Molecular Size of Garlic Fructooligosaccharides and Fructopolysaccharides by Matrix-Assisted Laser Desorption Ionization Mass Spectrometry

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Fructooligosaccharides and fructopolysaccharides were isolated from different cultivars of garlic. The garlic cultivars investigated included organically grown garlic, elephant garlic, garlic grown in California, and garlic from China. Garlic contained a high concentration of oligo- and polyfructosaccharides, ranging from 125 to 235 mg/g, on a wet weight basis. Fructose:glucose ratio in the fructooligo/fructopolysaccharides was about 15:1. The molecular weight distribution of garlic fructooligosaccharides and fructopolysaccharides was investigated by MALDI-MS. Molecular masses of fructans ranged from less than 1000 Da to around 4500 Da, corresponding to DP as high as 38. Differences in sugar size, among cultivars, were observed. Comparison was made with MALDI-MS spectra of fructose, sucrose, and inulin. Molecular masses of commercially available inulin, from Dahlia tubers, ranged from less than 1000 Da to 4000 Da with a peak value of distribution around 2500, corresponding to DP 14. Dahlia tubers contained mostly oligofructose while garlic cloves contained a mixture of oligofructose and polyfructose. We suggest that the health-enhancing properties ascribed to garlic should also include garlic high content of fructooligo/polysaccharides.

Keywords: *Fructooligo/polysaccharides; garlic; MALDI-MS*

INTRODUCTION

Fructans are non-reducing water-soluble fructooligo/polysaccharides that accumulate in temperate and cool zone grasses, such as garlic, as short-term carbohydrate storage materials used for osmoregulation, adaptation to low temperature photosynthesis, and protection from freezing stress (Darbyshire and Henry, 1978; Pollock, 1984; Nelson and Smith, 1986; Chatterton et al., 1990; Livingston, 1990).

Fructans resist hydrolysis and digestion in the human upper gastrointestinal intestinal tract and thus represent a low caloric sweetener (providing about 2 kcal/g) with effects similar to those of dietary fiber (Yamashita et al., 1984; Hidaka et al., 1986; Tokunaga et al., 1986). In the colon, fructans are almost quantitatively fermented by competitive microflora, such as bifidobacteria, which generate adverse conditions for *Salmonella* colonization (Roberfroid, 1993; Ebskamp, 1994; Oyarzabal et al., 1995). Fructooligosaccharide feeding improved recovery from anemia and increased Fe, Ca, Mg, and Pi absorption in normal and Fe-deficient anemic rats (Ohta et al., 1995).

Garlic, *Allium sativum*, has since millenia attracted attention as food with a wide range of culinary and medicinal values. Antiarrhythmic, antiatherosclerosis, antibacterial, anticancer, antidiabetic, antifungal, antihelminthic, antioxidant, antipathogenic, antiprotozoal, antithrombic, antiviral, antiradical, enzyme-inhibiting, larvicidal, and pesticidal properties have all been ascribed to garlic (Willis, 1956; Caragay, 1992; Lawson, 1993; Popov et al., 1994).

Allium species accumulate only fructans as their nonstructural carbohydrates (Darbyshire and Henry, 1981). Garlic sugar consists of a mix of homologous series of linear β (2→1) and β (2→6) linked oligofructoses connected to a terminal sucrosyl moiety with a molecular mass up to 9 kDa (Pollock, 1986). Starch and monosaccharides are absent in garlic; fructan and sucrose make up to 96% and 4% of total nonstructural carbohydrate, respectively (Pollock, 1986). Oligomers of at least DP 8 and polymers of up to DP 50 have been reported in garlic using gel permeation chromatography (Spiegel et al., 1994; Darbyshire and Henry, 1981). A selenium-containing polysaccharide with an average MW of 15 000, corresponding to DP 83, isolated from Hubei Enshi garlic inhibited human cytomegalovirus as shown by plaque reduction assays and cytopathogenic effect tests (Yang et al., 1992).

Interest in oligosaccharides is growing worldwide as they are being recognized as health-promoting food ingredients (O'Donnell, 1994; Harada et al., 1994). Ebskamp (1994) tested whether fructans could accumulate in transgenic crop plants by selecting the vacuolar constructs for introduction in potato plants. Such potato plants could accumulate up to 30% of their dry weight as fructan in leaves. Large quantities of these sugars are now being produced, synthetically or from natural sources, for use in health foods, processed foods, and pet foods.

Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) allows effective fingerprinting of the chemical composition of a food sample without resorting to time-consuming separation/enrichment/isolation methods (Stahl et al., 1991, 1994, 1997; Mock et al., 1991, 1995; Pasch and Rode, 1995). The advantages of MALDI-MS over conventional techniques of carbohydrate analysis are, among others, sensitivity, short analysis time, and direct access to molecular weights.

The main objectives of this study were to determine the concentration of total sugar and the molecular size

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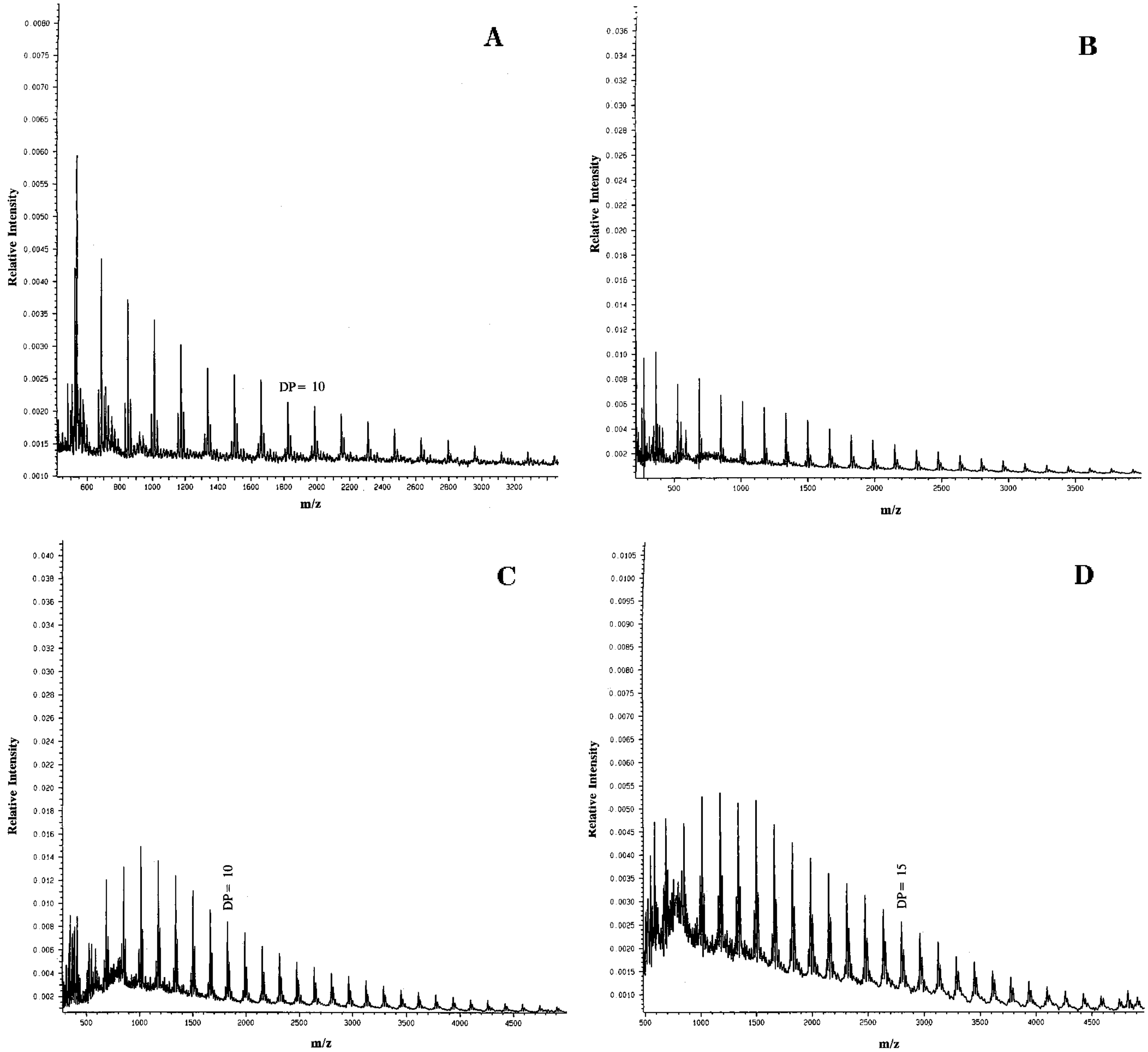

Figure 1. MALDI-MS spectra, in positive-ion mode, of garlic sugar. Each spectrum was obtained as the sum of 200 shots accumulated by rastering accross the sample to improve the signal to noise ratio. (A) Organically grown garlic, (B) elephant garlic. (C) California grown garlic, (D) garlic imported from China. The region of mass spectrum below 500 Da is mostly dominated by intense signals associated with the DHB matrix.

Table 1. Total Sugar Concentration in Different Garlic Cultivars

garlic cultivar	sugar concn (mg/g wet weight)
organically grown	161−200
elephant	141−189
California grown	125−216
China grown	180−235

distribution of fructooligosaccharides/fructopolysaccharides in garlic by MALDI-MS.

MATERIALS AND METHODS

Materials and Reagents. Garlic samples, *Allium sativum* (organically grown garlic, garlic grown in California, and garlic samples from China), and elephant garlic (*Allium ampeloprasum*) were purchased from different stores across Vancouver, BC. Fructose, sucrose, and inulin (from Dahlia tubers) were purchased from Sigma (St. Louis, MO). 2,5-Dihydroxybenzoic acid (DHB) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Ion exchange Diaion HP 20 was obtained from Supelco (Mississauga, ON). All other reagents were of analytical grade.

Total Sugar Isolation. Sugar extraction was performed according to Darbyshire and Henry (1981). A total of 100 g of bulbs was blended with 250 mL of 80% boiling ethanol (v/v). The supernatant was decanted, the pulp was blended twice with 100 mL of 100% ethanol and twice with 200 mL of water, and the extracts were combined. No more sugar was detected in the pulp. The combined supernatant was concentrated under vacuum to remove ethanol and the sugar was stored at −20 °C. The frozen sample was thawed and centrifuged at 10000*g* for 20 min to remove additional protein. The supernatant was deionized using Diaion HP 20 ion exchange resins and lyophilized.

Total Sugar Concentration in Garlic Cloves. Total sugar concentration of dry garlic cloves, expressed as fructose, was determined by the phenol−sulfuric acid method as modified by Saha and Brewer (1994).

Garlic Sugar Hydrolysis. Hydrolysis was carried out by heating sugar in 0.02 M HCl for 1 h at 100 °C (Wolf and Ellmore, 1975).

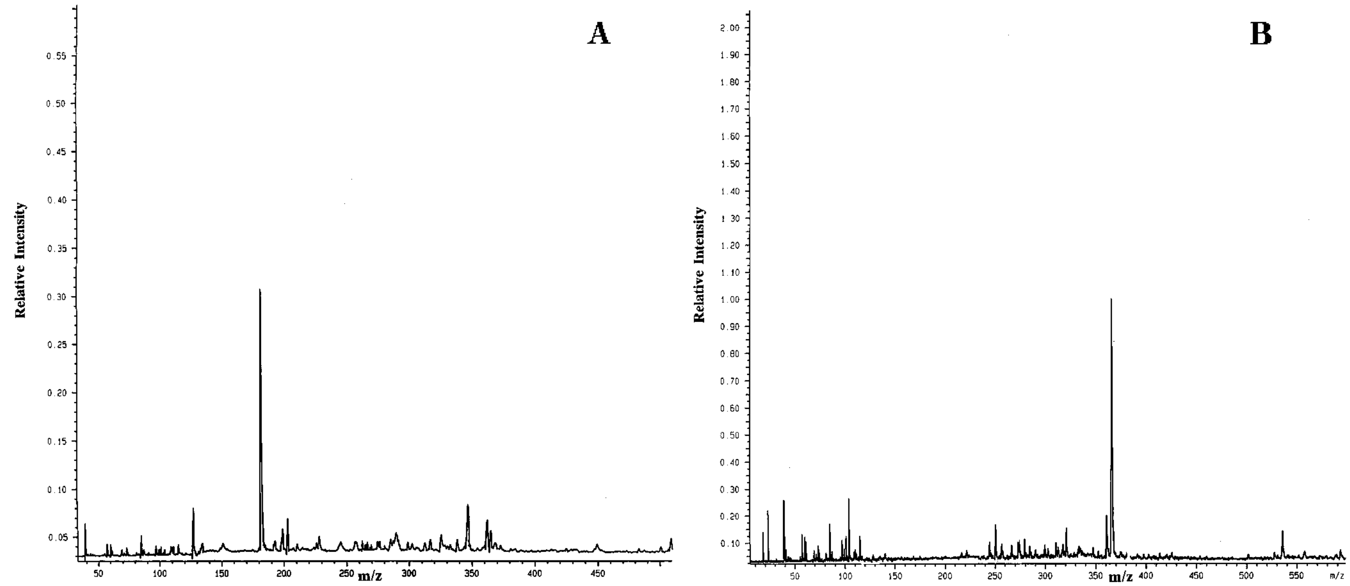

Figure 2. MALDI-MS spectra of (A) fructose and (B) sucrose. One microliter of 1 mg/mL fructose or sucrose was mixed with 1 μL of 10 mg/mL DHB, and an aliquot was applied on the target and air-dried. Two hundred shots were accumulated as described under Materials and Methods.

MALDI-MS. MALDI-MS was performed using reflectron-type time-of-flight mass spectrometer. Sample matrix consisted of 2,5-dihydroxybenzoic acid (DHB) dissolved at 10 mg/mL in doubly distilled water (Mohr et al., 1995). Isolated garlic sugars were dissolved in doubly distilled water to give a concentration of 1 mg/mL. Fructose and sucrose were dissolved at a concentration of 1 mg/mL each. Inulin, 1 mg/mL, was added to water and heated for 10 min to 50 °C. The sugar solution was mixed with a 5-fold volume of DHB. A volume of 1 μL of the sugar-matrix mixture was placed on the stainless target and air-dried. External calibration was provided by the $[M + H]^+$ ions of dextran 1000, insulin, and cytochrome C. MALDI-MS analysis was performed on a Bruker Biflex II operating at 20 keV accelerating potential and using 337 nm radiation from a nitrogen laser with the mass spectrometer operating in the reflectron mode. Spectra were obtained in positive ion mode. Data were obtained by averaging 200 shots acquired by rastering across the sample surface in order to improve the signal to noise ratio.

RESULTS AND DISCUSSION

Sugar concentrations of garlic samples analyzed are given in Table 1. High concentrations of sugars, on a wet weight basis, were found in garlic cloves. Fructose and glucose were found in a molar ratio of 15:1.

MALDI-MS. MALDI-MS of neutral garlic sugars, using DHB as matrix, are shown in Figure 1. The molecular mass distribution of garlic sugar comprised oligomers and polymers of *m/z* values ranging from less than 1000 to about 4000 Da. This value corresponds to a degree of polymerization of 21. The maximum abundance of molecular mass, in one cultivar, was as high as 6829 (not shown) corresponding to DP 38. Differences in sugar size were observed among cultivars. Figure 1 also shows that sugar size was distributed over a wide range of molecular weight and was not centered around some *m/z* values. In general, carbohydrates are determined as $[M + Na]^+$ and $[M + K]^+$ ions because sodium and potassium are ubiquitous contaminants of sample matrix and target. Table 2 gives the molecular weight distribution of sugars in different garlic cultivars. Most sugars and their fragments appeared as sodium, potassium, or sodium and potassium adducts.

MALDI-MS of fructose and sucrose were obtained under the same experimental conditions and are shown in Figure 2. The *m/z* values of 180 and 203 were associated with the presence of fructose and fructose plus sodium adduct, respectively. Peaks associated with sucrose were observed at *m/z* equal to 360 and 383 ($M + Na^+$), respectively. The *m/z* value of 349, 339, 333, and 154 were associated with DBH matrix adducts. Peaks associated with fructose or glucose, at *m/z* 180 or 203, were not present in all garlic cultivars investigated (Figure 3). Pollock (1986) reported no monomeric sugar present in garlic. Stahl et al. (1991) reported that spectral signals in the mass range below 500 Da exclusively represent ions of the matrix. In *Allium* species, fructan synthesis occurs via a transfer involving a sucrose:sucrose fructosyltransferase and a fructan: fructan fructosyltransferase (Darbyshire and Henry, 1981). Sucrose:sucrose fructosyltransferase specifically synthesizes trisaccharide from sucrose while fructan: fructan fructosyltransferase utilizes trisaccharide as

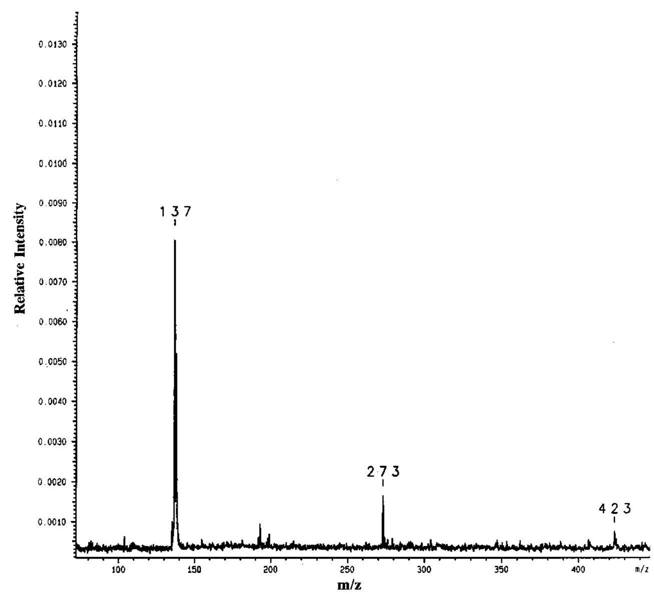

Figure 3. MALDI-MS spectrum of garlic sugar in the region of *m/z* value below 450 showing the absence of free fructose or glucose in the sugar mixture. Spectrum was obtained under the same conditions as described under Materials and Methods.

Table 2. Molecular Weights of Oligo/polyfructosaccharides from Garlic Cultivars by MALDI-MS

organically grown	elephant garlic	California grown	China grown	inulin
			3935	
			3773	
	3623		3623	3623
			3448	3449
	3279		3286	3286
	3120		3124	3124
	2962		2979	2977
			2962	2960
			2816	2814
2795	2797		2799	2779
			2724	2724
2633	2627		2637	2636
			2492	2489
2471	2474		2475	2474
2324		2328	2329	2328
2309	2304	2312	2313	2311
2163		2166	2167	2166
2146	2145	2141	2150	2149
2001		2004	2005	2003
1985	1986	1988	1988	1987
1938			1840	1839
1823	1825	1825	1824	1824
1683		1679	1680	1683
1660		1660	1664	1660
	1652	1646	1646	1645
1515		1517	1518	1517
1502		1502	1501	1502
1481		1479	1484	1481
1353		1355	1355	
	1335	1339	1339	1338
1319		1321	1322	1320
		1283		
		1263		
		1252		
		1236		
		1219		
1191		1192	1193	1193
1175	1176	1176	1176	1176
1159		1159	1159	1159
		1147		
1029				
1013	1014	1014		1014
995		997		996
867		868		
851		852		
833		834		
817				
795				
779				
758				

substrate to produce higher polymers. Darbyshire and Henry (1981) suggested that, in *Allium*, carbohydrate supply in the form of substrate sucrose may play a central role and control the ability of the plant to synthesize polymers having different degree of polymerization.

Comparison was made with the size distribution of sugars in commercially available inulin (Figure 4). The molecular size of inulin ranged from less than 1000 to 3600. Degree of polymerization was as high as DP 20 with a maximum peak intensity centered around 2500 Da. Dahlia tubers contained mostly oligofructose. Inulin is a polydisperse fructan, with a DP ranging from 2 to 60 or more, consisting mostly of linear chains of fructosyl moieties linked by β (2→1) (Roberfroid, 1993). Inulin fraction with a DP between 2 and 20 is considered as an oligofructose. Inulin is slightly soluble in water; heating at 50 °C is required for complete dissolution. Stahl et al. (1997) have recently published the MALDI-MS of onion, *A. cepa*. The molecular size of onion sugar

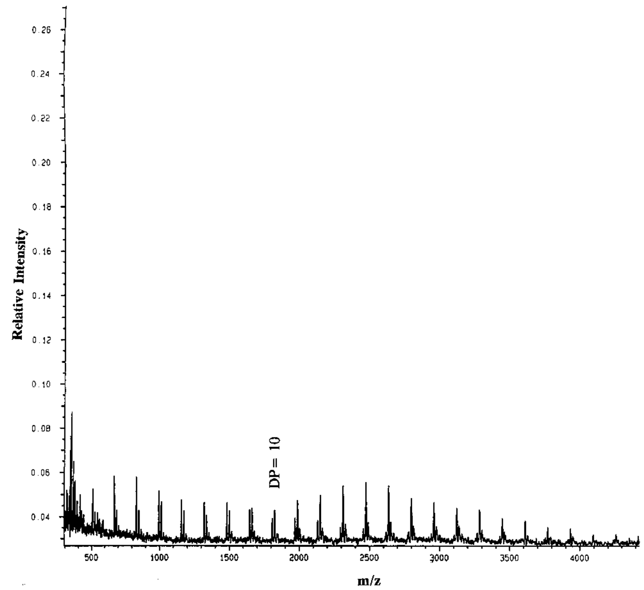

Figure 4. MALDI-MS of inulin from Dahlia tubers. Inulin was dissolved in water at 50 °C and mixed 1:1 with the matrix as described under Materials and Methods.

did not exceed the *m/z* value of 3000. Darbyshire and Henry (1981) reported onions to contain mostly oligosaccharides with a DP of up to 7. Polymer size difference between onion and garlic was also reported by Darbyshire and Henry (1981) using gel permeation chromatography. Onions accumulate no starch, and they accumulate less and small-size fructans, more sucrose, and more monosaccharides than garlic, which accumulates mostly higher concentrations of both fructooligosaccharides and fructopolysaccharides. Highly polymerized fructans are not efficiently utilized by bifidobacteria (Yazawa and Tamura, 1982).

Garlic accumulates more fructan than soybeans but less than Jerusalem artichoke on a dry wet basis (Yamazaki and Matsumoto, 1994). The health benefits associated with garlic consumption should also include garlic high content of fructooligosaccharides and fructopolysaccharides. The use of garlic sugar in food products may open up interesting new fields of applications or add up to existing fields of applications with additional health benefits. A wide range of applications awaits garlic sugar.

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Received for review May 28, 1997. Accepted September 2, 1997.[⊗]

JF970433U

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1997.