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In vitro digestibility and starch content, predicted glycemic index and potential *in vitro* antidiabetic effect of lentil sprouts obtained by different germination techniques

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ABSTRACT

The study focuses on changes in starch content and expected glycemic index (eGI) caused by different sprouting methods of lentil. On germination, a decrease was observed in total starch content (TS), α-amylase inhibitors activity (αAI) and eGI values. After elicitation, the highest TS content was determined in 3-day-old control sprouts (100.9 mg/g f.m.), whereas the lowest was in 4-day-old sprouts induced with 300 mM NaCl (57.8 mg/g f.m.). Resistant starch (RS) content was most effectively increased by induction with 600 mM mannitol. The highest eGI values were determined for 3-day-old sprouts induced with 300 mM NaCl, whereas the lowest were for 6-day-old sprouts induced with 100 mM NaCl. In treated sprouts starch digestibility was connected with αAI activity and RS content. Sprouting conditions can modify starch content, its potential bioavailability and eGI values. Optimization of this process will allow for the maximum nutritional benefit.

1. Introduction

Within the context of the increasing interest in leading a healthy life, the use of functional food and directed nutrition is nowadays presented as an alternative way to stay fit and healthy. There is much evidence confirming the fact that legume-rich food may significantly alter people's overall health and quality of life. Legumes provide powerful nutrition as well as proven anticancer, chemopreventive antioxidant and antimicrobial properties. These diets also lower cholesterol levels and improve blood glucose control in diabetics (Dixon, 2001).

All over the world legumes are consumed after different types of processing (e.g., cooking, frying) but, as has been shown, the nutraceutical and nutritional quality is most significantly improved by sprouting (Cevallos-Casals & Cisneros-Zevallos, 2010; Gawlik-Dziki & Świeca, 2011; Urbano et al., 1995). Legume sprouts are a good source of highly bioavailable proteins, starch, lipids and minerals. Additionally, germinated seeds contain significant amounts of polyphenols with well-documented pro-health properties. Among sprouted legumes, lentil sprouts contain many functional, health-promoting components, the consumption of which is linked to reduced risk of cardiovascular diseases, obesity, diabetes, inflammation and cancer (Caccialupi et al., 2010; Losso, 2003). Lentil seeds and sprouts are also good sources of high quality starch and proteins. Lentil sprouts are very high in fibre and low in fat. They have high levels of iron, potassium, folate, and niacin and are often recommended for vegetarians who need to supplement their diet with additional iron and protein. Additionally, they have a low glycemic index, slowly release glucose into the blood stream and secure a more stable insulin response. For these reasons lentils are suited to people with diabetes and those who are overweight (Chung, Liu, Hoover, Warkentin, & Vandenberg, 2008; Garcia-Alonso, Goni, & Saura-Calixto, 1998).

Additionally, in the last few years many trials have been undertaken to modify the chemical composition of functional foods including legume sprouts. It is well known that the chemical composition of lentil sprouts is generally affected by sprouting, environmental (e.g. illumination and temperature) and genetic factors (e.g. cultivar). There is some evidence that the quality of sprouts may be improved by modification of the metabolism of germinated seeds with elicitors (Eyaru, Shrestha, & Arcot, 2009; Zhao, Lawrence, & Verpoorte, 2005). Additionally, the application of such minimal processing methods may preserve the shelf life of sprouts, and reduce food microorganisms without affecting the sensory and nutritional quality. Thus, elicitation of seedlings seems to be a promising alternative for other conventional biotechnological techniques used for improving the yield of plant secondary

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metabolites and the nutraceutical and nutritional potential of low-processed food.

So far, little has been reported about the effect of different germination conditions on *in vitro* antidiabetic properties, starch content and composition of legume sprouts. Thus, the objectives of this study were to determine the influence of sprouting modification (elicitation by solution with high osmotic potential) on the starch content, composition and *in vitro* digestibility and predicted glycemic index. The information provided in this study will be valuable for the selection of lentil germination conditions (elicitors) to develop functional foods.

2. Materials and methods

2.1. Materials

Lentil seeds var. Tina were purchased from the PNOS S.A. in Ozarów Mazowiecki, Poland. Seeds were sterilized in 1% (v/v) sodium hypochloride for 10 min, then drained and washed with distilled water until they reached neutral pH. They were placed in distilled water and soaked by 6 h at 25 °C. Seeds were dark germinated 6 days in a growth chamber on the Petri dishes (∅125 mm) lined with absorbent paper. Seedlings were watered with 5 ml of Milli-Q water daily.

For the elicitation experiments mannitol (200 mM and 600 mM – Os1 and Os2, respectively) and NaCl (100 mM and 300 mM – S-Os1 and S-Os2, respectively) solutions were selected as abiotic elicitors. All solutions were freshly prepared before each application. Mannitol (Os1, Os2) and NaCl (S-O1, S-O2) treatments were applied by watering daily (not as soaking) 2-days-old sprouts with 5 ml of test solution. Sprout samples were gently collected dried at 50 °C for 24 h, milled and kept in polyethylene bags at −20 °C according to Ghavidel and Prakash (2007). For each treatment, three replicates were taken for analysis.

2.2. Sensory evaluation

An expert panel of researchers/scientists conducted the sensory evaluation. The sensory panel consisted of 12 men and 16 women (aged 25–50 years) and the members were trained to recognize and score different quality attributes including appearance, colour, texture, odour, and taste of the fresh, minimally processed sprouts. A five-point hedonic scale was used for marking, ranging from very poor to excellent. Plain water was used for mouth rinsing before and after each sample testing (Hajare, Saroj, Dhokane, Shashidhar, & Bandekar, 2007).

2.3. Total starch content

Total starch (TS) content was determined after dispersion of the starch granules in 2 M KOH (50 mg sample, 6 ml KOH) at room temperature (30 min, constant shaking) and hydrolysis of the solubilized starch with 80 μl (1 mg/ ml) amyloglucosidase (14 U mg^{-1}; EC 3.2.1.3) at 60 °C for 45 min (Goni, Garcia-Alonso, & Saura-Calixto, 1997). Glucose content was determined by using the standard dinitrosalicylic acid (DNSA) method (Miller, 1959). Total starch was calculated as glucose × 0.9. The free reducing sugar content of the samples was determined in order to correct the obtained total starch values obtained. The sucrose content of the samples was also determined in order to correct the obtained total starch values. The samples dispersed in sodium acetate buffer, pH 5.0 were treated with 200 μl of (10 mg in 1 ml of 0.4 M sodium acetate buffer, pH 5.0) invertase (EC 3.2.1.26; 300 U mg^{-1}) for 30 min at 37 °C. After centrifugation, reducing sugars were analyzed in the supernatants, using the DNS reagent.

After centrifugation (3000*g*, 15 min) and removal of supernatant, the pellet was dispersed with 2 M KOH, hydrolyzed with amyloglucosidase and liberated glucose was quantified, as described above, for total starch (TS).

2.4. Resistant and potentially bioavailable starch content

The resistant (RS) and potentially bioavailable (AS) starch content was analyzed on the basis of results obtained after simulated gastrointestinal digestion. Simulated mastication and gastrointestinal digestion was performed according to Elles, Blaylock, Huang, and Gussman (2000). Lentil sprouts (150 mg of dry weight) were homogenized in 3.5 ml of simulated salivary fluid (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8 g NaCl, 200U α-amylase (E.C. 3.2.1.1 in 1 l H_2O, pH 6.75) and shaken for 10 min at 37 °C. Next, the samples were adjusted to pH = 1.2 with HCl (5 mM), suspended in 1.25 ml of simulated gastric fluid (300 U ml^{-1} of pepsin A, EC 3.4.23.1 in 0.03 M HCl, pH = 1.2) and shaken for 120 min. at 37 °C. After simulated gastric digestion, samples were adjusted to pH = 6 with 0.1 M $NaHCO_3$ and suspended in simulated intestinal juice (0.05 g of pancreatin (activity equivalent 4 × USP) and 0.3 g of bile extract in 2.0 ml 0.1 M $NaHCO_3$; adjusted to pH = 7 with 1 M NaOH and finally 1.25 ml of 120 mM NaCl and 5 mM KCl was added to the sample. The prepared samples underwent *in vitro* intestinal digestion for 120 min.

After centrifugation (3000*g*, 15 min) and removal of supernatant, the pellet was dispersed with 2 M KOH, hydrolyzed with amyloglucosidase and liberated glucose was quantified, as described above, for total starch (TS). Resistant starch (RS) was calculated as glucose × 0.9. The potentially bioavailable starch (AS) content was calculated as the differences between TS and RS.

2.5. In vitro starch digestibility

The *in vitro* digestibility of starch was evaluated on the basis of total starch content (TS) and resistant starch (RS) determined after digestion *in vitro*.

$$SD[\%] = 100\% - \left(\frac{RS}{TS} \times 100\%\right)$$

where *SD* is the *in vitro* digestibility of starch, TS the total starch content, and RS is the resistant starch content.

2.6. In vitro starch digestion rate and expected glycemic index

The digestion kinetics and expected glycemic index (eGI) of the lentil sprouts were calculated in accordance with the procedure established by Goni, Garcia-Alonso, & Saura-Calixto (1997). A non-linear model following the equation [$C = C \propto (1 - e^{-kt})$] was applied to describe the kinetics of starch hydrolysis, where C, C1 and *k* were the hydrolysis degree at each time, the maximum hydrolysis extent and the kinetic constant, respectively. The hydrolysis index (HI) was calculated as the relation between the areas under the hydrolysis curve (0–240 min) of the sprout sample and the area of standard material from white bread. The expected glycemic index (eGI) was calculated using the equation proposed by Granfeldt, Björck, Drews, and Tovar (1992): eGI = 8.198 + 0.862HI.

2.7. Activity of α-amylase inhibitors

For extraction, 100 mg of lentil sprouts were suspended in 4 ml of 50 mM phosphate buffer, pH 7.6, then stirred for 2 h at 4 °C and centrifuged at 3000*g* for 60 min. α-amylase inhibitor activity (αAI) was measured according to the modified method of Deshpande and Cheryan (1984). Porcine pancreatic α-amylase (220 U ml^{-1})