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# Effect of domestic processes and water hardness on soluble sugars content of chickpeas (Cicer arietinum L.)

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#### Abstract

An HPLC method for sugar analysis on legumes has been applied to three different Spanish cultivars of chickpeas, to evaluate changes on the soluble sugar fraction (with special attention to  $\alpha$ -galactosides) during domestic processing (soaking and cooking) using two types of water with different hardness. Processing liquids were also analysed. Ciceritol, was the main sugar in all the samples. Raffinose and stachyose concentrations were from 1.312 to 1.947  $g/100 g$  in raw samples. Soaking liquids extracted less than 4.86% of the  $\alpha$ -galactosides of raw seeds with no considerable amounts of flatogenic sugars. Decreases of raffinose + stachyose after processing were between 24.7 and 42.6%. Cooking liquid contents of  $\alpha$ -galactosides ranged from 0.282 to 0.565 g/100 ml,  $(0.119-0.302 \text{ g}/100 \text{ ml of flatogenic sugars})$ . Statistical tests  $(ANOVA)$  showed that water hardness has no significant effect  $(p \le 0.05)$  on flatogenic sugar contents of final processed chickpeas.  $\odot$  1999 Elsevier Science Ltd. All rights reserved.

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# 1. Introduction

After the ingestion of legumes,  $\alpha$ -galactosides reach the lower intestine without degradation, where they are fermented by microbial a-galactosidase. Gas expulsion occurs  $(H_2, CO_2$  and  $CH_4$ ), causing flatus effect, and sometimes osmotic diarrhoea and abdominal pain (Rackis, 1975; Reddy et al., 1980; Olsen et al., 1982; Cummings et al., 1986). This phenomenon is not a toxic effect of legumes, but undesirable for people with intestinal problems.

 $\alpha$ -Galactosides derived from sucrose (raffinose, stachyose and verbascose) have been the most studied in legumes. Another group of  $\alpha$ -galactosides in legumes are glucose galactosides (melibiose and manninotriose), and inositol galactosides (galactinol, galactopinitol, ciceritol). Ciceritol (a-D-galactopyranosil-6-a-D-galactopyranosil-2-(1D)-4-O-methyl-chiro-inositol) has been reported previously as a characteristic trisaccharide in chickpeas (Quemener & Brillouet, 1983; Bernabé et al.,

1993), being the most abundant sugar in these seeds (Sánchez-Mata et al., 1998).

There are some differences between flatulence-inducing potential of  $\alpha$ -galactosides. Quemener & Brillouet (1983) reported that the in vitro hydrolysis rate of ciceritol by  $\alpha$ -D-galactosidase is much lower than for raffinose, stachyose and verbascose. These authors considered the presence of pinitol as a factor that reduces the sensitivity of the  $\alpha$ -galactoside to hydrolysis by a-galactosidase. In vivo studies have shown the ability of raffinose, stachyose and verbascose to produce flatulence (Reddy et al., 1980; Fleming, 1981; Phillips & Abbey, 1989). Fleming (1981) found a decrease in the correlation coefficient between hydrogen production in rats and  $\alpha$ -galactosides content of pulses when the trimer, identified subsequently as ciceritol, was added to the raffinose family sugars. For this reason there is no evidence of ciceritol as a significant faltogenic sugar.

Some beneficial properties of dietary fiber have recently been attributed to  $\alpha$ -galactosides (raffinose and stachyose): these compounds tend to normalise bowel habits, increase lactobacilli and bifidobacteria (decreas-

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ing enterobacteria in intestinal microflora) and reduce potentially carcinogenic N-nitroso compounds levels in the gut (Rowland et al., 1998; Van Loo, 1998).

Domestic or technological processes may affect  $\alpha$ galactoside content in legumes by dilution in the medium or/and transformations in the carbohydrate fraction which may include hydrolysis of  $\alpha$ -galactosides. Reddy et al. (1980) attributed the reduction on verbascose content and increase of sucrose and stachyose during cooking of beans, to enzymatic hydrolysis during the first stages of cooking. Ku et al. (1976), Jood et al. (1986) and Vijayakumari et al. (1997) detected higher diffusion of soluble sugars into the soaking liquid when  $NaHCO<sub>3</sub>$  was added. Díaz Pollán (1994) detected a higher reduction in  $\alpha$ -galactosides content when the cooking process was performed under pressure. Vijayakumari et al. (1997) reported low reductions of stachyose after soaking an Indian legume (Prosopis chilensis (Molina) Stunz). Wang et al. (1997) studied the combined processes of soaking and water blanching on cowpeas, with higher reductions in  $\alpha$ -galactosides when soaking and steam-blanching were used.

Most of these works studied different processes for legumes, using distilled water (sometimes treated with  $NaHCO<sub>3</sub>$  or other salts). Domestic processing of legumes includes soaking and cooking with tap water. The composition of tap water is very unstable, and this may affect the composition of processed legumes. Water with high amounts of calcium and magnesium salts induces the formation of calcium and magnesium pectates (with lower solubilities than sodium and potassium ones) and hinders the softening of the seeds (Bhatty, 1984; Hincks & Stanley, 1987; Aguirre-Terrazas, 1992). The longer processing time that is necessary to soften the seeds, could induce more transformations. For this reason, this study compares the effect of water hardness on soluble sugar contents of processed chickpeas.

Since legumes are usually ingested together with their cooking liquids, the study of the soluble sugar compositions of the processing liquids have been considered in this work, in order to know the real amount of  $\alpha$ -galactosides ingested.

This work is aimed to establish the soluble sugar composition of three spanish cultivars of chickpeas (Cicer arietinum L.) with special attention to  $\alpha$ -galactoside content, and the influence of domestic soaking and cooking processes using tap water. The effect of processing water hardness was evaluated, and the soluble sugar composition of the processing liquids was also studied.

# 2. Materials and methods

#### 2.1. Samples

Mature seeds of three spanish cultivars of chickpea (Cicer arietinum L. var. macrocarpum Jaub et Sp.), cv. Castellano (C), cv. Blanco lechoso (BL) and cv. Pedrosillano (P), were analysed.

The study was performed using two types of tap water with different contents of calcium and magnesium salts: soft water, from Madrid (M) and hard water, from Zaragoza (Z). Calcium and magnesium contents of water used for processing were measured by an EDTA titrimetic method (AOAC, 1990, 973.52; Rodier, 1981 ) and the results are shown in Table 1.

#### 2.2. Processing conditions

Raw material was soaked and then cooked in boiling water. Time to cook the samples was longer when hard water was used. Processing conditions were similar to domestic practice, and they are shown in Table 2. Soaking and cooking processes were triplicated for each chickpea sample and type of water.

# 2.3. Analytical procedure

Free sugar content in the samples, was determined by HPLC. The analytical method applied was based on those reported by Labaneiah and Luh (1981) and Kuo et al. (1988), and modified by Sánchez-Mata et al. (1998). 1.5 g of raw chickpea or 3 g of processed chickpea was extracted with 40 ml of 80% ethanol, during 45 min, twice. The supernatants were collected, evaporated under vacuum at  $40^{\circ}$ C and made up to 10 ml with distilled water. Samples were passed through a Sep-Pak C18 cartridge (Waters, Mildford, MA, USA). 2 ml of filtrate was mixed with  $8$  ml of acetonitrile and filtered

Table 1 Hardness characteristics of processing waters

Water	Total hardness	Cа	Mg		
	(mg CaCO <sub>3</sub> /liter)	(mg/liter)	(mg/liter)		
	$X \pm SD$	$X \pm SD$	$X \pm SD$		
Madrid	$37.3 \pm 1.15$	$11.7 \pm 0.92$	$1.9 \pm 0.28$		
Zaragoza	$408 \pm 11.31$	$134 \pm 7.35$	$19.6 \pm 2.66$		

 $X=$  mean of three measurements.

 $SD =$  standard deviation  $(n-1)$ .

Table 2 Chickpea processing conditions

	Soaking	Cooking
Seeds/water $(w/w)$	1/5	1/10
Temperature	Ambient	$100^{\circ}$ C
Pressure	Atmospheric	Atmospheric
Time	12 <sub>h</sub>	40 min (soft water: Madrid) 95 min (hard water: Zaragoza)

through a 0.45 µm Millex membrane (Millipore, Bedford, MA, USA), prior to injection of 250 µl into the chromatographic system.

Quantification of peaks was performed using the external standard method. An approach to the amount of ciceritol (with no comercial standard available) and unknown peaks  $(U_1$  and  $U_2)$  was made, using the calibration curve of the previous peak (maltose for  $U_1$  and raffinose for  $U_2$  and ciceritol), corrected by molecular weights.

Processing liquids were also analysed. They were passed through a Sep-Pak cartridge, mixed with acetonitrile  $(2:8)$ , and filtered through Millex  $(0.45 \mu m)$ prior to injection.

# 2.4. Apparatus

A Waters chromatographic system was used for sugar analysis, supplied with an amino bonded column  $(\mu$ Bondapack carbohydrate), isocratic pump (mod. 6000 A), refraction index detector (mod. R401) and Data Module (mod. 745) register. The mobile phase was acetonitrile/water  $(80/20)$ , at a flow rate of 0.9 ml/min and ambient temperature.

#### 2.5. Statistical analysis

An ANOVA test was applied to the obtained data, in order to know the influence of processing water hardness on the soluble sugar content of chickpea samples. The statistical F (Fischer) test, was used to compare the experimental and theoretical F values, with a confidence level of 95%.

# 3. Results and discussion

#### 3.1. Raw samples

Fig. 1 shows the chromatographic profile of raw chickpea, cv. Castellano. Monosaccharides (ribose, fructose, glucose and galactose), disaccharides (sucrose and maltose) and oligosaccharides (raffinose and stachyose) were identified and quantified. The presence of ciceritol was also considered, although it could not be confirmed, due to the lack of a commercial standard available.

Two unknown compounds appeared in the chromatograms. Their retention times did not correspond with any of the commercial standards assayed (melibiose, maltotriose). Considering their elution order, the first one may be a di- or trisaccharide, and the second one, a trisaccharide. In chromatograms and tables, they appear as  $U_1$  and  $U_2$  and have been quantified using the calibration curves of the previous peak (maltose and raffinose, respectively).

Table 3 shows the soluble sugar content of chickpeas analysed (mean values of triplicate analyses). In all of them, monosaccharides represented only  $2-8\%$  of the total sugar content, and the main one was fructose (3.21% of the total) . Among disaccharides, sucrose is the main one (about 28% of the total sugar content), followed by maltose.

The  $\alpha$ -galactoside group was around 60% of the total sugar content in raw samples. The main sugar in samples of chickpea was that whose peak appears after raf finose and  $U_2$ , and before stachyose. Its position in the chromatograms and its quantity in the samples, supported by the studies of Quemener and Brillouet (1983), Knudsen (1986); Bernabé et al. (1993); Díaz Pollán (1994) and Frias et al. (1996), suggest that this sugar is ciceritol. These authors used a similar chromatographic method, but the analytical conditions were not the same, and, for this reason, the retention time obtained cannot be compared with ours. This sugar was also reported in a previous study of other raw legumes (Sánchez-Mata et al., in press).

Ciceritol comprises about 40% of total sugars in chickpeas analysed. Other  $\alpha$ -galactosides identified were stachyose and raffinose. Stachyose was always in higher amount than raffinose, as corresponds to legumes (Kuo et al., 1988).

Verbascose was not identified in our chickpea samples, nor in the studies of Quemener and Brillouet  $(1983)$  and Díaz Pollán  $(1994)$ .

The  $\alpha$ -galactoside contents are higher in Blanco Lechoso chickpea, followed by Castellano and Pedrosillano ones. The sum of flatogenic sugars (raffinose and stachyose) in raw chickpeas analysed were 1.95 mg/100 g (BL), 1.80 mg/100 g (C) and 1.31 mg/100 g (P), which represents, respectively, 19.4, 21.9 and 22.3% of the total amount of sugars.

#### 3.2. Processed samples

Table 4 shows soluble sugar contents of processed samples. Total sugar contents in soaked and cooked samples with hard water  $(Z)$  were around 1.3 times higher than with a soft water (M). They were between 2.68 and 5.03 g/100 g in soaked samples and 1.57 and 2.22 g/100 g in cooked samples.

The main sugars were ciceritol, sucrose and stachyose, which represent together more than 69.7% of the total sugar content in processed chickpeas. This percent increased in all the cooked samples as the smaller sugars transfer to processing liquid easier than those of higher molecular weight, such as ciceritol and stachyose. These results agree with Vidal-Valverde et al. (1993).

In cooked samples, low amounts of monosaccharides were detected. The major monosaccharide in processed samples was fructose, as in raw ones.



Fig. 1. Chromatographic profile of sugars in raw chickpea (cv. Castellano). Ri: ribose; F: fructose; Gl: glucose; Ga: galactose; Su: sucrose; Ma: maltose;  $U_1$ : unknown; Ra: raffinose;  $U_2$ : unknown; Ci: ciceritol; St: stachyose.

Table 3 Soluble sugar contents in raw chickpea samples (g/100 g)

Sample		Ribose	Fructose	Glucose	Galactose	Sucrose	Maltose	$U_1$	Raffinose	U,	Ciceritol	Stachyose	Total
	Х	0.033	0.231	0.065	0.050	2.28	0.568	0.020	0.629	0.318	2.79	1.17	8.21
	SD	0.008	0.056	0.020	0.011	0.242	0.049	0.001	0.095	0.007	0.204	0.145	0.195
BL	X	0.019	0.196	Traces	Traces	3.25	0.479	Traces	0.864	Traces	3.93	1.08	10.1
	SD.	0.000	0.045			0.011	0.015		0.039		0.756	0.242	0.647
P	X	0.191	0.288	Traces	Traces	1.09	0.613	0.066	0.569	-	2.51	0.743	5.89
	SD.	0.041	0.009			0.077	0.051	0.008	0.088		0.277	0.093	0.328

 $X=$  mean value of three determinations.

SD = standard deviation  $(n-1)$ .

The  $\alpha$ -galactoside group represented 45.2–58.5% of the total sugars in soaked samples and  $64.9-74.2\%$  in cooked samples. The major  $\alpha$ -galactoside in processed samples was always ciceritol, followed by stachyose and raffinose, as in raw samples. The amounts of raffinose and stachyose in processed samples were from 0.381 to 0.911 g/100 g, which represented about  $17.2-37.8\%$  of its total sugar content.

### 3.3. Processing liquids

Table 5 shows the soluble sugar content in processing liquids. Sugar contents were about 1.6 times higher when Z water was used for processing. The fact that sugar content was higher in both seeds and liquids processed with Z water could be due to the longer time necessary to cook these samples. Rao and Belavady (1978) considered that

Table 4 Soluble sugar contents of processed chickpea samples (g/100 g)

Chickpea Water Process						Ribose Fructose Glucose Galactose Sucrose Maltose $U_1$				Raffinose	$U_2$		Ciceritol Stachyose Total	
C	М	Soaking $X$ 0.075		0.088	0.053	0.063	1.05	0.243	0.021	0.209	0.069	0.731	0.479	2.82
			SD 0.021	0.023	0.006	0.007	0.199	0.053	0.001	0.019	0.010	0.143	0.101	0.310
		Cooking $X$	$\overline{\phantom{a}}$	0.041	Traces	0.012	0.533	0.145	Traces	0.099	0.044	0.473	0.448	1.57
		<b>SD</b>		0.001		0.000	0.113	0.037		0.017	0.000	0.109	0.103	0.230
	Ζ	Soaking $X$ 0.068		0.176	0.122	0.054	1.25	0.234	0.003	0.236	0.028	0.930	0.470	3.619
			SD 0.011	0.040	0.030	0.011	0.317	0.055	0.000	0.045	0.000	0.056	0.110	0.069
		Cooking $X$	$\overline{\phantom{a}}$	0.027	$\overline{\phantom{0}}$	0.011	0.331	0.059	Traces	0.126	Traces	0.634	0.546	1.78
		<b>SD</b>		0.003		0.000	0.070	0.017		0.014		0.115	0.126	0.203
BL	M	Soaking $X$ Traces		0.097	Traces	Traces	1.28	0.210	0.041	0.220	Traces	1.06	0.415	3.35
		<b>SD</b>		0.019			0.241	0.054	0.010	0.034		0.208	0.082	0.481
		Cooking $X$ Traces		0.013	Traces	$\qquad \qquad -$	0.444	0.169	Traces	0.135	Traces	0.760	0.422	1.89
		SD.		0.005			0.082	0.035		0.029		0.182	0.100	0.452
	Z	Soaking $X$ 0.029		0.163	0.129	Traces	1.717	0.330	0.054	0.314	Traces	1.85	0.597	5.03
			SD 0.006	0.039	0.000		0.174	0.072	0.007	0.025		0.192	0.088	0.438
		Cooking $X$ Traces		0.040	Traces	$\overline{\phantom{m}}$	0.552	0.065	Traces	0.149	Traces	1.035	0.373	2.22
		<b>SD</b>		0.019			0.068	0.014		0.031		0.114	0.069	0.148
P	M	Soaking $X$ 0.079		0.102	0.104	$\overline{\phantom{m}}$	0.645	0.230	0.050	0.213	Traces	1.11	0.249	2.68
			SD 0.020	0.017	0.010		0.097	0.030	0.010	0.040		0.184	0.086	0.051
		Cooking $X$	$\hspace{0.1mm}-\hspace{0.1mm}$	0.025	$\overline{\phantom{0}}$	—	0.270	0.086	Traces	0.097	Traces	0.911	0.284	1.74
		<b>SD</b>		0.006			0.020	0.014		0.017		0.097	0.060	0.386
	Ζ	Soaking $X$	0.080	0.120	Traces		0.730	0.367	0.042	0.273	Traces	1.38	0.368	3.56
			SD 0.009	0.024			0.158	0.063	0.010	0.066		0.194	0.050	0.604
		Cooking $X$	0.051	0.018	Traces	$\overline{\phantom{0}}$	0.333	0.100	0.057	0.120	Traces	1.09	0.273	2.09
		SD	0.010	0.003			0.076	0.020	0.014	0.021		0.303	0.051	0.413

 $X$ = mean value of three processes analysed in triplicate.

 $SD =$  standard deviation  $(n-1)$ .





 $X$ = mean value of three processes analysed in triplicate.

SD = standard deviation  $(n-1)$ .

Table 6

Z S.: 3.16 4.87 g. : 67.2% Ci. 22.8% St. 10.0% Ra. 1.60 g : 0.837 g. on S. (52.44%)





 $S =$ chickpea seeds; CL. = cooking liquid; SL. = soaking liquid.

 $Ci = Ciceritol$ ; St. = Stachyose; Ra. = Raffinose.

sugars can be joined to proteins and macromolecules in the seeds, or be present as constituents of high molecular weight polysaccharides. Technological or domestic processes modify cellular microstructure and can induce release of sugars from these molecules.

CL.: 0.894 SL.: 0.100

CL.: 1.71 SL.: 0.000

In soaking liquids, only the most soluble sugars were detected. Mono and disaccharides such as fructose and sucrose were the most abundant, and ribose, glucose, galactose and maltose were at low levels. The only  $\alpha$ galactoside quantified was ciceritol, due to its high amount in the initial sample. In some cases raffinose and stachyose appeared in trace levels.

Soluble sugar contents of cooking liquids were always higher than soaking liquids, as it could be expected. The main sugars were ciceritol and sucrose, followed by stachyose. These major sugars represented the 72.5–88.4% of total sugar content in cooking liquids. Monosaccharides were at low levels, due to their previous dissolution in soaking liquid; as in the initial sample, fructose was the main one.  $\alpha$ -galactosides represented 46.3–66.6% of the total soluble sugar content in cooking liquids of chickpeas.

### 3.4. Influence of the whole process

Calculated percent losses of total soluble sugars in the samples (Murphy et al., 1975) showed a higher decrease in cooked chickpeas than in soaked ones.

In many cases, mono and disaccharides increased after processing. Reddy et al. (1980) also reported an increase in sucrose content in processed legumes, due to hydrolysis of oligo- and polysaccharides in the samples, during the cooking process. Onigbinde and Akyniele (1983) also supported heat hydrolysis of oligosaccharides. The  $\alpha$ -galactoside total contents decreased in the analysed samples; however, ciceritol and stachyose showed an increase in some cases. These results agree with Rao and Belavady (1978), Alberti et al. (1981), Reddy et al. (1980) and Liu and Markakis (1987), who detected an increase in stachyose, raffinose and verbascose contents in cooked legumes, probably due to the interaction with macromolecules.

 $Ra. (S. + CL.)$ 

0.758 g. on L. (47.56%)

The reduction detected in stachyose  $+$  raffinose, as flatulence-inducing sugars after soaking and cooking, was from 22.0 to 42.8%. Similar results were obtained by Silva and Braga (1982), Attia et al. (1994), Vijayakumari et al. (1997) and Wang et al. (1997), who studied this phenomenon in other legumes. Labaneiah and Luh (1981) reported a higher reduction of flatulent sugars in different types of beans.

In soaking liquids, a maximum of  $4.86\%$  of the  $\alpha$ galactoside content of the initial samples was detected, corresponding almost totally to ciceritol, with no evidence of flatulence-inducing ability.

As it can be seen in Table 6, the product obtained from 100 g of raw chickpea, soaked and cooked (cooked seeds and cooking liquid, discarding the soaking liquid), has a total amount of  $1.40-2.14$  g of stachyose + raffinose. If cooked liquid is discarded,  $0.837-1.36$  g of these sugars are ingested per 100 g of raw product. This content is similar for Castellano and Blanco Lechoso chickpeas, but lower for Pedrosillano chickpea. From this, a reduction of  $26-47\%$  of flatogenic sugars can be obtained if the cooking liquid is discarded before ingestion.

The statistical analysis applied showed that, although sugar content in samples processed with Z water were higher than those processed with M water, these variations were significant ( $p \le 0.05$ ) only for monosacharides and ciceritol (in soaking process), but not for disaccharides and flatogenic sugars. For this reason, processing water hardness cannot be considered as an influencing factor on the flatulence-inducing sugar content of the final processed chickpeas.

### 4. Conclusions

From the analysed Spanish cultivars of chickpeas, cv. Pedrosillano showed lower flatogenic  $\alpha$ -galactoside content, in both raw and cooked form.

Soaking and cooking processes induce a reduction of soluble sugar in samples, by dilution in the processing liquid. However, soaking liquid does not extract appreciable amounts of raffinose and stachyose. Discarding cooking liquid may be advisable for people with digestive problems, with a reduction of  $26-47\%$  in the ingestion of flatogenic sugars, but not for the general population, because of the presence of other nutrients in this liquids and also because of the functional properties of these carbohydrates.

Water hardness did not show a significant influence ( $p \le 0.05$ ) on the final content of flatogenic  $\alpha$ -galactosides in processed chickpea.

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