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High-performance liquid chromatographic determination of dietary fibre in raw and processed carrots☆

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Abstract

An HPLC method was developed to determine dietary fibre in carrots (*Daucus carota* L.). Primary hydrolysis of dietary fibre residue was performed with 12 *M* H_2SO_4 for 2 h at 40°C and secondary hydrolysis with 0.414 *M* H_2SO_4 for 3 h at 100°C. For the neutralization step prior to injection, AG4-X4 resin (Bio-Rad) was used. Neutral monosaccharides were separated using an HPX-87P column (Bio-Rad). This method was applied for evaluation of the quantitative variation of dietary fibre content in carrots during autoclaving.

1. Introduction

There are several definitions of dietary fibre which differ mainly in the components considered. One of them refers to this component of food as non-starch polysaccharides (NSP) [1]. This definition has been traditionally defended by Englyst and Cummings [1] in terms of providing a clear scope for the analyst: NSP can be clearly measured and are responsible of some physiological effects.

Dietary fibre has been related to some beneficial effects against several diseases, e.g., constipation, diabetes, cancer of the colon and high levels of cholesterol. Some of the mechanisms are not well defined at present, but some others such as absorption of fibre in the foregut, modification of sterol metabolism and caecal fermentation [2] are being elucidated nowadays.

There have been numerous studies on the determination of dietary fibre in carrots by different types of methods, e.g. spectrophotometric, gravimetric and gas chromatographic (GC). HPLC methods for the determination of dietary fibre are very scarce, and the existing ones use refractive index detection. Although cell wall hydrolysates were determined by this technique nearly 20 years ago [3], dietary fibre was not measured by HPLC until some time later. One of the first studies was carried on by Barton et al. [4] and related to the determination of neutral detergent fibre (NDF) in forages. For human food Slavin and Marlett [5] developed an interesting method for the analysis of different kinds of materials. All these HPLC systems

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included refractive index detection and silica, ion-exchange and amino columns.

In this work, we developed a method for the determination of dietary fibre by HPLC that is fundamentally similar to those of Englyst and Cummings [1] and Garleb et al. [6]. We used it to evaluate quantitative changes produced during autoclaving of carrots (*Daucus carota* L.).

2. Experimental

2.1. Equipment

The chromatographic equipment consisted of a Waters chromatograph equipped with a Model 6000 A pump, a U6K injector and a Data Module Model 701 recorder. An Erma ERC-7522 refractive index detector was incorporated (attenuation of sensitivity ×4). A Haake W_{19} water-bath with a Haake D_8 thermostat was used. An HPX-87P column (300 × 7.8 mm I.D.) (Bio-Rad, Richmond, CA, USA) with particle size 9 μm was used. The mobile phase was deionized water filtered through a Millipore membrane (0.45 μm) and degassed in an ultrasonic bath. The optimum temperature was 85°C and the flow-rate was 0.5 ml/min.

2.2. Preparation of sample

Three different lots of carrots were purchased at local markets in Madrid. The samples were peeled in the laboratory and cut into pieces of similar mass. They were separated into three groups and each group was divided into two parts, one of which was kept to be analysed raw and the other was processed. As a result, thermal processing was applied three times to each lot. The conditions of autoclaving were 121°C for 15 min. When thermal treatment was finished, the processed sample and liquid were separated. Raw and processed samples were freeze-dried (Terruzzi Mevilsa TP-3 freeze-drier), homogenized and kept in hermetically closed bottles.

2.3. Procedure

The method is divided into three steps: isolation of dietary fibre residue from the original material, chemical hydrolysis of the residue and neutralization of the hydrolysate for determination of each component by HPLC.

Isolation of dietary fibre residue

The method of Englyst and Cummings [1] was applied, using 300 mg of freeze-dried sample. Starch was dispersed by adding 2 ml of dimethyl sulfoxide (DMSO) and hydrolysed by enzymatic treatment with α-amylase (EC 3.2.1.1) and pullulanase (EC 3.2.1.41) for 16 h. The residue free from starch was washed with ethanol and acetone and then dried.

Chemical hydrolysis of dietary fibre residue

The residue was hydrolysed with 0.3 ml of 12 *M* H_2SO_4 on a hot magnetic plate with stirring for 2 h at 40°C (primary hydrolysis). A volume of 8.4 ml of deionized water was added, followed by stirring for 3 h in a boiling water-bath (secondary hydrolysis).

Neutralization of hydrolysate and chromatographic conditions

An aliquot of 5 ml of hydrolysate was passed through a column of resin (Bio-Rad) 4 cm high and 0.9 cm wide. After the hydrolysate had eluted from the column the latter was washed with 5 ml of deionized water three times. The combined eluates were evaporated to dryness and the residue was dissolved with 2 ml of water. The liquids were filtered through a Millex filter of pore size 0.45 μm and through a Sep-Pak C_{18} cartridge. The purified liquids were kept in a vial and a volume of 75 μl was injected.

Extraction of pectic substances

Galacturonic acid was extracted from freeze-dried samples with hot oxalic acid–ammonium oxalate (pH 4) in two steps following the method described by Dekker and Richards [7].

Determination of galacturonic acid

Pectic substances were measured as galacturonic acid by spectrophotometry using 3,5-dimethylphenol as the chromogenic reagent [8], the method being adapted to carrot samples in our laboratory [9].

3. Results and discussion

Enzymatic treatment for the isolation of a dietary fibre residue free of starch and protein has frequently been studied. In this work, α-amylase and pullulanase were used as highly effective enzymes for starch hydrolysis [1]. In some recent studies [10] the incubation times were drastically reduced owing to the incorporation of Termamyl in the enzymatic treatment in all kinds of food. The elimination of enzymatic treatment in samples with a low proportion of starch has also been proposed [11], because the results obtained were very similar for treated and untreated samples.

In this work, the mass of freeze-dried sample to be used was studied. An amount of 300 mg of carrot proved to give satisfactory results. This is the maximum amount considered in the method for the indicated concentration of enzymes.

For validating the method different assays were carried out and different hydrolysis conditions were compared [12]. Those chosen for the analysis of carrots were applied to standards of cellulose (Sigmacell Type 20) and xylan (Fluka Biochemika), resulting in good recoveries of 91.88 ± 2.50 g/100 g for cellulose and 81.09 ± 2.38 g/100 g for xylan.

An important percentage of unrecovered cellulose corresponds to cellobiose (approximately 6%) and a minor percentage to a different oligosaccharide, probably cellotriose, but unconfirmed owing to lack of a standard [5]. Incomplete hydrolysis of cellulose requires further investigation in order to understand it better. With respect to xylan, the lower recovery obtained is probably due to the important degree of degradation experienced by xylose during secondary hydrolysis. This was clearly revealed during the study of the recovery of monosaccharides in this work and has also been reported by several other workers [5,6,13,14].

Table 1
Monomeric composition of polysaccharides that constitute dietary fibre in raw carrot (g per 100 g dry matter)

Constituent	Mean[a]	R.S.D.[a]
Cellobiose	0.5567	0.5064
Glucose	3.9375	0.1844
Xylose	0.4180	0.0947
Galactose/rhamnose	2.2252	0.1587
Arabinose	1.6330	0.2718
Mannose	0.3546	0.1153
Galacturonic acid	5.1833	0.0868
Total	14.3083	0.0936

[a] $n = 18$.

The resolution of the column was checked using mixtures of standard sugars in different proportions. All the monosaccharides were well separated except rhamnose and galactose, which co-eluted.

It has been reported that the extent of losses of monosaccharides varies depending on the hydrolysis conditions [13]. A solution of standard monosaccharides was treated under selected hydrolysis conditions, obtaining satisfactory recoveries for all the sugars: cellobiose 89.21%, glucose 91.24%, xylose 88.39%, galactose/rhamnose 89.34%, arabinose 95.28% and mannose 98.12%. This assay was carried out in parallel with each batch of samples and the correction

Table 2
Monomeric composition of polysaccharides that constitute dietary fibre in processed carrots (g per 100 g dry matter)

Constituent	Mean[a]	R.S.D.
Cellobiose	0.8123	0.3882
Glucose	5.2812	0.1158
Xylose	0.5399	0.5051
Galactose/rhamnose	3.9278	0.2910
Arabinose	3.3835	0.2773
Mannose	0.4130	0.1770
Galacturonic acid	5.8344	0.1121
Total	20.1921	0.0990

[a] $n = 18$.

Table 3
Comparison of values for monomeric components of dietary fibre in raw and processed carrots (g per 100 g fresh matter)

	Raw				Processed			
	Lot A	Lot B	Lot C	Overall	Lot A	Lot B	Lot C	Overall
Cellobiose	0.037 ± 0.002	0.039 ± 0.002	0.108 ± 0.027	0.061 ± 0.039	0.043 ± 0.007	0.059 ± 0.015	0.069 ± 0.031	0.057 ± 0.014
Glucose	0.449 ± 0.039	0.286 ± 0.020	0.531 ± 0.014	0.422 ± 0.122	0.411 ± 0.065	0.366 ± 0.039	0.347 ± 0.034	0.374 ± 0.032
Xylose	0.044 ± 0.007	0.039 ± 0.002	0.049 ± 0.002	0.044 ± 0.049	0.025 ± 0.005	0.028 ± 0.003	0.061 ± 0.010	0.038 ± 0.020
Galactose/rhamnose	0.247 ± 0.005	0.178 ± 0.012	0.287 ± 0.054	0.237 ± 0.054	0.208 ± 0.003	0.267 ± 0.041	0.353 ± 0.056	0.276 ± 0.071
Arabinose	0.168 ± 0.020	0.113 ± 0.015	0.248 ± 0.048	0.176 ± 0.066	0.214 ± 0.032	0.194 ± 0.032	0.308 ± 0.041	0.238 ± 0.059
Manose	0.033 ± 0.003	0.033 ± 0.003	0.047 ± 0.002	0.038 ± 0.008	0.036 ± 0.005	0.027 ± 0.005	0.026 ± 0.005	0.030 ± 0.005
Galacturonic acid	0.551 ± 0.025	0.514 ± 0.037	0.571 ± 0.044	0.545 ± 0.029	0.471 ± 0.017	0.425 ± 0.014	0.347 ± 0.034	0.414 ± 0.061

Results are mean values ± standard deviations ($n = 6$), corresponding to three processings in duplicate applied to each lot of carrot.

factors obtained in each instance were applied to the sample.

The efficacy of the extraction of pectic substances with oxalic acid–ammonium oxalate was tested and satisfactory results were obtained [15]. The accuracy, precision and inteferences with neutral sugars were also tested. 3,5-Dimethylphenol gave a better sensitivity and selectivity than *m*-phenylphenol, frequently used in the determination of galacturonic acid [9].

Dietary fibre of raw and processed carrots was characterized by HPLC and a spectrophotometric method. Mean values corresponding to the three lots and general statistics are summarized in Tables 1 and 2. The component monosaccharides were expressed as polysaccharides (×0.88 pentoses; ×0.90 hexoses and galacturonic acid and ×0.95 cellobiose). Results are expressed in grams per 100 g of dry matter.

Table 1 shows that glucose is the major monosaccharide in raw carrot samples, followed by galactose/rhamnose, arabinose, cellobiose, xylose and mannose. Galacturonic acid is the most representative monomer in the dietary fibre fraction in this product.

The results for processed carrot samples, summarized in Table 2, show that the values for different sugars are higher than in the raw product. However, the order of quantitative importance is maintained: glucose, galactose/rhamnose, arabinose, cellobiose, xylose and mannose. Galacturonic acid is, as in raw product, the most representative monomer.

Studying both materials (grams per 100 g dry matter) and calculating the proportion of each sugar in NSP, the increase observed in each sugar could be explained in terms of the different nature of raw and processed materials. If the sum of all sugars plus galacturonic acid is considered (NSP), the distribution of each component in both materials is as follows: glucose, raw 27.52% and processed 26.15%; galactose/rhamnose, raw 15.55% and processed 19.45%; arabinose, raw 11.41% and processed 16.76%; cellobiose, raw 3.89% and processed 4.02%; xylose, raw 2.92% and processed 2.67%; mannose, raw 2.48 and processed 2.04%; and galacturonic acid, raw 36.23% and processed 28.89%.

The comparative distribution of monomers shows that galacturonic acid is the main component in both raw and processed carrots, but in the latter the proportion of galacturonic acid has diminished in relation to the unprocessed material, giving a large increase in the proportion of galactose/rhamnose and arabinose.

For studying the influence of thermal treatment on carrots, the results are expressed in grams per 100 g of fresh matter (Table 3). Losses of soluble solids during processing result in an increase in the concentration of insoluble components. Among the soluble compounds is galacturonic acid, which is the most abundant monomer of pectic polysaccharides. This explains why this monomer did not experience the same increase as neutral monosaccharides. To establish a correct comparison with the results for processed samples, a correction factor was applied to avoid errors due to the losses of soluble solids mentioned above.

The experimental design developed to study modifications in carrots during processing allows statistical treatment with the analysis of variance (ANOVA) test to determine significant differences that could be attributed to the autoclaving conditions. Although some differences were observed when comparing raw and processed material in each lot, these were not statistically significant (level of significance, $\alpha = 0.05$) because they are small and the different characteristics of each lot of material should be considered. From a quantitative point of view no statistical differences were observed when the global mean value for raw material was compared with the global mean value for processed material in any monosaccharide. For galacturonic acid the differences observed were statistically significant ($\alpha = 0.05$). This component is solubilized during autoclaving of carrots. Similar results were found by Waldron and Selvendran [16] during boiling of other vegetables. The monosaccharide fraction of NSP of carrots remained unmodified during processing under the conditions mentioned above and the value of NSP considered as the sum of monosaccharides plus galacturonic acid did not experience any variation as a result of autoclaving.

Other workers did not find any difference in cooked or tinned carrots using a GC method for the determination of monosaccharides and a spectrophotometric method for the determination of galacturonic acid [17].

The HPLC method developed here for dietary fibre determination is rapid and easy to perform and gives good results for samples with different amounts of monomeric components of NSP. This method shows no statistically significant changes in dietary fibre during autoclaving of carrots.

References

[1] H.N. Englyst and J.H. Cummings, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 808.
[2] M.A. Eastwood and E.R. Morris, *Am. J. Clin. Nutr.*, 55 (1990) 436.
[3] E.C. Conrad and J.K. Palmer, *Food Technol.*, October (1976) 84.
[4] F.E. Barton, W.R. Windham and D.S. Himmelsbach, *J. Agric. Food Chem.*, 34 (1982) 1119.
[5] J.L. Slavin and J.A. Marlett, *J. Agric. Food Chem.*, 31 (1983) 467.
[6] K.A. Garleb, L.D. Bourquin and G.C. Fahey, Jr., *J. Agric. Food Chem.*, 37 (1989) 1287.
[7] R.F.H. Dekker and G.N. Richards, *J. Sci. Food. Agric.*, 23 (1972) 475.
[8] R.W. Scott, *Anal. Chem.*, 51 (1979) 936.
[9] M.D. Rodríguez, A. Redondo and M.J. Villanueva, *Alimentaria*, 232 (1992) 79.
[10] H.N. Englyst, M.E. Quigley, G.J. Hudson and J.H. Cummings, *Analyst*, 117 (1992) 1707.
[11] B.W. Li and M.S. Cardozo, *J. AOAC Int.*, 45 (1992) 372.
[12] M^a.D. Rodríguez, A. Redondo and M^a.J. Villanueva, in D.A.T. Southgate, K. Waldron, I.T. Johnson and G.R. Fenwick (Editors), *Dietary Fibre: Chemical and Biological Aspects*, Royal Society of Chemistry, Cambridge, 1990, Part 3, p. 130.
[13] J.H. Sloneker, *Anal. Biochem.*, 43 (1971) 539.
[14] R.R. Selvendran, J.F. March and S.G. Ring, *Anal. Biochem.*,, 96 (1979) 282.
[15] M.J. Villanueva, A. Redondo and M.D. Rodríguez, *Anal. Bromatol.*, 42 (1990) 57.
[16] K.W. Waldron and R.R. Selvendran, in D.A.T. Southgate, K. Waldron, I.T. Johnson and G.R. Fenwick (Editors), *Dietary Fibre: Chemical and Biological Aspects*, Royal Society of Chemistry, Cambridge, 1990, Part 2, p. 44.
[17] H.N. Englyst, S.A. Bingham, S.A. Runswick, E. Collinson and J.H. Cummings, *J. Hum. Nutr. Diet.*, 1 (1988) 247.