Effect of Processing on the Soluble Carbohydrate Content of Lentils

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(Received for publication July 18, 1991)

ABSTRACT

Lentils (Lens culinaris), like other legumes, are important both nutritionally and agriculturally. Soaking of lentil drastically reduce the quantities of α-galactosides present in the raw material (45-100% reduction). Analysis of the soaking medium (distilled water, 0.1% citric acid solution, 0.07% sodium bicarbonate solution) showed that these losses could not be explained by leaching alone, since the α-galactosides in the soaking medium amounted to only 1-10% of the recorded losses. Other monosaccharides (fructose and glucose) underwent a conspicuous increase (120 and 280% respectively) after soaking. The results indicate that during the 9 h soaking period the sugars in the lentils underwent a metabolic mobilization reminiscent of the changes taking place during germination. Cooking of the soaked lentils modified the α-galactoside content only slightly. On the other hand fructose, glucose, and sucrose decreased significantly during cooking. Soaking and cooking in water led to removal of a substantial proportion of the flatulence-causing oligosaccharides.

MATERIALS AND METHODS

Lentils (Lens culinaris var. vulgaris) were obtained from commercial sources.

Soaking

This process was carried out by mixing lentil seeds with distilled water or a solution of citric acid (0.1% w/v) or sodium bicarbonate (0.07% w/v) in a ratio of 1:3 w/v seeds:soaking medium. The lentils were allowed to absorb water at room temperature for 9 h. This soaking period was chosen so as to attain maximum weight and hydration values in the lentils (12). The soaking medium was drained from the legume seeds using a strainer. The volume of soaking liquid was measured and weighed, and the soaked seeds was weighed and comminuted in a blender. The material was stored in a freezer at -20°C until to be analyzed.

Cooking

After soaking and draining as above, the lentil seeds were weighed, placed in a pot with distilled water (seed:water ratio 1:6.7 w/v), and boiled for 35 min. After cooking, the lentils and cooking water were separated using a strainer. The cooked seeds were weighed and comminuted and stored in a freezer at -20°C.

Determination of soluble carbohydrates

Apparatus. A Waters Associates chromatograph equipped with a Model 6000 A pump, a Model U6K injector, and a Model R410 differential refractometer was used. The detection signal was processed on a Baseline 810 Chromatography Workstation. A precolumn (3.2 mm i.d. x 4.0 cm) packed with C18 Porasil B from Waters Associates and a μBondapack/carbohydrate column (3.9 mm i.d. x 30 cm) were employed. The chromatographic conditions were: mobile phases acetonitrile/water (75/25) and acetonitrile/water (85/15), flow rates 2 ml/min and 3 ml/min, column temperature 35°C, injection volume 100 μl.

Standard carbohydrate solutions

Varying amounts of fructose, sucrose, raffinose, and stachyose (Merck) were dissolved in distilled water and filled up with acetonitrile to obtain a similar composition to that of the mobile phase. These standard solutions were prepared daily and filtered through a Millipore FH (0.45 μm) membranes.

Manninotriose was prepared by enzymatic hydrolysis of stachyose following a similar procedure to the one used by Wight.
and van Niekerk (22) to prepare glucose from sucrose. Stachyose was dissolved in 25 ml of distilled water; 25 ml of 0.1 M acetate buffer (pH 4.65) and 4 mg of β-fructosidase (β-D-Fructofuranoside fructohydrolase EC 3.2.1.26 from Bochinger-Mannheim) were added. The solution was incubated at 25°C for 1 h. Neutral lead acetate (Merck) was added and the precipitate filtered. A mixed bed resin (Bio-Rad AG 50-X8) was converted to its bicarbonate form and air-dried. This resin (2 g) was added to the above filtrate (10 ml), and the mixture was allowed to stand for 30 min with occasional shaking, and filtered again. An aliquot of this solution was used to carry out thin-layer chromatographic analysis (TLC).

Sample preparation

Extraction of soluble carbohydrates was carried out as described elsewhere (18). Aliquots of these extracts were used in chromatographic analysis. Identification of the soluble carbohydrates was performed on silica-gel TLC plates as indicated previously (19) and by high-pressure liquid chromatography (HPLC), spiking with standards. Quantitation of each sugar was accomplished by comparing the peak heights or areas of the samples to those for the standard solutions curve. Pure manniotriose obtained by hydrolysis of stachyose was used to identify the manniotriose in our samples by TLC. We were unable to prepare sufficient amounts of manniotriose to obtain a standard curve for HPLC determination and this sugar was therefore identified based on its retention time as reported in the literature (15) and quantified taking raffinose as the standard.

Determination of water content

Aliquots of the raw, soaked, and cooked legume were dried to constant weight in a vacuum oven (20 mm/35°C) to determine the water content in the raw and processed legume.

RESULTS AND DISCUSSION

The soluble carbohydrate (fructose, glucose, sucrose, raffinose, manniotriose, and stachyose) content of raw, soaked, cooked lentils appears in Table 1. The identity of these carbohydrates was established by monodimensional and bidimensional TLC using known standards (19).

Quantitation of the various carbohydrates was obtained by HPLC (20). Standard curves were obtained for each sugar and fit using a method of least square. The regression coefficients for the curves were always >0.990. The quantity of the individual sugars was calculated by comparison of peak areas and concentrations.

According to the data presented in Table 1, soaking in water eliminated most of the oligosaccharides of the raffinose family; the raffinose itself was completely removed. The pH of the soaking solution had no significant effect on the amount of α-galactosides removed. Sucrose, another water-soluble carbohydrate also underwent a significant loss, though less pronounced than that for the galactosides; more strikingly, glucose was found in the lentils after soaking, despite the fact it was not detected in the raw lentils. The fructose content also increased, during soaking.

The sugar content of the soaking solution is shown in Table 2. Based on the data for each sugar for the raw and soaked lentils and the soaking solution (weight of sugar in the raw lentils + weight of sugar in the soaking solution), it was found that the losses of the galactosides (raffinose, manniotriose, and stachyose) through leaching into the soaking solution did not account for the total losses measured in the soaked seeds. Generally speaking, only 1-10% of the galactosides losses detected could be accounted for by the galactosides actually leaching into the soaking solution. In other words, although on the average soaking reduced the amount of galactosides by half, only 1-10% of this reduction was attributable to the galactosides that had leached out into the soaking solution. A different picture emerged for the other soluble sugars (fructose, glucose, and sucrose). The sum of the quantities of fructose in the soaking solution and in the soaked lentils practically doubled the sugar present in the raw lentils. The presence of glucose was still more noteworthy, since it was not initially present in the raw legume. Finally, sucrose levels also showed a net loss, though much less pronounced than that for the α-galactosides.

When raw lentils are soaked and cooked (2) their nutrient content can be affected in one or more of the following ways: a) dilution, in which as a consequence of rehydration the moisture content increases from 6% to 52-70%; b) leaching, in which some nutrients pass from the lentils into the soaking water; and c) chemical reaction which may take place at any temperature but especially at those higher than refrigeration temperatures. According to Augustin and Klein (2), very few studies have been carried out to ascertain whether any positive alterations might occur during the soaking period, similar to ascorbic acid synthesis during the sprouting of legumes. The present data indicate that during the soaking period of the lentils, there was an acute decrease (45-90%; see Table 2, column D) in the galactosides along with an increase in glucose and fructose. These changes have a positive connotation from a nutritional standpoint.

### TABLE 1. Changes in soluble carbohydrate in processed lentils.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fructose</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Raffinose</th>
<th>Manniortiose</th>
<th>Stachyose</th>
<th>Water (g/100 g dry matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>0.09 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.26 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.22 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.01 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.96 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.0</td>
</tr>
<tr>
<td>Soaking in:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>water</td>
<td>0.12 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>0.54 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.68 ± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>51.7</td>
</tr>
<tr>
<td>citric acid 0.1%</td>
<td>0.12 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.88 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.07 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.49 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.75 ± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.7</td>
</tr>
<tr>
<td>sodium bicarbonate 0.0%</td>
<td>0.13 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.22 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.84 ± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.09 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.48 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.69 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54.7</td>
</tr>
<tr>
<td>Cooking in water after soaking in:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>water</td>
<td>0.06 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.41 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.08 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.45 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.01 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>67.0</td>
</tr>
<tr>
<td>citric acid 0.1%</td>
<td>0.05 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>0.42 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.08 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.51 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.16 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>68.4</td>
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<tr>
<td>sodium bicarbonate 0.07%</td>
<td>0.07 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>0.44 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.08 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.49 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.12 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70.1</td>
</tr>
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</table>

ND = Not detected.

* g/100 g dry matter. Values are the means of four determinations ± standard deviation.

Different superscripts in the same column indicate significant differences (P<0.05).
TABLE 2. Effect of soaking process on soluble sugars in lentils.

<table>
<thead>
<tr>
<th>Soaking process in</th>
<th>Fructose (mg)</th>
<th>Glucose (mg)</th>
<th>Sucrose (mg)</th>
<th>Raffinose (mg)</th>
<th>Manninotriose (mg)</th>
<th>Stachyose (mg)</th>
</tr>
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<tbody>
<tr>
<td>Distilled water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B) Soaking liquid</td>
<td>63</td>
<td>52</td>
<td>105</td>
<td>25</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>C) Soaked lentil</td>
<td>124</td>
<td>228</td>
<td>953</td>
<td>ND</td>
<td>539</td>
<td>681</td>
</tr>
<tr>
<td>D) Effect of soaking (%)**</td>
<td>+120</td>
<td>+280</td>
<td>-16</td>
<td>-89</td>
<td>-45</td>
<td>-64</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soaking process in</th>
<th>Fructose (mg)</th>
<th>Glucose (mg)</th>
<th>Sucrose (mg)</th>
<th>Raffinose (mg)</th>
<th>Manninotriose (mg)</th>
<th>Stachyose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% citric acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B) Soaking liquid</td>
<td>82</td>
<td>73</td>
<td>54</td>
<td>23</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>C) Soaked lentil</td>
<td>124</td>
<td>198</td>
<td>880</td>
<td>69</td>
<td>491</td>
<td>746</td>
</tr>
<tr>
<td>D) Effect of soaking (%)**</td>
<td>+142</td>
<td>+271</td>
<td>-26</td>
<td>-59</td>
<td>-50</td>
<td>-61</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soaking process in</th>
<th>Fructose (mg)</th>
<th>Glucose (mg)</th>
<th>Sucrose (mg)</th>
<th>Raffinose (mg)</th>
<th>Manninotriose (mg)</th>
<th>Stachyose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.07% Na bicarbonate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B) Soaking liquid</td>
<td>59</td>
<td>53</td>
<td>73</td>
<td>32</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>C) Soaked lentil</td>
<td>133</td>
<td>174</td>
<td>841</td>
<td>86</td>
<td>484</td>
<td>689</td>
</tr>
<tr>
<td>D) Effect of soaking (%)**</td>
<td>+126</td>
<td>+227</td>
<td>-27</td>
<td>-47</td>
<td>-50</td>
<td>-64</td>
</tr>
</tbody>
</table>

* B = Content (in mg) of the soaking liquid used for 100 g (dry matter) of raw lentil.
** D = Modification of the sugar on a percentage basis; D = [(B+C)-A] x 100.

Kataria et al. (9) reported that during interspecific hybridization of black gram and mung beans, soaking for 6 to 18 h reduced the levels of total soluble sugars, reducing sugars, and nonreducing sugars. They pointed that the losses in the sugars during soaking could be due to simple diffusion of the sugars after solubilization. Similar effects of soaking on the soluble carbohydrate content in certain pulses have also been reported previously (5-7). However, Ologhobo and Fetuga (11) recorded an increase in glucose, fructose, and sucrose and a decrease in galactosides in lima beans.

Soaking, an integral part of traditional methods of processing and reducing energy costs by shortening cooking time, thus affords the additional advantage of rendering seeds nutritionally superior by removing antinutritional factors (e.g., phytic acid, polyphenols) and by enhancing the digestibility of the proteins in mung bean seeds (8) and lentils (1).

According to the data on glucose and fructose in this study (Table 2), the sum of the sugars detected in the soaking medium and the soaked lentils (B and C) far exceeded the amount present in the raw legume (A). On the contrary, the α-galactosides present after soaking failed to account for the initial levels recorded in the raw material. It is therefore clear from the data in Table 2 that soaking is also a metabolic process. It, in fact, signifies the onset of the metabolic changes that take place during germination. In the case of lentils (21), these changes actually bring about an appreciable increase in fructose and glucose (particularly this latter sugar, which was not detected in the raw legume), a slight decrease in sucrose, and the disappearance of all the α-galactosides. The raffinose family oligosaccharides are metabolized during germination. Nigam and Giri (10) observed increased α-galactosidase activity in germinated pulses, and Dey (3) and Reddy and Salunkhe (14) reported an increase in α-D-galactosidase and β-D-fructo-furanoside levels during germination. Although large amounts of galactose and fructose might be expected from the enzymatic hydrolysis of α-galactosic oligosaccharides according to Dey (4) the D-hexoses that are released during germination are rapidly metabolized, and D-galactose is barely detectable.

A similar trend was observed after soaking the lentils for 9 h, and these changes cannot be accounted for simply by leaching into the soaking medium.

The soaking process would thus appear to initiate a metabolic process similar to sprouting rather than mere water absorption resulting in some structural changes in the proteins and starches and slight losses in certain water-soluble components (minerals, sugars, pectic substances, and proteins) as reported by Varriano-Marston and Omana (17) (among others) in black beans.

Cooking of soaked lentils did not significantly alter the manninotriose and raffinose contents when an acid and basic medium was used for soaking. In the lentils soaked in distilled water, there was a slight but significant decrease in manninotriose, and a small amount of raffinose (0.08%) was again detected. The stachyose content increased slightly with cooking. Fructose, glucose, and sucrose decreased significantly during cooking.

The changes that took place during cooking were much less pronounced than the changes that occurred during soaking and can be attributed to the particular balance for each individual sugar attained between leaching into the cooking water (which tends to decrease the sugar content on a percentage basis) and losses of other nutrients (which causes a relative increase in the sugar content on a percentage basis).

Soaking and cooking in water led to the removal of a substantial proportion of the flatulence-causing oligosaccharides, and consequently these conditions of preparation would be preferred from a nutritional standpoint, considering that Shunku et al. (16) previously showed protein loss in soybean seeds was minimal (0.9%) when these seeds were boiled in water. Boiling in a sodium bicarbonate solution increased the protein loss slightly (2.7%).

Kataria et al. (9) noted that total soluble sugars, reducing sugars, and nonreducing sugars in amphidiploids underwent an upward trend in concentration after cooking as compared to the values obtained after soaking. This increase was nevertheless less than that in raw seeds. Cooking of unsrowned seeds also raised the level of total soluble sugars, reducing sugars, and nonreducing cont. on p. 306
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sugars. Cooking increased the content of reducing sugars to a greater extent than that of nonreducing sugars.

Rao and Belavady (13) observed lower values of glucose, fructose, and sucrose in four pulses (red gram, bengal gram, black gram, and green gram) after cooking. Ologhobo et al. (11) obtained similar findings for cooked lima bean seeds, but there were higher amounts of galactosides than in the raw lima bean seeds. This trend is similar to that observed for the cooked soaked lentils (Table 1). Compared with the values for the soaked lentils there was an increase in stachyose, a negligible or small increase in raffinose and manninotriose, and a decrease in glucose, fructose and sucrose.

According to Rao and Belavady (13) a possible explanation for this is that a part of oligosaccharides could be present in bound form, bound either to proteins or to other macromolecules, or as constituents of high molecular weight polysaccharides. Cooking may affect these bonds and thus result in release of the oligosaccharides.

ACKNOWLEDGMENTS

This study was supported by CICYT ALI 88-046-C02.01 and is part of the Ph.D. dissertation work of J. Frías.

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JOURNAL OF FOOD PROTECTION, VOL. 55.