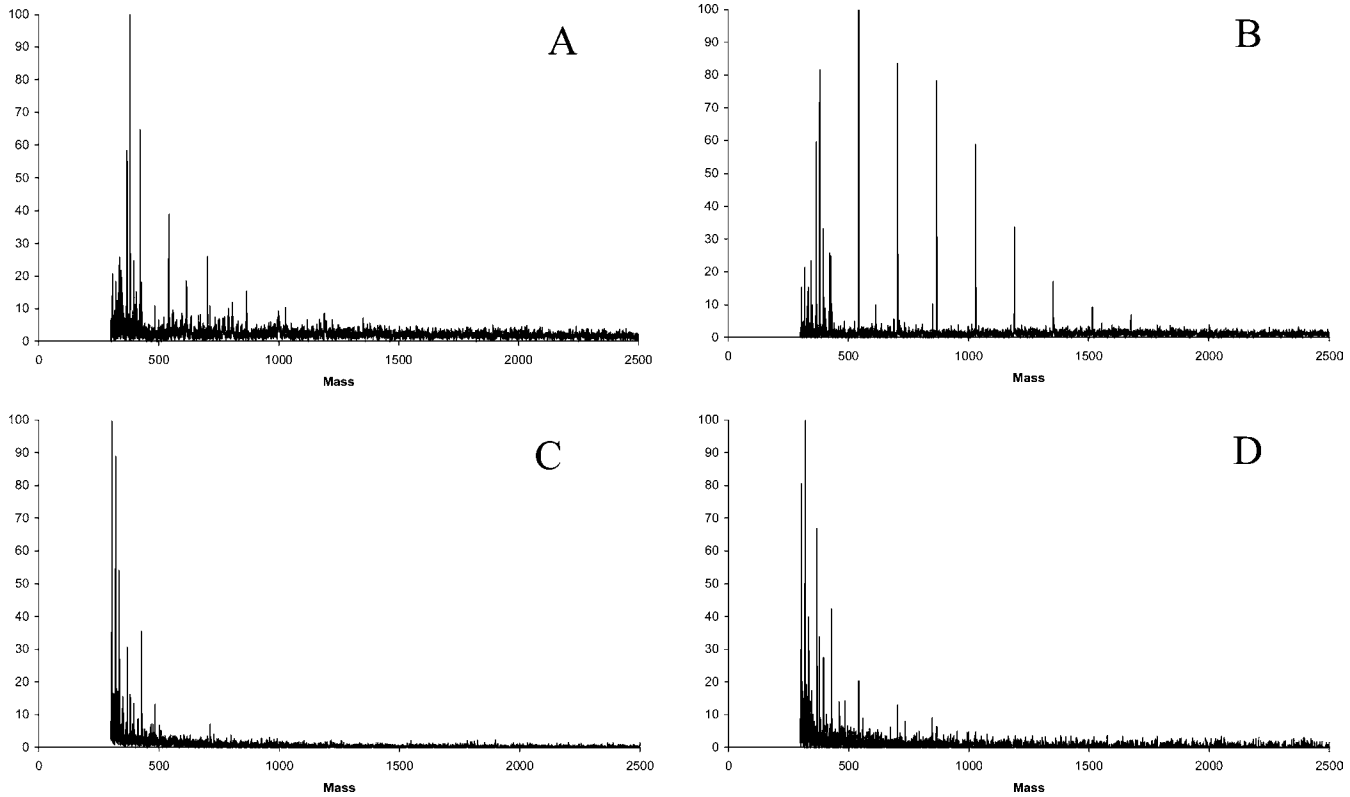

Figure 3. Temporal change in fructan in typical MALDI profiles (O'Donoghue-based extraction procedure) for two onion cultivars from the 2003 onions, which have been stored for differing lengths of time: (**A**) cv. SS1, no storage; (**B**) cv. Radar, no storage; (**C**) cv. SS1, 2 months of storage at 2 °C; (**D**) cv. Radar, 2 months of storage at 2 °C. Note that the profile is scaled such that the highest peak in the profile has a height of 100.

ethanol. The other two methods utilized within this study were both based on the use of 80% (v/v) ethanol as a solvent. Both methods have advantages and disadvantages that could affect the efficiency of extraction. However, the Kahane method, which is very labor intensive, utilizes refluxing solvent, thereby meaning the extraction both takes place at a higher temperature and is agitated through boiling. Following the initial extraction and evaporation of the samples, the flask is, however, washed with only 1 mL of water. The effectiveness of this process in redissolving precipitated matter is not known. This process could easily have led to the poor correlations noted within this paper (**Table 5**).

The implications of this work are that the type of extraction procedure is vital for accurate investigation of nonstructural carbohydrate composition in onion bulbs. Not only is the total sugar extracted affected by the method chosen but also the relative ratio of glucose, fructose, and sucrose within and across cvs. is dependent on the extraction procedure used. A less suitable extraction procedure could lead to an underestimation of sugar content, especially for monosaccharides. Therefore, discrepancies between nonstructural carbohydrates presented in this study suggest that some previous work which has quantified nonstructural carbohydrates in onion bulbs (**Table 1**) should be treated with some caution when ethanol-based extractions have been employed.

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