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High pressure treatment of germinated chickpea *Cicer arietinum* L. seeds

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Abstract

The 2 days germinated chickpea *Cicer arietinum* L. seeds in citric acid pickle (pH 2.5) were treated in press CYX 6/0103, ZDAS joint stock co., Czech Republic, using pressure 500 MPa, time 10 min. The treated samples were stored in refrigerator at temperatures 5–8 °C. The total number of microorganisms of germinated chickpea seeds in citric acid pickle decreased from 1.6×10^5 CFU/g to less than 10 CFU/g by pressurization and varied between less than 10 and 80 CFU/g during 21 days of storage. The number of yeasts, coliform bacteria, *Escherichia coli* and fungi were decreased by pressure treatment to near zero and exhibited no changes during the further 21 days of storage. The content of α-galactosides causing flatulence was decreased to 7% of original value (content in dry seeds) by germination, pressurization and storage.

Keywords: High pressure treatment; Germinated seeds; Chickpea; Microbial evaluation; α-galactosides

1. Introduction

Germination of grain legume seeds is the most effective way to decrease of high content of α-galactosides, which affects undesirably digestibility and acceptability of legume seeds. The methods of germination and changes in contents of soluble carbohydrates during pea germination were presented in our previous paper (Skulinová et al., 2002). The potential microbial contamination of germinated grain legume seeds is however the main reason of their short shelf life and possible unsuitability for healthy food and dishes preparation. The total number of microorganisms can increase during germination of grain legume seeds up to high-order 10^9 CFU/g. Sprouting seeds are susceptible to contamination by human pathogens (e.g. *Escherichia coli*, *Salmonella spec*., *Giardia*, *Cryptosporodium*, *Enterobacter* spp., *Kleibsella*, etc.) due to high temperature and humidity typically used during production (Robertson, Johannessen, Gjerde, & Loncarevic, 2002). Consumption of raw sprouted seed has been associated with many outbreaks of foodborne disease (Bremer, Fielding, & Osborne, 2003; Robertson, Greig, Gjerde, & Fazil, 2005; Taormina, Beuchat, & Slutsker, 1999; Thomas, Palumbo, Farrar, Farver, & Cliver, 2003; Warriner, Spaniolas, Dickinson, Wright, & Waites, 2003). Legumes proteins were pointed out as potential source of *Clostridium perfringens* and the following gastrointestinal symptoms after consuming of minestrone soup (Roach & Sienko, 1992).

The high pressure processing is technology which could be suitable for preservation of germinated legume seeds. It was demonstrated that microorganisms in treated food products (milk, fruit juice, meat and a variety

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of fruits and vegetables) were destroyed by pressures of 600 MPa for 10 min (Fellows, 2000). This technology ensures the high quality of food products (flavour, colour, vitamin contents, biological active components, etc.) similar to that of the fresh raw materials (Čapek, Houška, & Strohalm, 2000; Knorr, 1995, 1999).

The goal of this paper was the verification of the influence of high-pressure preservation upon microorganisms and α-galactosides contents in germinated chickpea seeds. The legume seeds can be in contact with spores of *Clostridium* spp. from soil. Germination of that spores can be regarded as the potential risk during germination of chickpea seeds. Therefore, we have decided to use the acidification of the pickle as preventative measure together with high pressure treatment that enhances the effect of acidification.

2. Experimental

2.1. Plant material

We have used the white chickpea seeds (*Cicer arietinum* L.)—year of harvest 2002, country of origin Turkey. The seeds were germinated in aerated water media. The seeds were incubated 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 1–5 days.

2.2. High pressure equipment

Laboratory press CYX 6/0103, ZDAS joint stock co., Czech Republic, was used having working pressure up to 600 MPa. Size of chamber: diameter—90 mm, length—320 mm, chamber volume—2 l, power input—7.5 kW (Čapek et al., 2000).

2.3. Pressurization and storage of treated material

The pickle with pH 2.5 was added to germinated chickpea seeds. After pressure treatment the pickle pH value increased immediately to value about 3.5 and continuously slowly was increasing during storage. We have selected the initial pH pickle and pickle/seeds ratio so to prevent the pickle pH to overcome value 4.0. Germinated seeds have rinsed with tap water after germination and have been placed into the plastic bags (foil PA/PE), corresponding amount of pickle was added into the bag and the bag was closed by welding. The air was removed by hand compression of the bag before welding. Pickle with pH 2.5 composed of 0.6 g of citric acid per 100 ml of water. The bags (50–100 g) have been treated in press CYX 6/0103, pressure 350–500 MPa, time of pressurization 3–15 min. Initial temperatures of samples were about 10–14 °C. During compression their temperature increased by adiabatic heating to about 3 °C per each 100 MPa (increase by 10.5–15 °C). Nearly the same decrease of the temperature was observed during decompression. The samples were input immediately after pressure treatment into the refrigerator. The treated samples were stored in refrigerator at temperature 5–8 °C, time of storage 21 days.

2.4. Extraction and determination of soluble carbohydrates

Approximately 5 g of ground dry sample was homogenized in 20 ml of ethanol:water (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 on 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.

2.5. HPLC determination

Soluble carbohydrates (galactose, glucose, sucrose and α-galactosides (rfo): raffinose—raf, verbascose—ver, stachyose—sta) were determined on column CarboPac PA1 (2×250 mm) with guard column PA1 (2×50 mm) by high performance anion-exchange chromatography with pulsed amperometry detection (HPAEC–PAD) using gold working electrode with the waveform setting E_1: 0.05 V for 0.40 s; E_2: 0.75 V for 0.19 s; E_3: −0.15 V for 0.39 s, with integration from 0.20 to 0.40 s (all Dionex system, Sunnyvale, USA). Sugars were eluted at a mobile phase flow rate of 0.25 ml/min by gradient elution of NaOH (linear ramp from 16 to 200 mM NaOH over 40 min), at temperature 25 °C. All analyses were carried out in duplicate.

2.6. Microbial evaluation

The total number of microorganisms (ISO 4833), yeasts (ISO 7954), fungi (ISO 7954), coliform bacteria (ISO 4832), *E. coli* (ISO 16649-2) and *Salmonella* (ISO 6579) were determined before pressurization, after pressurization (0 days) and after 7, 14 (18) and 21 days of storage. For storage experiment the pickle and seeds have been analysed separately. Separated seeds have been homogenized before microbial analysis.

3. Results and discussion

The influence of acidification itself on microbial counts can be regarded. We have observed decay of about 2-log decades corresponding to the type of