Changes of $\alpha$-galactosides in grain legume seeds during germination, high pressure processing and storage

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ABSTRACT

High pressure processing of germinated grain legume seeds is technology which enables the important decrease of high content of $\alpha$-galactosides and the microbial contamination of germinated grain legume seeds and to prolong their shelf life. The objective of this paper was to evaluate the changes of soluble carbohydrates of pressurized germinated grain legume seeds during their storage. Grain legume seeds: pea ($Pisum sativum$ L.), chickpea ($Cicer arietinum$ L.), lentil ($Lens esculenta$ MOENCH) and mung bean (green gram) seeds ($Vigna radiata$ (L.) WILCZEK) were germinated 3 days and then pressurized in water or in pickle from citric acid (pH 2.5). The commonly used parameters for pressurizing were: pressure 500 MPa, time 10 minutes. The pressurized germinated grain legume seeds were stored 1, 7 and 14 days. Carbohydrates (fructose, glucose, galactose, sucrose and $\alpha$-galactosides - raffinose, verbascose and stachyose) were determined by means of HPLC with refractometric detection. Germination is the most effective process from point of view the reduction of high content of indigestible $\alpha$-galactosides in grain legume seeds. Content of $\alpha$-galactosides was decreased after high pressure processing of germinated seeds in the acidified pickle and 14 days storage at temperatures 5-8 °C for all tested seeds next to zero. The pressurization of germinated seeds in the acidified pickle magnifies the effect of germination and pressure, ensures reduction of $\alpha$-galactosides and inhibits of spores germination and growth. The shelf life of pressurized germinated seeds is prolonged by this way and the seeds keep their sensory properties like fresh germinated seeds.

Keywords: grain legume seeds; $\alpha$-galactosides; germination; high pressure processing; storage

INTRODUCTION

Consumption of grain legumes in the Czech Republic is relatively low compared with the rest of Europe. The main reasons for this include an unappealing, sometimes slightly bitter, taste and, in particular, the digestive problems often experienced after consumption. An effective way of reducing the problems associated with the digestion of grain legumes is to germinate their seeds because, during the germination process, the indigestible $\alpha$-galactosides that induce flatulence are broken down.

High pressure treatment as a method of preservation can inactivates microorganisms when a pressure of 400-600 MPa is applied for 10 minutes. The application of such technology preserves the original quality of the treated products, with particular respect to colour, aroma, vitamin content and biologically active substances [1], [2].

Our previous results in the treatment of germinated grain legume seeds with high pressure were described in earlier papers [3], [4]. Our last paper at ICEF 10 [5] was concentrated on model to calculate the parameters of a baroinactivation model for the total number of microorganisms inactivated during the pressurization of germinated lentil, chickpea and mung bean seeds. High pressure processing of germinated grain legume seeds is technology which enables the important decrease of high content of $\alpha$-galactosides and the microbial contamination of germinated grain legume seeds and to prolong their shelf life. The objective of this paper was to evaluate the changes of soluble carbohydrates of pressurized germinated grain legume seeds during their storage.

MATERIALS & METHODS

Samples
Chickpea (*Cicer arietinum* L.); country of origin: Turkey.

Lentil (*Lens esculenta* MOENCH); country of origin: Canada.

Mung bean (green gram) seeds (*Vigna radiata* (L.) WILCZEK); country of origin: Burma.

Pea (*Pisum sativum* L.); country of origin: Czech Republic

**Germination**

The samples were germinated at the Czech Agricultural University, Prague, in the Department of Plant Production. They were germinated in aerated bottles, connected in sequence for 3 days at temperature 20 °C. One such bottle containing 50 g of substrate can produce approximately 100 g of germinated sample. The water in the bottles was changed every 24 h. After three days, the germinated samples were taken for simultaneous analysis and high pressure processing.

**Determination of soluble carbohydrates by HPLC**

Sucrose and α-galactosides (raffinose, ciceritol, stachyose and verbascose) were separated by means HPLC.

Detector: Refractive Index Detector, Shodex RI SE-61, Showa Denko K.K., Japan

Autosampler: Basic Marathon type 816, Spark Holland B.V., The Netherlands

Pump: LCP 4000, Ecom s.r.o., Czech Republic

Column: SGX NH2 5 μm, 4x250mm, Tessek Ltd., Czech Republic

Treatment of signal: Chromatographic set CSW version 1.7, Data Apex, Czech Republic

**Conditions of determination:**

Mobile phase: mixture of acetonitril and dematerialized water (65:35, v/v),

Flow of mobile phase: 0.8 ml/min, pressure 9.8 MPa,

Injection: 100 μl, laboratory temperature.

Standards of stachyose and raffinose from Sigma-Aldrich (Germany), standard of verbascose from Fluka (Switzerland).

**Treatment by high pressure**

Germinated seeds were weighted into transparent laminated bags (PA/PE 80 VAC STAR, Germany). Seeds were washed down by citric acid pickle or water in the given ratio (see Table 1)

<table>
<thead>
<tr>
<th>Grain legume seeds</th>
<th>Citric acid pickle (pH)</th>
<th>Ratio seeds:pickle for pressurization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea</td>
<td>2.5</td>
<td>2:3</td>
</tr>
<tr>
<td>Chickpea</td>
<td>2.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Lentil</td>
<td>2.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Mung bean</td>
<td>2.0</td>
<td>1:2</td>
</tr>
</tbody>
</table>

The bags were closed with minimal air content and pressurized in high-pressure press A CYX 6/0103 (ŽĎAS a.c., Czech Republic), volume of pressure chamber 2 liters.

Used pressure 500 MPa, time of pressurization 10 min. Average rate of pressure strain 8 MPa/s, rate of pressure decline about 150 MPa/s.

Temperature of pressurized medium – portable water 18 - 22 °C, the same as temperature of samples.

**RESULTS & DISCUSSION**

Changes in contents of soluble carbohydrates were monitored after pressurization of germinated seeds in citric acid pickle and in water and then during storage. The treated samples were stored in plastic bags in refrigerator at temperature 5-8 °C, time of storage 14 days. Reduction of contents of α-galactosides (in relative % to the germinated seed) is shown in Table 2.

Table 2. Changes of α-galactosides after 14 days storage of pressurized germinated grain legumes seeds in water bath and in citric acid pickle (relative % to the germinated seed)

<table>
<thead>
<tr>
<th>Grain legume seeds</th>
<th>In water bath</th>
<th>In citric acid pickle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea</td>
<td>12.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Chickpea</td>
<td>22.7</td>
<td>12.3</td>
</tr>
<tr>
<td>Lentil</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mung bean</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figures 1 and 2 present the changes of soluble carbohydrates in pressurized chickpea seeds (Fig. 1) and lentil seeds (Fig. 2) in citric acid pickle and in water bath during storage.
Figure 1. Changes of carbohydrates during storage of pressurized germinated chickpea seeds in water bath (CW1, CW7, CW14) and in citric acid pickle (CP1, CP7, CP14)

Legend: CG – germinated chickpea seeds; CW1 – pressurized germinated chickpea seeds in water bath, 1st day of storage; CW7 – pressurized germinated chickpea seeds in water bath, 7th day of storage; CW14 – pressurized germinated chickpea seeds in water bath, 14th day of storage; CP1 – pressurized germinated chickpea seeds in citric acid pickle, 1st day of storage; CP7 – pressurized germinated chickpea seeds in citric acid pickle, 7th day of storage; CP14 – pressurized germinated chickpea seeds in citric acid pickle, 14th day of storage.

Course of monitored changes of soluble carbohydrates is very similar for all sorts of grain legumes. Maximal reduction is caused by germination. The effect of germination on reduction of $\alpha$-galactosides (expressed in relative % to the original seeds before germination) is presented in Table 3.
Table 3. Effect of germination on reduction of α-galactosides (relative % to the original seeds before germination)

<table>
<thead>
<tr>
<th>Grain legume seeds</th>
<th>Raffinose</th>
<th>Ciceritol</th>
<th>Verbascose</th>
<th>Stachyose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lentil</td>
<td>66.0</td>
<td>77.1</td>
<td>84.7</td>
<td>90.8</td>
</tr>
<tr>
<td>Mung bean</td>
<td>75.7</td>
<td>83.8</td>
<td>75.6</td>
<td></td>
</tr>
<tr>
<td>Chickpea</td>
<td>87.4</td>
<td>64.9</td>
<td>48.0</td>
<td></td>
</tr>
</tbody>
</table>

General results of decrease α-galactosides contents during storage of pressurized germinated seeds in water bath and in citric acid pickle are presented on Figure 3. The contents of α-galactosides in high pressure treated germinated seeds were reduced for lentil and mung bean seeds up to zero. Content of α-galactosides in pea seeds was decreased up to 4.9 % in water bath and up to 2.1 % in citric acid pickle. Decrease of α-galactosides in chickpea seeds was up to 16 % in water bath and up to 11.4 % in citric acid pickle.

Figure 3. Changes of α-galactosides during storage of pressurized germinated seeds in water bath and in citric acid pickle
Legend: 0 – seeds before germination; G – germinated seeds; W1 – pressurized germinated seeds in water bath, 1st day of storage; W7 – pressurized germinated seeds in water bath, 7th day of storage; W14 – pressurized germinated seeds in water bath, 14th day of storage; P1 – pressurized germinated seeds in citric acid pickle, 1st day of storage; P7 – pressurized germinated seeds in citric acid pickle, 7th day of storage; P14 – pressurized germinated seeds in citric acid pickle, 14th day of storage

Reduction of α-galactosides is possible to explain by means:

- enzyme hydrolysis to lower carbohydrates (monosaccharides, sucrose)
- acid hydrolysis in citric acid pickle
- maceration of soluble carbohydrates from seeds to the bath by means of high pressure

CONCLUSION

The germination is very effective way to decrease α-galactosides contents in the grain legume seeds. The microflora of germinated grain legumes can be effectively destroyed by high pressure treatment which ensures the high quality of food products from sensory point of view as well [4], [5]. During the high pressure treatment (500 MPa for 10 minutes) the further α-galactosides are decomposed. The contents of α-galactosides in high pressure treated germinated seeds were reduced for lentil and mung bean seeds up to zero during 14 days storage at the temperature 5-8 °C. Such treatment extends the shelf life of germinated seeds, enabling them to maintain sensory qualities comparable to those of fresh seeds.

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REFERENCES


