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Determination of Free Inositols and Other Low Molecular Weight Carbohydrates in Vegetables

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ABSTRACT: Different low molecular weight carbohydrates including saccharides, polyalcohols, sugar acids, and glycosides have been identified and quantified in different edible vegetables from Asteraceae, Amarantaceae, Amarylidaceae, Brassicaceae, Dioscoreaceae, and Solanaceae families by gas chromatography−mass spectrometry. Apart from glucose, fructose, and sucrose, other saccharides such as sedoheptulose in chicory, spinach, cabbage, purple yam, eggplant, radish, and oak leaf lettuce, rutinose in eggplant skin, and a glycosyl-inositol in spinach have been identified. *chiro*-Inositol was found in all vegetables of the Asteraceae family (3.1−32.6 mg 100 g^{-1}), whereas *scyllo*-inositol was detected in those of purple yam, eggplant, artichoke, chicory, escarole, and endive (traces−23.2 mg 100 g^{-1}). α-Galactosides, kestose, glucaric acid, and glycosyl-glycerols were also identified and quantified in some of the analyzed vegetables. Considering the bioactivity of most of these compounds, mainly chicory leaves, artichokes, lettuces, and purple yam could constitute beneficial sources for human health.

KEYWORDS: Vegetables, low molecular weight carbohydrates, *chiro*-inositol, *scyllo*-inositol, *myo*-inositol

INTRODUCTION

Vegetables have been considered healthy foods because of their vitamin and fiber content, as well as a source of other bioactive substances such as antioxidants (e.g., spinach,[1] chicory,[2] artichokes,[3] cabbage,[4] and lettuces [5]). Nevertheless, the carbohydrate fraction also deserves a detailed study.

Carbohydrates in vegetables mainly consist of cellulose and other polysaccharides, with important roles as structural materials and energy reserves. Free low molecular weight carbohydrates (LMWC) are biologically important constituents of vegetables and include saccharides, mainly fructose, glucose, and sucrose. Minor compounds in this fraction are oligosaccharides such as raffinose or kestose, as well as cyclitols, alditols, and acid sugars, many of them with positive properties.

Besides their role in plant metabolism, inositols have been shown to have favorable consequences in human health[6−8] and to present substantial beneficial effects for the treatment of certain diseases.[9] They have been proposed for treating conditions associated with insulin resistance, which can result in disorders such as diabetes mellitus, obesity, atherosclerosis, hypertension, etc.[10] Special attention has been paid to their effect on the treatment of polycystic ovary syndrome.[6] The administration of inositol to premature infants with respiratory distress syndrome who received parenteral nutrition during the first week of life is also associated with increased survival and a decrease incidence of retinopathy of prematurity.[11]

The best known and ubiquitous member of this family is *myo*-inositol;[12] other interesting and less extended inositols are *chiro*-inositol, which has been detected in soybeans,[13] citrus fruits,[14] and black rice,[15] and *scyllo*-inositol, which has been found in fruits[14,16] and also in vegetables from *Apiaceae* family.[17] The beneficial properties of these carbohydrates and their derivatives have promoted the study of their concentration in human diet. Alditols have also shown many physical properties similar to sugars but with lower caloric content, noncariogenicity, low glycemic index, and low insulin response.[18,19]

Data about saccharides in edible vegetables have been focused on common sugars (glucose, fructose, and sucrose) and a few polyalcohols (mannitol, sorbitol, and *myo*-inositol),[20−23] whereas the presence of other LMWC with possible functional properties has not been considered.

Special attention has been paid to fructans (FOS) considering their prebiotic properties.[24] Some α-galactosides such as raffinose, stachyose, and galactosyl-cyclitols have been considered as non-nutritional carbohydrates,[25] but at present, they are considered as prebiotics.[21] These carbohydrates are characteristic compounds of legumes, although some of them appear in other vegetal sources such as beet root,[26] potato,[27] and safflower.[28]

Gas chromatography coupled to mass spectrometry (GC-MS) is a powerful technique for the separation, structural elucidation, and quantification of volatile compounds, including carbohydrates previously submitted to a derivatization process.

In the present work, GC-MS has been used for the determination of LMWC in different vegetables from the market, considering the beneficial properties of these compounds and the influence of their content in the diet. Besides the more common soluble saccharides, other carbohydrates including inositols, alditols, sugar acids, heptuloses, and glycosides have been identified and quantified.

MATERIALS AND METHODS

Standards. Fructose, galactinol, galactose, glucose, *chiro*-inositol, *myo*-inositol, *scyllo*-inositol, isomaltose, kestose, maltose, mannitol, mannose, phenyl-β-D-glucopyranoside, raffinose, rutinose, and sucrose were acquired from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO), and mannoheptulose was obtained from Biosynth (Staad, Switzerland).

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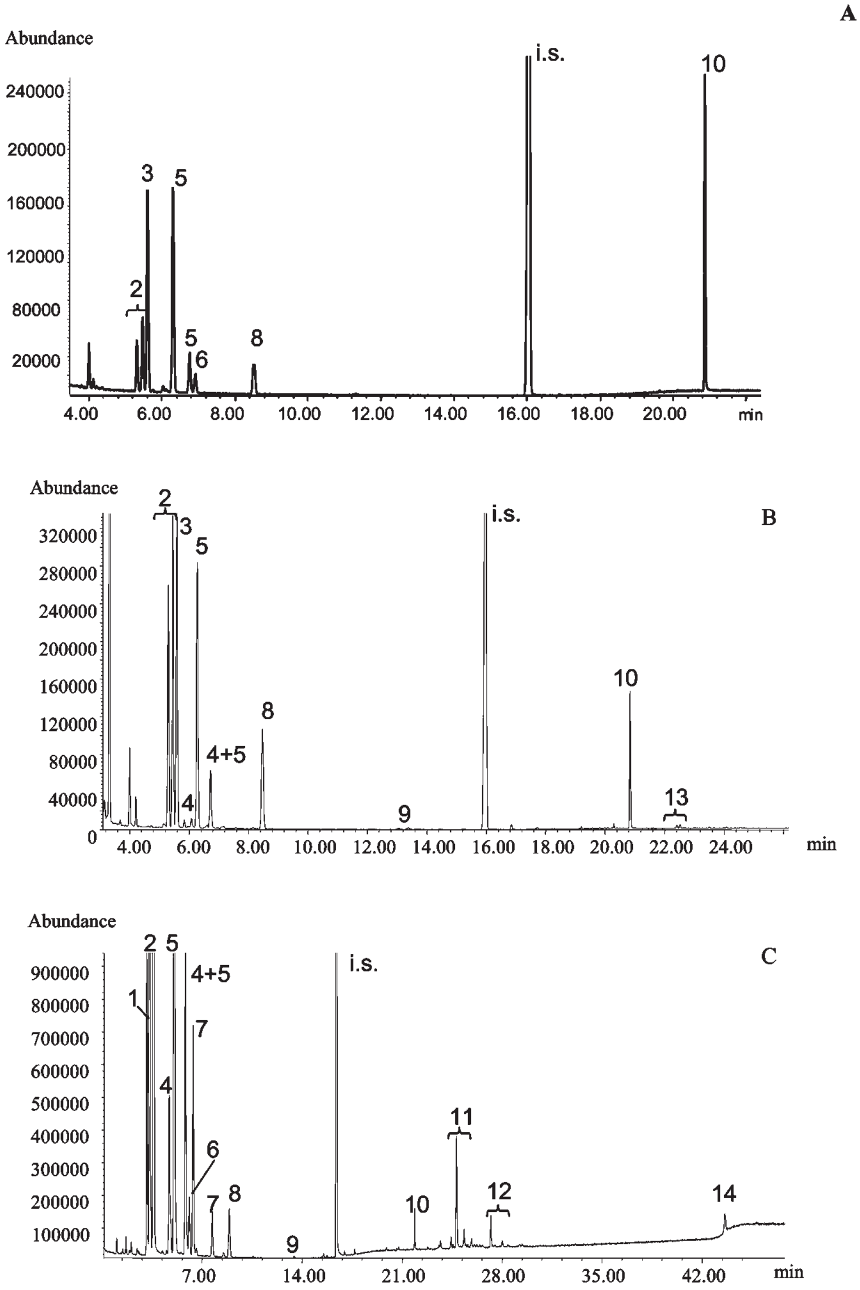

Figure 1. GC-MS profiles of TMSO of low molecular weight carbohydrates of (A) artichoke, (B) oak leaf lettuce, and (C) purple yam. Peaks: 1, mannitol; 2, fructose; 3, *chiro*-inositol; 4, galactose; 5, glucose; 6, *scyllo*-inositol; 7, glucaric acid; 8, *myo*-inositol; 9, sedoheptulose; i.s., phenyl-β-glucoside; 10, sucrose; 11, maltose; 12, isomaltotriose; 13, other disaccharides; and 14, kestose.

Samples. Good quality fresh vegetables (artichoke, chicory leaves, endive, escarole, spinach, beet, cabbage, radish, eggplant, purple yam, and lettuces) were purchased at local markets in Madrid (Spain). As the sugar content in vegetables depends on several factors including cultivar, season, and agricultural and postharvest treatments, seven different cultivars of lettuces were purchased in different seasons. The extraction was carried out using edible parts of the samples. Two or three units of each vegetable were chopped and mixed. Five grams of them was immediately extracted with 25 mL of ultrapure water +0.1% acetic acid at 60 °C for 1 h, using constant agitation. The different extracts obtained were filtered with Whatman #1 filter paper and kept at −20 °C until analysis. Edible skins from eggplant and radish were also used for analysis. All extractions were carried out in duplicate.

GC-MS Analysis. One milliliter of vegetable extract was mixed with 0.5 mL of phenyl-β-D-glucoside (1 mg mL^{-1}) and evaporated under vacuum. Dried samples were treated with 350 μL of 2.5% hydroxylamine hydrochloride in pyridine (30 min at 75 °C), and 350 μL of hexamethyldisilazane plus 35 μL of trifluoroacetic acid (45 °C for 30 min).[29,30]

Derivatized samples were centrifuged, and 1 μL of supernatant was injected into the injection port of a Hewlett-Packard 7890 gas chromatograph coupled to a 5975 quadrupole mass detector (both from Agilent, Palo Alto, CA), using helium as carrier gas (average linear velocity ~20 cm s^{-1}). A 30 m × 0.25 mm i.d. × 0.25 μm film thickness fused silica column coated with TRB-1 (cross-linked methyl silicone) from Teknokroma (Barcelona, Spain) was used. The oven temperature was held at 200 °C for 20 min, then programmed to 270 °C at a heating rate of 15 °C min^{-1}, then programmed to 290 at 1 °C min^{-1}, and finally

Table 1. Saccharide Concentrations (mg/100g of Product) in Analyzed Vegetables[a]

family	genus	common name	fructose	galactose	glucose	sedoheptulose	sucrose	other disaccharides	raffinose	kestose
Asteraceae	*Cichorium*	chicory leaves	345.5 (48.9)	6.5 (1.5)	229.5 (25.8)	1.0 (0.2)	125.8 (51.5)			
		endive	687.1 (3.9)		829.8 (4.0)	tr[b]	32.5 (0.1)			
		escarole	358.6 (8.2)		239.3 (4.8)		31.2 (0.9)			
	Cynara	artichoke	8.9 (1.0)		15.7 (1.7)	tr	21.2 (2.7)			
	Lactuca	Batavian lettuce	294.9 (29.3)	3.3 (0.1)	194.2 (12.9)	tr	28.1 (1.9)			
		iceberg lettuce	485.6 (7.6)	1.4 (0.8)	389.8 (6.5)		23.8 (4.1)			
		oak leaf lettuce	38.4 (1.1)	1.3 (0.1)	25.3 (0.6)	0.8 (0.0)	8.7 (0.2)	0.7 (0.0)		
		Lollo Rosso lettuce	6.4 (0.3)	0.9 (0.1)	3.4 (0.2)		1.0 (0.1)	3.6 (0.2)		
		Romaine lettuce	216.1 (2.0)	tr	144.3 (1.5)		29.5 (0.6)			
		Cresta lettuce	361.3 (6.0)	2.0 (0.5)	255.6 (2.4)		38.8 (0.9)			
Amarantaceae	*Spinacia*	spinach	47.5 (6.4)	2.2 (0.1)	74.8 (10.7)	0.4 (0.3)	54.4 (14.7)	0.5[c] (0.1)		
	Beta	beet root	140.0 (58.42)	10.5 (0.5)	220.7 (75.5)	tr	10697.5 (1010.9)	30.5[d] (9.2)	37.7 (8.6)	16.9 (2.6)
Amarylidaceae	*Allium*	onion	1760.1 (434.2)	12.7 (0.9)	1538.9 (352.7)		220.6 (7.6)	2.2 (0.2)		
Brassicaceae	*Raphanus*	radish[e]	797.6 (80.9)	4.3 (0.5)	799.9 (108.3)	2.1 (0.4)	46.4 (12.6)			
	Brassica	cabbage	614.6 (4.2)	61.1 (0.5)	693.7 (19.9)	1.6 (0.0)	251.6 (50.1)		1.3 (0.1)	
Dioscoreaceae	*Dioscorea*	purple yam	2622.1 (170.7)	181.7 (3.5)	913.8 (47.4)	tr	1.6 (0.1)	145.8[f] (30.2)		19.0 (1.0)
Solanaceae	*Solanum*	eggplant	827.0 (96.8)	2.6 (0.1)	965.9 (115.7)		147 (11.93)	[g]		

[a] Standard deviations are in parentheses. [b] tr, traces. [c] Isomer of galactinol. [d] Galactinol and an isomer of sucrose (about 13 and 23 mg 100 g^{-1}, respectively). [e] Radish also contained 0.5 mg/100 g mannose. [f] Mainly maltose and isomaltose. [g] Free rutinose was detected in skin (1.9 mg 100 g^{-1}).

programmed to 300 °C at 15 °C min^{-1} and held for 40 min.[15] The injector temperature was kept at 300 °C, and injections were made in split mode with a split ratio of 1:20. The mass spectrometer was operated in electronic impact (EI) mode at 70 eV, scanning the 35−700 m/z range. The interface and source temperature were 280 and 230 °C, respectively. Acquisition was done using a HPChem Station software (Hewlett-Packard, Palo Alto, CA).

RESULTS AND DISCUSSION

The GC-MS method allowed the analysis of soluble carbohydrates (free saccharides, cyclitols, alditols, sugar acids, and glycosides) along with small amounts of free amino acids, phenolic acids, and other low molecular weight substances. Figure 1 shows the chromatographic profile of artichoke, oak leaf lettuce, and purple yam. The identity of peaks was assigned by GC-MS and confirmed by comparison of retention time and mass spectra with those of standard substances, when available. Otherwise identities were given as tentative.

Quantitative results (average values expressed as mg 100 g^{-1} and standard deviations) are shown in Tables 1 and 2. The detection (LOD) and quantitation (LOQ) limits of the method were calculated for each compound according to Foley and Dorsey.[31] Mean values of 0.13 and 0.4 mg 100 g^{-1} were obtained for LOD and LOQ, respectively.

Saccharides. Average values (mg 100 g^{-1} of product) and standard deviations of saccharides found in the vegetables are shown in Table 1. Predominant sugars in most vegetable in this study were fructose and glucose, these values being higher in onion (1.8 and 1.5 g 100 g^{-1}, respectively) and purple yam (2.6 and 0.9 g 100 g^{-1}, respectively). Sucrose appeared in all samples, ranging from 1 mg 100 g^{-1} in Lollo Rosso lettuce to 11 g 100 g^{-1} in beet root. Galactose occurred as a minor component in most samples, the highest values being found in cabbage and purple yam (61 and 181 mg 100 g^{-1}, respectively), whereas small amounts of mannose were found only in radish (0.5 mg 100 g^{-1}).

A carbohydrate with a mass spectrum compatible with a heptulose was also detected in chicory, spinach, cabbage, radish, and oak leaf lettuce at low levels (less than 2.1 mg 100 g^{-1}) and at trace levels in a few more. This saccharide was identified as sedoheptulose by comparison with a hot water extract of *Sedum spectabile* leaves. Heptuloses are common in Crassulaceae and have been detected in 26 plant families[32] and tropical fruits.[33] Soria et al.[17] have recently reported the presence of sedoheptulose in carrots ranging from 1.5 to 5.8 mg 100 g^{-1} of product, values slightly higher than those found in the vegetables analyzed in the present work. Besides sedoheptulose, other heptulose (probably manno-heptulose on the basis of its mass spectrum and retention time) appeared in spinach (0.8 mg 100 g^{-1}).

It is worth noting the presence of free rutinose (6-*O*-L-rhamnopyranosyl-D-glucose) in eggplant skin. This disaccharide is commonly present in different vegetal sources as a rutinoside. However, it is not frequently found in free form.

Apart from the small amounts of sucrose found in purple yam, some disaccharides, mainly maltose and isomaltose, were detected. The presence of maltose in yam has been previously reported.[34] The only trisaccharide detected in this plant was kestose.

A small peak eluting close to sucrose with a mass spectrum very similar to this disaccharide and differing only in the slightly higher relative abundance of the ion at m/z 271 was also detected in beet root. Taking into account its retention time and mass spectrum, it could correspond to a diastereomer of sucrose. However, it could not be confirmed considering that GC and MS data about diastereomers of sucrose are very scarce.[35] Galactinol, raffinose, and kestose, which have been reported in this tuber,[26,36] were also found in the present work.

Cyclitols. Cyclitol concentrations of analyzed vegetables are shown in Table 2. As expected, *myo*-inositol (which is the most abundant inositol in nature, occurring in both vegetal and animal kingdoms) was observed in all analyzed samples, ranging from 0.5 mg 100 g^{-1} in Lollo Rosso to 24.6 mg 100 g^{-1} in purple yam.

chiro-Inositol was found in all vegetables of *Cynara*, *Cichorium*, and *Lactuca* genus (chicory, endive, escarole, artichoke, and lettuces), all belonging to Asteraceae family. This cyclitol is a secondary messenger in insulin signal transduction, and different

Table 2. Polyalcohol and Inositol Concentrations (mg/100 g of Product) in Analyzed Vegetables[a]

family	genus	common name	mannitol[b]	*chiro*-inositol	*scyllo*-inositol	*myo*-inositol
Asteracea	*Cichorium*	chicory leaves	0.8 (0.5)	19.9 (2.3)	5.3 (0.1)	18.2 (0.4)
		endive	1.8 (0.4)	3.1 (0.2)	0.9 (0.1)	3.0 (0.0)
		escarole		4.4 (0.3)	tr[c]	4.1 (0.2)
	Cynara	artichoke		21.6 (1.7)	2.1 (0.3)	3.0 (0.2)
	Lactuca	Batavian lettuce		8.2 (1.1)		10.9 (0.9)
		iceberg lettuce		8.3 (0.2)		8.4 (1.1)
		oak leaf lettuce		32.6 (1.0)		8.0 (0.3)
		Lollo Rosso lettuce		9.0 (0.3)		0.5 (0.0)
		Romaine lettuce		3.5 (0.1)		4.1 (0.2)
		Cresta lettuce		13.2 (0.3)		18.4 (0.3)
Amarantacea	*Spinacia*	spinach	0.4 (0.5)			1.2 (0.1)
	Beta	beet root				1.7 (0.2)
Amarylidacea	*Allium*	onion				22.2 (2.1)
Brassicacea	*Raphanus*	radish	1.9 (1.7)			4.4 (0.9)
	Brassica	cabbage	3.2 (0.5)			18.1 (1.2)
Dioscoreacea	*Dioscorea*	purple yam	141.1 (7.2)		28.3 (0.1)	24.6 (1.6)
Solanacea	*Solanum*	eggplant			1.6 (0.0)	21.5 (0.2)

[a] Standard deviations are in parentheses ($n = 2$). [b] Mannitol was overlapped with small amounts of an unknown product with m/z fragments 157, 219, 244, and 375. [c] tr, traces.

studies have shown that it can help to treat women with polycystic ovarian syndrome by improving insulin sensitivity.[6,37] Previous studies have reported that *chiro*-inositol is frequently found in the Asteraceae family,[38] but to the best of our knowledge, its presence in the vegetables studied here has not been previously reported. Concentrations of *chiro*-inositol ranged from 3 mg 100 g^{-1} in endive to 33 mg 100 g^{-1} in oak leaf lettuce. It is also worth pointing out the relatively high values observed in artichoke where it was the most abundant LMWC detected along with sucrose. Its content in vegetables is lower than in citrus juices,[14] where it varied from 7 mg 100 mL^{-1} in lemon juice to 108 mg 100 mL^{-1} in mandarin orange juice.

Therapeutic properties related to cognitive deficit in AD pathologies have been attributed to *scyllo*-inositol.[39] Small amounts of *scyllo*-inositol were found in chicory, endive, escarole, artichoke, purple yam, and eggplant, varying from traces in escarole to 28.3 mg 100 g^{-1} in purple yam. This figure was lower than that reported by Soria et al.[17] in carrot, parsley, coriander, and fennel (2 mg g^{-1}). Quantitative differences in cyclitol concentrations among diverse cultivars of lettuces examined can be attributed to factors such as cultivar, season, and agricultural and postharvest treatments.

Alditols. Alditol concentrations of vegetables studied are also shown in Table 2. These polyalcohols play various physiological roles in vegetables.[18,19] Small amounts of mannitol appeared in chicory leaves, endive, spinach, radish, cabbage, and eggplant; however, its chromatographic peak was overlapped with an unknown product with fragments at m/z 157, 219, 244, and 375 in its mass spectrum. The highest level of mannitol (141.1 mg 100 g^{-1}) was found in purple yam, where the interference was not detected.

Other Compounds. Glucaric acid also appeared in a small concentration in cabbage and spinach, as previously reported;[41,42] this sugar acid was also detected in purple yam in this work at notably higher levels (about 36 mg 100 g^{-1} of product). This acid has been shown to promote some beneficial effects on health.[40,41]

Small amounts of glycosyl glycerols were detected in cabbage (1.0 mg 100 g^{-1}), purple yam (2.5 mg 100 g^{-1}), eggplant (0.17 mg 100 g^{-1}), and spinach (0.22 mg 100 g^{-1}). Minute amounts (not quantified) of sugar phosphates were found in chicory and cabbage.

Final Remarks. The exhaustive analysis of LMWC in natural sources is a difficult task, due to the high number of isomers present and the scarce availability of standards. However, the chosen GC-MS method has afforded the simultaneous quantification of saccharides, polyalcohols, acid sugars, and glycosides in different vegetables. It has also allowed the identification for the first time of several remarkable and/or bioactive compounds such as *chiro*-inositol, *scyllo*-inositol, sedoheptulose, free rutinose, α-galactosides, kestose, glucaric acid, and glycosyl-glycerols in the vegetables studied.

A recent review by Fardet[42] offered new perspectives about the health-protective effects of whole grain cereals, considering the high number of bioactive compounds present. Similar hypotheses can be set out about the vegetables studied here. They contain different bioactive LMWC, besides fiber, vitamins, antioxidants, and prebiotics.

Considering the therapeutic potential of cyclitols (treatment of conditions associated with insulin resistance, polycystic ovary syndrome, respiratory distress syndrome, AD-like pathologies, etc.), some of the vegetables such as chicory leaves, artichokes, and lettuces should be included in special diets to increase their consumption. Moreover, purple yam has shown to be a valuable source of bioactive carbohydrates. Removal of nonbioactive mono- and disaccharides (glucose, fructose, and sucrose) from these vegetable sources to obtain special ingredients for diabetics could be a new route for future investigations.

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