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Effects of processing conditions on soluble sugars content of carrot, beetroot and turnip

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Abstract

The effect of processing conditions on the soluble sugars fraction of three vegetables, carrot, beetroot and turnip, was evaluated by high-performance liquid chromatography (HPLC) and by a colorimetric method using potassium ferricyanide. Processing was performed at 121°C during 15 min and three different lots of each vegetable were studied. To verify whether the behaviour was homogeneous, each lot was processed three times, consecutively, under the same conditions. Results were statistically evaluated by two-way ANOVA to elucidate the effects of processing on each vegetable. A significant reduction was observed of soluble sugars, fructose, glucose and sucrose, in the processed samples. A high correlation was observed between the results of total sugars obtained by HPLC and by the colorimetric method.

1. INTRODUCTION

Consumption of fresh and processed vegetable foods is very high and it is expected that in the near future these commodities will increase in the daily diet of industrialized countries. This tendency is due to the continuous effort towards persuading consumers to moderate the intake of proteic foods of animal origin and, especially, to take advantage of the healthy effects of a diet rich in vegetables which cannot be supplanted. Another factor that has contributed to the increase of consumption of vegetables is the expansion of the food processing industry (Desai & Salunkhe, 1991).

Processing of foods is, in most cases, an obligatory practice due to the effects produced. Heating in an aqueous medium gelatinizes starch granules, partially hydrolyzes hemicelluloses and solubilizes protopectins after depolymerization, modifying the texture of these products (Conning, 1991). The rest of the constituents, among which sugars are found, may also be affected by thermal treatment. The effect of heat treatment, applied under specified conditions of temperature and duration, on vegetable food components, depends on crop, cultivar, maturity, freshness and season (Salunkhe & Desai, 1984). Therefore, knowledge of modifications allows one to optimize conditions for proper processing and handling of vegetables (Milaszewski, 1985). A need for such information is particularly important in the case of sugars, due to the increasing interest of nutritionists in the role of these components in the diet and to the fact that they constitute the main energy source in vegetarian diets (Li & Schuhmann, 1983).

In the present work, effects of processing conditions on the content and composition of soluble sugars of three roots commonly used for human consumption, carrot, beetroot and turnip, was studied.

Several methods are described in the literature for the analysis of soluble sugars: volumetric, spectrophotometric (chemical and enzymatic), chromatographic (gas-chromatography, cation-exchange chromatography, high performance liquid chromatography) (Callul, Marcé, & Borull, 1992; Prodolliet, Bugner, & Feinberg, 1995; Weiss, 1995). In this work, two methods have been used to analyze the soluble sugars fraction: high-performance liquid chromatography (HPLC) (Ball, 1990) and the colorimetric potassium ferricyanide method (Gaines, 1973).

2. Materials and Methods

2.1. Materials

Samples selected for this work were: carrot (*Daucus carota* L., Nantesa), beetroot (*Beta vulgaris* L., Cruenta

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Alef.) and turnip (*Brassica napus* L., Martillo). Each vegetable was purchased at local markets in sufficient amounts at three different times (lots 1, 2 and 3).

2.2. Processing

Once in the laboratory, each sample was divided into three groups (A, B and C) with the aim of repeating the processing three times under the same conditions. In each group the different units were washed, peeled and cut into pieces of homogeneous weight, which were divided into two fractions: in one of them the material was analyzed raw and in the other one after processing. Processing was performed by autoclaving at 121 °C during 15 min after adding 300 ml of water to 200 g of sample. Fresh and processed samples were freeze-dried, and cooking liquids were kept at −18°C until analysis was performed.

2.3. Methods of analysis

Moisture content of samples was determined according to the AOAC method (AOAC, 1980).

2.3.1. Determination of soluble sugars content

Extraction of soluble sugars present in vegetable samples was performed in two steps with hot 85% methanol. The extracts were combined and concentrated in a rotary evaporator. Concentrates were redissolved with distilled water in varying volumes depending on the different samples.

2.3.1.1. Individual soluble sugars. Individual soluble sugars were analyzed by HPLC (Rodríguez, 1993). An aliquot of 2.5 ml of the above mentioned extract was mixed with 7.5 ml of acetonitrile and was filtered through a Sep-pak C_{18} cartridge (Waters, Milford, USA). Before injection, this solution was filtered through a 0.45 μm Millipore filter (Millipore Corporation, Milford, USA). The injection volume was 50 µl. The analysis was performed using a Waters modular instrument equipped with a 6000 A pump and a U6K injector, and a refractive index detector (mod. ERC 7522, Erma CR. Inc., Tokyo, Japan), employing a μBondapak/carbohydrate analysis column of stainless steel (300×3.9 mm) (Waters, Milford, USA). The eluent was a mixture of acetonitrile/water (75:25 v/v) at a flow rate of 0.9 ml/min at room temperature. The external standard method was employed using a solution with a mixture of sugars in different proportions depending on the sample studied.

2.3.1.2. Total soluble sugars. To determine the total soluble sugars, the colorimetric potassium ferricyanide method was followed (Gaines, 1973). Once acid hydrolysis of sucrose was performed, the aqueous solution was neutralized, conveniently diluted and the potassium ferricyanide added. Finally absorbance was read ($\lambda = 380$ nm) in a spectrophotometer (Ultrospec Plus. Pharmacia LKB Biochrom Ltd, Cambridge, UK). The calibration curve was produced for the range between 0 and 500 μg/ml. The correlation coefficient obtained was 0.9998.

2.3.2. Verification of the methods

Accuracy and precision assays were performed for analytical methods. Accuracy assays were performed by adding to the samples known quantities of sugars. Precision assay was carried out by applying the methods on ten different days for the same materials and experimental conditions.

2.3.3. Statistical analysis

Data were statistically analyzed using the BMDP program (Biomedical Computers Program, Berkeley, CA). Differences between methods were tested using a paired *t*-test ($\alpha = 0.05$), and regression analysis. Two-way ANOVA was used to determine significant differences that could be attributed to processing conditions.

3. Results and discussion

Accuracy and precision assays gave satisfactory results for all sugars with recoveries between 94.0 and 104% (Table 1) and low variation coefficients, between 1.00 and 5.17% (Table 2).

3.1. Soluble sugars content of vegetables studied

Soluble sugar contents of raw and processed samples are included in Table 3. The mean values correspond to the analysis of three different lots of each vegetable.

Table 1
Accuracy assay for HPLC and colorimetric methods

	Recovery			
	HPLC method		Colorimetric method	
	Mean	CV[a] (%)	Mean	CV[a] (%)
Carrot				
Fructose	103	3.05		
Glucose	100	4.03	101	1.32
Sucrose	103	0.29	97.6	6.68
Beetroot				
Fructose	98	2.09		
Glucose	102	3.08	101	3.68
Sucrose	102	2.97	97	1.27
Turnip				
Fructose	100	0.28		
Glucose	104	0.25	97	2.50
Sucrose	94	1.06	101	2.68

[a] CV = coefficient of variation.

Table 2
Precision assay for HPLC and colorimetric methods

	CV[a] (%)
HPLC method	
Fructose	5.17
Glucose	3.17
Sucrose	2.86
Colorimetric method	
Sucrose	1.00

[a] CV = coefficient of variation.

Soluble sugars found were fructose, glucose and sucrose. In these roots, fructose and glucose were found in similar proportions in each of the three vegetables, although fructose was always in lower amounts. Sucrose presented very different amounts compared with fructose and glucose. In raw carrot, sucrose was the major soluble sugar and represented 56.9% of total value, followed by glucose (24.6%) and fructose (18.5%). The soluble sugars fraction of raw beetroot was represented almost exclusively by sucrose (91.6%). Raw turnip presented a major content of fructose and glucose, 40.6 and 51.9%, respectively, against sucrose that represented 7.6%. In processed samples the percentages obtained had similar distributions to the raw samples.

The results for total soluble sugars obtained by HPLC and by the colorimetric method were statistically compared. A paired *t*-test indicated that mean values are not significantly different ($\alpha = 0.05$). Regression analysis confirm a high correlation between both analytical methods ($r = 0.9727$) ($y = 0.979620x + 0.0345002$).

3.2. Effect of processing conditions on soluble sugars content

Results for soluble sugars content of raw and processed samples are summarized in Table 4 and Table 5. In each lot, processing was applied to three groups of samples (A, B and C) under the same conditions to assess possible variations. With the aim of knowing the incidence of processing on the soluble sugars fraction of samples analyzed, results for processed samples have been corrected by a factor to take into account the soluble solids loss and the modification of moisture after processing (Redondo, Villanueva, Rodríguez, & Saco, 1997). The factor was calculated by the following formula:

$$F = \frac{\mathrm{TWPM}(100 - \mathrm{WPM})}{\mathrm{TWRM}(100 - \mathrm{WRM})}$$

where:
F: correction factor
TWRM: total weight of raw material
WPM: water content of processed material
TWPM: total weight of processed material
WRM: water content of raw material

Zyren, Elkins, Dudek, and Hagen (1983) used a parameter they called "retention" calculated by the Murphy, Criner, and Gray (1975) formula, which takes into account the variation in water content originating

Table 3
Soluble sugars content in raw and processed samples (expressed as g% fresh weight)[a]

	Raw samples		Processed samples	
	Mean ± SE	RSD[b]	Mean ± SE	RSD[b]
Carrot				
Fructose	0.919 ± 0.134	0.252	0.603 ± 0.058	0.167
Glucose	1.22 ± 0.132	0.187	0.782 ± 0.042	0.093
Sucrose	2.83 ± 0.187	0.114	1.98 ± 0.127	0.111
Total (HPLC method)	4.97 ± 0.346	0.120	3.36 ± 0.103	0.053
Total (Colorimetric method)	4.89 ± 0.260	0.092	3.43 ± 0.298	0.150
Beetroot				
Fructose	0.127 ± 0.029	0.394	0.085 ± 0.024	0.499
Glucose	0.488 ± 0.094	0.335	0.267 ± 0.008	0.049
Sucrose	6.68 ± 0.934	0.242	4.34 ± 0.076	0.030
Total (HPLC method)	7.30 ± 1.016	0.241	4.69 ± 0.106	0.039
Total (Colorimetric method)	7.14 ± 1.091	0.265	4.72 ± 0.265	0.097
Turnip				
Fructose	1.10 ± 0.240	0.376	0.695 ± 0.144	0.360
Glucose	1.41 ± 0.060	0.073	0.991 ± 0.126	0.220
Sucrose	0.206 ± 0.026	0.219	0.091 ± 0.008	0.159
Total (HPLC method)	2.7 ± 0.280	0.178	1.78 ± 0.243	0.236
Total (Colorimetric method)	2.65 ± 0.391	0.256	1.75 ± 0.305	0.303

[a] Values are the mean ± standard error of three lots (1, 2 and 3), processed in triplicate (A, B and C) and analyzed at least in duplicate.
[b] RSD: relative standard deviation.

Table 4
Effects of processing on soluble sugars of raw and processed samples (expressed as g% fresh weight)[a]

Sample	Lot	Fructose		Glucose		Sucrose	
		Raw	Processed[b]	Raw	Processed[b]	Raw	Processed[b]
Carrot	1	0.661 ± 0.017	0.474 ± 0.023	0.982 ± 0.033	0.634 ± 0.056	2.90 ± 0.118	1.97 ± 0.136
	2	0.998 ± 0.050	0.632 ± 0.053	1.25 ± 0.067	0.757 ± 0.075	2.48 ± 0.040	1.63 ± 0.070
	3	1.11 ± 0.040	0.489 ± 0.087	1.44 ± 0.045	0.678 ± 0.070	3.11 ± 0.027	1.64 ± 0.061
Beetroot	1	0.173 ± 0.012	0.126 ± 0.014	0.345 ± 0.039	0.264 ± 0.032	5.06 ± 0.202	4.30 ± 0.070
	2	0.134 ± 0.011	0.069 ± 0.005	0.666 ± 0.043	0.258 ± 0.045	8.30 ± 0.262	4.05 ± 0.106
	3	0.075 ± 0.010	0.050 ± 0.009	0.453 ± 0.006	0.257 ± 0.026	6.68 ± 0.211	4.31 ± 0.102
Turnip	1	1.51 ± 0.059	0.916 ± 0.028	1.44 ± 0.025	1.07 ± 0.103	0.255 ± 0.022	0.075 ± 0.006
	2	0.914 ± 0.012	0.499 ± 0.004	1.30 ± 0.005	0.709 ± 0.035	0.167 ± 0.006	0.103 ± 0.012
	3	0.818 ± 0.013	0.632 ± 0.045	1.50 ± 0.043	1.18 ± 0.042	0.194 ± 0.004	0.094 ± 0.005

[a] Values are the mean ± standard error for processing repeated three times in each lot.
[b] Data corrected for losses of soluble solids.

Table 5
Effects of processing on total soluble sugars content of raw and processed samples (expressed as g% fresh weight)[a]

Sample	Lot	HPLC method		Colorimetric method	
		Raw	Processed[a]	Raw	Processed[a]
Carrot	1	4.54 ± 0.158	3.07 ± 0.067	5.07 ± 0.063	3.54 ± 0.081
	2	4.72 ± 0.113	3.02 ± 0.133	4.37 ± 0.017	2.83 ± 0.036
	3	5.66 ± 0.101	2.80 ± 0.094	5.21 ± 0.018	2.71 ± 0.020
Beetroot	1	5.58 ± 0.183	4.69 ± 0.061	5.23 ± 0.092	4.67 ± 0.170
	2	9.10 ± 0.278	4.37 ± 0.068	9.01 ± 0.175	3.94 ± 0.081
	3	7.203 ± 0.226	4.62 ± 0.119	7.19 ± 0.063	5.20 ± 0.729
Turnip	1	3.28 ± 0.021	2.07 ± 0.126	3.41 ± 0.049	2.19 ± 0.145
	2	2.38 ± 0.009	1.31 ± 0.023	2.11 ± 0.032	1.27 ± 0.058
	3	2.50 ± 0.054	1.91 ± 0.085	2.43 ± 0.111	1.71 ± 0.145

[a] Values are the mean ± standard error for processing repeated three times in each lot.
[b] Data corrected by soluble solids loss.

during processing. Nyman, Palsson and Asp (1987), Nyman (1995) and Svanberg, Nyman, Andersson, and Nilsson (1997) calculated the percentage of soluble solid loss expressed on a dry weight basis and used it to correct their results.

Variations of each monosaccharide, expressed as percentage of the initial monosaccharide value, showed that losses of each sugar were characteristic of each one and of the sample because conditions of processing were kept constant along the study. Fructose descended in a very similar proportion in the three samples (42.3% in carrot, 35.4% in beetroot and 36.9% in turnip). Glucose presented similar falls in carrot and beetroot (43.6 and 46.7%) while, in turnip, the reduction was of lesser magnitude (30.0%). Sucrose losses were 36.8% for beetroot, 38.4% for carrot and 55.6% for turnip. Variation coefficients calculated for mean value of experimental losses in each lot indicate a low dispersion for the three vegetables, especially beetroot.

Solubilization of cooking liquids was found in all the samples. The proportion of sugars found was similar to the losses experienced by the samples. In a previous work (Redondo, Villanueva, & Rodríguez, 1988) related to cooking of beetroot (100°C), it was observed that soluble sugar losses were not only due to solubilization in cooking liquids, but also due to hydrolysis of sucrose, because a proportional increase of fructose and glucose was detected.

The statistical study of variations during processing was carried out using two-way ANOVA (Table 6). The effect of processing and the possible influence of the lot which was constituted by the product purchased on different days and with different origins were considered.

For carrot, the processing caused statistically significant reductions of the soluble sugars fraction. The influence of the lot was also significant, which indicates the possible effects of origin on the characteristics of the carrot. The interaction between both factors was significant, except for sucrose, which indicates that the processing occurred with different intensities depending on the lot.

For beetroot, statistically significant reductions of the different soluble sugars were found as a consequence of the processing and depending on the lot. The interaction between both factors was significant, except for fructose.

Table 6
Two-way ANOVA: F values and significance level for the effect of processing and lot on soluble sugars

Sample	Factor	Fructose	Glucose	Sucrose	Total sugars (HPLC method)	Total sugars (colorimetric method)
Carrot	Processing (P)	55.17***[a]	132.72***	162.49***	329.57***	2475.77***
	Lot (L)	35.25***	9.03*	6.54*	12.88**	115.80***
	P×L	5.84*	6.70*	3.89 ns	14.99**	74.47***
Beetroot	Processing (P)	69.98***	227.75***	306.48***	319.48***	117.56***
	Lot (L)	21.24**	6.39*	36.41***	48.59***	10.88**
	P×L	4.75 ns	40.07***	51.51***	52.59***	32.33***
Turnip	Processing (P)	320.13***	130.68***	251.37	453.66***	98.48***
	Lot (L)	135.24***	18.54**	2.73 ns	58.00***	130.00***
	P×L	34.23***	5.09 ns	22.11**	16.53**	4.11 ns

[a] $*p \leqslant 0.05$; $**p \leqslant 0.01$; $***p \leqslant 0.001$; ns: non-significant.

For turnip, the losses of soluble sugars during the processing were statistically significant. There were significant differences between sugar value, depending on the lot. However, in some cases, the interaction between factors was not significant.

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