# The Changes of α-Galactosides during Germination and High Pressure Treatment of Legume Seeds

*J. DOSTÁLOVÁ[1]*, P. KADLEC[2], J. BERNÁŠKOVÁ[1], M. HOUŠKA[3] and J. STROHALM[3]*

*[1]Department of Food Chemistry and Analysis and [2]Department of Carbohydrate Chemistry and Technology, Institute of Chemical Technology in Prague, 166 28 Prague, Czech Republic; [3]Food Research Institute Prague, 102 31 Prague, Czech Republic*

**E-mail: jana.dostalova@vscht.cz*

**Abstract**: The α-galactosides negatively affect of grain legumes digestibility. The most effective way of α-galactosides content decreasing is germination. The contents of α-galactosides in legume seeds were decreased by germination up to 16% of original value (lentil). During the germination, the contents of microorganisms are arising to high values. The effective method for decreasing of microorganisms content is high pressure treatment. During the high pressure treatment (500 MPa for 10 min) the further α-galactosides are decomposed up to 5% of original value (mung bean). The contents of α-galactosides in high pressure treated germinated seeds were reduced up to 0 (lentil, mung bean) during 14 days storage at the temperature 4–8°C.

**Keywords**: α-galactosides; cold storage; germination; high pressure treatment; legume seeds

## INTRODUCTION

Grain legumes are very valuable food. They are source of proteins with high nutritive value (20–25 g/100 g), vitamins B (thiamine, riboflavin, niacin, folic acid), minerals (Ca, Fe, P, K, Zn, Mg, etc.) and dietary fibre. They have further nutritional advantages (low fat content, cholesterol absence, low GI, presence of various protective factors). The grain legume consumption is very low in many developed countries (average in Europe about 3 kg per capita per year), most of all, due to digestive troubles after consumption which are caused by no digestible oligosaccharides – α-galactosides. The most effective way to decrease the high content of α-galactosides in legume seeds is germination (Skulinová *et al*. 2002; Dostálová *et al*. 2004). The potential microbial contamination of germinated legume seeds is however main reason of their short shelf life and possible unsuitability for healthy food. Sprouting seeds can be contaminated by human pathogens and consumption of raw sprouted seeds has been associated with many outbreaks of food-borne diseases (Bremer *et al*. 2003; Robertson *et al*. 2005).

The high pressure processing is technology which could be suitable for preservation of germinated legume seeds. It was demonstrated that microorganisms in many food commodities were destroyed by pressures 600 MPa for 10 min (Fellows 2000). The positive effect on microorganisms destruction in germinated legume seeds we confirmed in our previous studies (Kadlec *et al*. 2006, 2007; Dostálová *et al*. 2007).

The aim of this study was to determine the changes of α-galactosides during germination and high pressure treatment of grain legume seeds (pea, chickpea, lentil and mung bean – green gram).

## MATERIALS AND METHODS

***Material.*** Pea (*Pisum sativum* L.), country of origin Czech Republic; lentil (*Lens esculenta* MOENCH), country of origin Canada; chickpea (*Cicer arietinum* L.), country of origin Turkey; mung bean (green gram) (*Vigna radiata* (L.) WILCZEK, country of origin Burma. All grain legume seeds were obtained from company Beskyd Fryčovice Ltd., Czech Republic.

### Technological processes

***Germination.*** The legume seeds were germinated in aerated water media in aeration bottles, 50 g of seeds and 100 ml of tap water in each bottle, water was changed every 24 h, temperature 20°C, and time of germination 3 days.

***High pressure treatment.*** Laboratory press CYX 6/0103, ZDAS joint stock Co., Czech Republic, having working pressure up to 600 MPa, was used. Size of chamber: diameter – 90 mm, length – 320 mm, chamber volume – 2 l, power input – 7.5 kW (Čapek *et al*. 2000). Germinated seeds were rinsed with tap water after germination and have been placed into the plastic bags (foil PA/PE 80, VAC STAR). The pickle from citric acid or water was added to germinated seeds, the air was removed and the bag was closed by welding. The pickle from citric acids with pH 2.5 was used for pea and chickpea seeds and the pickle with pH 2.0 was used for lentil and mung bean seeds. Seeds:pickle ratio was 2:3 for pea, chickpea and lentil and 1:2 for mung bean germinated seeds. The bags (50–100 g) have been treated in the laboratory press, pressure 500 MPa, time of pressurisation 10 minutes. The temperature during pressurisation was 18–22°C. The samples were input immediately after pressure treatment and decompression into the refrigerator.

***Storage of high pressure treated germinated seeds.*** The treated samples were stored in plastic bags in refrigerator at temperature 5–8°C, time of storage 14 days.

***Extraction and determination of soluble carbohydrates.*** Approximately 5 g of ground dry sample were homogenised in 20 ml of ethanol:water mixture (80:20, v/v) and refluxed (boiled) for 60 min. Extract was cooled down and filtered through a membrane filter 0.45 μm pore size, the rest was rinsed out by distilled water. The filtrate was evaporated in vacuum evaporator (temperature 60°C, pressure max. 0.9 kPa) and the rest was diluted by demineralised water (5 g to 1000 ml), repurified by filtration on microfilter C18 (Maxi-Clean Cartridges) and analysed by HPLC.

***HPLC determination.*** Soluble carbohydrates (sucrose and α-galactosides: raffinose, verbascose, stachyose and ciceritol) were determined by HPLC with refractive index detector (Shodex RI SE-61, Showa Denko K.K., Japan), autosampler (Basic Marathon type 816, Spark Holland B.V., the Netherlands) and pump LCP 4000, Ecom, Czech Republic on column SGX $NH_2$ 5 μm (4 × 250 mm) (Tessek Ltd., Czech Republic). Sugars were eluted at mobile phase (acetonitrile: demineralised (water 65:35, v/v)) flow rate of 0.8 ml/min, pressure 9.8 MPa and laboratory temperature. The method of external standard was used for quantification. Standards of stachyose and raffinose were obtained from Sigma-Aldrich (Germany), verbascose from Fluka (Switzerland) and sucrose from Lachema (Czech Republic).

## RESULTS AND DISCUSSION

The changes of soluble carbohydrates (sucrose and α-galactosides: raffinose, verbascose and stachyose) during germination, high pressure treatment and cold storage of germinated high pressure treated legume seeds are given in Figure 1. The changes of soluble carbohydrates during high pressure treatment and cold storage of germinated high pressure treated legume seeds were followed at seeds treated and stored in the water and in the pickle containing citric acid as well.

The changes in the contents of all carbohydrates were similar at all kinds of legume seeds. All α-galactosides contents were decreasing during 3-days germination. The lowest degradation during germination was in chickpea seeds (up 87.4%, 64.9%, and 48% of original value of raffinose, ciceritol and stachyose contents, respectively). The highest degradation of α-galactosides proceeded in lentil (up 34% raffinose, 22.9% ciceritol, 9.2% stachyose, and 15.3% verbascose of original value) and mung bean seeds (up 24.3% raffinose, 24.4% stachyose, and 16.1% verbascose). The contents of α-galactosides in high pressure treated germinated seeds were reduced nearly up to 0% during 14 days storage at the temperature 4–8°C. The decrease of α-galactosides in the pickle with citric acid was faster. The changes of total α-galactosides are presented in Figure 2. The contents of α-galactosides

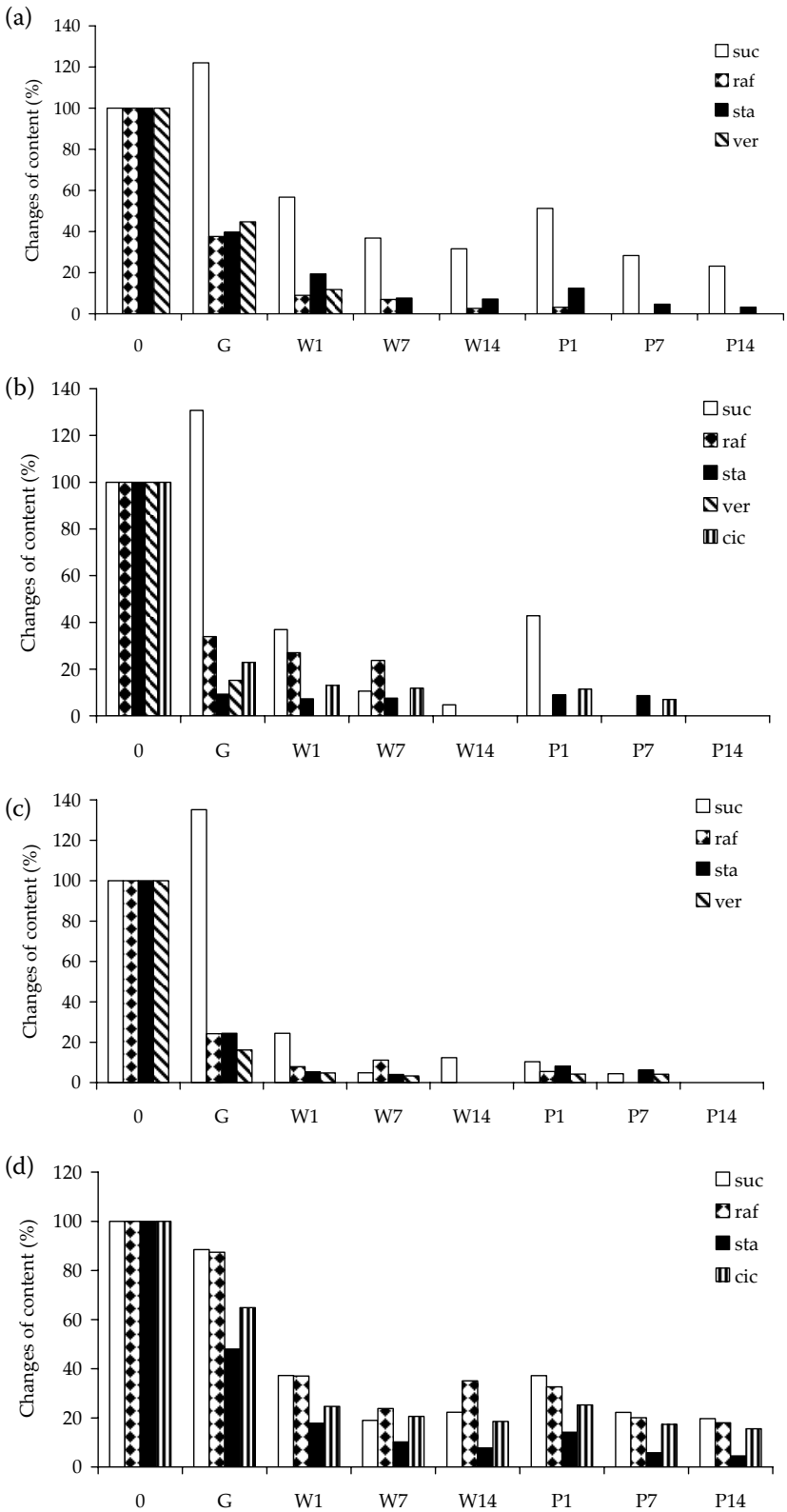


Figure 1. Relative changes of carbohydrates after germination, high pressure treatment, and storage of treated (a) pea, (b) lentil, (c) mung bean (green gram), and (d) chickpea seeds

suc – sucrose; raf – raffinose; sta – stachyose; ver – verbascose

0 – original seeds; G – after 3 days of germination; W1 – after high pressure treatment in water, 1st day of storage; W7 – after high pressure treatment in water, 7th day of storage; W14 – after high pressure treatment in water, 14th day of storage; P1 – after high pressure treatment in pickle from citric acid, 1st day of storage; P7 – after high pressure treatment in pickle from citric acid, 7th day of storage; P14 – after high pressure treatment in pickle from citric acid, 14th day of storage

in legume seeds were decreased by germination up to 16% of original value (lentil). During the high pressure treatment (500 MPa for 10 min) the further α-galactosides are decomposed up to 5% of original value (mung bean). The contents of α-galactosides in high pressure treated germinated seeds were reduced up to 0 (lentil, mung bean) during 14 days storage at the temperature 4–8°C.

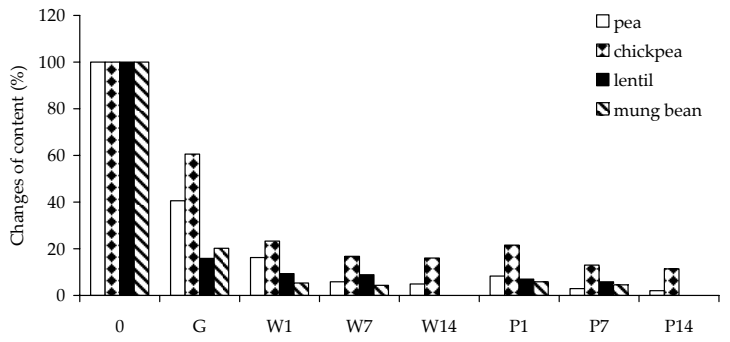


Figure2. Relative changes of α-galactosides after germination, high pressure treatment and storage of treated legume seeds

For explanation see Figure 1

The decrease of α-galactosides in germinated seeds during high pressure treatment and cold storage could be explained by hydrolysis and by extraction into the pickle.

## CONCLUSIONS

The germination is very effective way to decrease α-galactosides contents (up to 16% of original value) 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. During the high pressure treatment (500 MPa for 10 min) the further α-galactosides are decomposed up to 5% value before pressuring. The contents of α-galactosides in high pressure treated germinated seeds were reduced up to 0 during 14 days storage at the temperature 4–8°C.

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