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Efeito das condições de maceração da soja no teor de açúcares e na atividade de α-galactosidases

Effect of soybean soaking conditions on sugars content and α-galactosidases activity

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Resumo: Os efeitos do tempo e da temperatura de maceração da soja foram avaliados sobre as alterações no conteúdo de açúcares e atividade de α-galactosidase. A maceração da soja foi realizada a uma proporção de 1:1,5 (g:g, soja: água deionizada) a 25, 40, 55 ou 70 °C por 0, 1, 2, 3, 4, 5, 6 e 7 h, e o conteúdo e perfil de açúcares e a atividade de α-galactosidase foram determinadas por cromatografia de troca iônica de alta eficiência (HPAEC) e espectrofotometria, respectivamente. A soja macerada a 25 ou 40 °C não apresentou alterações significativas nos conteúdos de rafinose e estaquiose. A maceração da soja por 3 h a 25, 40, 55 ou 70 °C influenciou a atividade de α-galactosidases, que hidrolisou os oligossacarídeos dos grãos de soja macerados a 55 ou 70 °C. A maceração da soja a 55 °C por mais de 3 h apresentou reduções médias de 45% e 25% nos conteúdos de rafinose e estaquiose, respectivamente. Contudo, a soja macerada às temperaturas de 25, 40, 55 ou 70 °C não apresentou alterações significativas ($p > 0.05$) no conteúdo de sacarose. Portanto, a soja macerada a 40 ou 55 °C pode ser usada para promover a hidrólise parcial de rafinose e estaquiose em galactose e sacarose. Portanto, a maceração nessas condições pode ser utilizada para o desenvolvimento de alimentos à base de soja com níveis reduzidos de oligossacarídeos. Essa estratégia pode aumentar a digestibilidade desses produtos, sem erradicar completamente os benefícios prebióticos associados aos oligossacarídeos remanescentes.

Palavras-chave: Rafinose. Estaquiose. Hidrólise. Hidratação. Tratamento Hidrotérmico.

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Abstract: The effects of the time and temperature of soybean soaking on the changes in sugar content and α-galactosidases activity were evaluated. Soybean soaking was performed at a 1:1.5 (g:g, soybean: deionised water) ratio at 25, 40, 55 or 70 ºC for 0, 1, 2, 3, 4, 5, 6 and 7 h, and the content and profile of sugars and α-galactosidases activity were determined by high performance anion exchange chromatography (HPAEC) and spectrophotometry, respectively. Soybean soaked at 25 or 40 °C did not show significant changes in raffinose and stachyose contents. Soybean soaking for 3 h at 25, 40, 55 or 70 ° C influenced the activity of α-galactosidases, which hydrolysed the oligosaccharides of soaked soybeans at 55 or 70 °C. Soybean soaking at 55 °C for longer than 3 h showed mean reductions of 45% and 25% in raffinose and stachyose contents, respectively. However, soybean soaking at temperatures of 25, 40, 55 or 70 ºC showed no significant changes ($p > 0.05$) in the sucrose content. Therefore, soybean soaking at 40 or 55 ºC can be used to promote the partial hydrolysis of raffinose and stachyose to galactose and sucrose. Therefore, maceration under these conditions can be used for the development of soy-based foods with reduced levels of oligosaccharides. This strategy may enhance the digestibility of these products, without completely eradicating the prebiotic benefits associated with the remaining oligosaccharides.

Keywords: Raffinose. Stachyose. Hydrolysis. Hydration. Hydrothermal treatment.

1 INTRODUCTION

The soybean [*Glycine max* (L.) Merrill] is a legume widely investigated because of its bioactive compounds, particularly isoflavones and oligosaccharides, which are associated with beneficial effects on human health (Chen et al., 2012; Zhang et al., 2015).

Sucrose and the oligosaccharides raffinose and stachyose are the major soluble sugars found in soybeans. These two oligosaccharides contain one and two molecules of galactose bound to sucrose by means of α-(1→ 6) linkages, respectively, and, they are not digested in the gastrointestinal tract, causing abdominal discomfort and flatulence due to the formation of gases during their fermentation in the colon (Suarez et al., 1999). However, these oligosaccharides have been considered as prebiotics because they selectively stimulate the growth of and/or activity of microorganisms that promote health benefits to the host (Chen et al., 2012; Gibson & Roberfroid, 1995; Li et al., 2013; Saad et al., 2013). Fei, Ling, Hua and Ren (2014) reported that soy oligosaccharides were able to reduce oxidative stress and attenuate insulin resistance in patients, indicating that these sugars may play an important role in the management of complications of gestational diabetes mellitus. Moreover, an intake of 0.83 g of α-galactooligosaccharides per kg of body weight was shown to have a relevant prebiotic effect in mice (Li et al., 2013).

Soybean soaking is a pre-processing operation used for the preparation of various products, such as soymilk, tofu, tempeh and others. The time and temperature are important factors that should be monitored in this process because they influence the grinding and cooking of the grains, the leaching of some substances into the soaking medium and the activation of endogenous enzymes of the soybean (Fabbri & Crosby, 2016; Lima et al., 2014; Pan & Tangratanavalee, 2003).

The α-galactosidase enzymes convert the oligosaccharides raffinose and stachyose to galactose and sucrose by hydrolyzing α-(1→6) linkages between the galactosyl residue and the C-6 of the glucose moiety of sucrose (Viana et al., 2005). However, the relationship between changes in the oligosaccharide content and activity of the α-galactosidases during soybean soaking at different temperatures has not yet been investigated. Soybean seeds with a high sucrose content are desired because sucrose is a sweetness-imparting component and thus helps in the wider acceptance of soy-derived food products (Kumar et al., 2010).

The impact of the processing of soy products on the oligosaccharide contents have been described by Egounlety and Aworh (2003) and Mulimani et al. (1997). However, to define the best conditions of this step for the hydrolysis and partial leaching of raffinose and stachyose, the conditions should be investigated based on joint changes in the profiles of these compounds throughout the soybean soaking as a function of the temperature and time. Thus, the hydrothermal treatment of soybeans can serve as an effective pre-treatment for producing soy-based foods with reduced levels of oligosaccharides. This strategy can improve the digestibility of these products while maintaining the prebiotic contribution of the remaining oligosaccharides.

Therefore, the objective of this study was to investigate the effects of the time and temperature of soybean soaking on the changes in the contents of sugars and the activity of α-galactosidases.

2 MATERIALS AND METHODS

2.1 Sample and reagents

Soybeans [*Glycine max* (L.) Merril], lipoxygenase-null cultivar BRS 257 (Empresa Brasileira de Pesquisa Agropecuária, Londrina/Paraná, Brazil), were used in this study.

Calibration curves for the sugars were constructed from standard solutions of glucose, fructose, galactose, sucrose, raffinose and stachyose (Sigma-Aldrich Co., St. Louis, MO, USA). The substrate p-nitrophenyl-α-D-galactopyranoside (Sigma-Aldrich Co., St. Louis, MO, USA) was used to determine the α-galactosidase activity (EC 3.2.1.21) and p-nitrophenol (Sigma-Aldrich Co., St. Louis, MO, USA) was used to construct the calibration curve. All of the reagents used in the analyses were of analytical grade or liquid chromatography grade.

2.2 Effects of the time and temperature of the soybean soaking on the sugar contents

Soybean soaking was investigated to evaluate the effects of time (0, 1, 2, 3, 4, 5, 6 and 7 h) and temperature (25, 40, 55 and 70 ºC) on the contents and profiles of different sugars as well as the activity of α-galactosidases. Soybean soaking was performed as described by Lima et al. (2014). In this step, 50 g of soybean grains in a 1:1.5 (g:g, soybean:deionised water) ratio was used for each soaking condition. Afterwards, the soaked soybeans were drained, frozen, lyophilised (Christ Alpha 2-4 LD plus, Osterode am Harz, Germany) and ground (Ika A11 basic, St. Louis, MO, USA). These soaked soybean flours were stored at -22 °C until use for the determination of different sugars and α-galactosidase activity. The residual soaking solution was discarded.

2.3 Determination of the sugars by HPAEC-PAD

Prior to the extraction of the sugars from the raw soybeans and soaked soybean flours, these sample were defatted as described by Lima et al. (2014). Approximately 0.2 g of the defatted sample was transferred into a 50 mL flask, and 8 mL of ethanol solution (80 mL: 20 mL, absolute ethanol:ultra-pure water) was added, followed by continuous stirring (orbital shaker, 305 rpm) for 1 h at 25 °C. The mixture was centrifuged at 2070 × *g* for 15 min at 25 °C (Centrifuge 5804R-Eppendorf, Hamburg, Germany), and 0.5 mL of the extract was transferred into a microcentrifuge tube and submitted to centrifugal vacuum concentration at 927 × *g* at 30 °C (Jouan®, model RC 10.22, Jouan, Inc., Winchester, VA, USA) until evaporation of the solvent. The concentrated material was

solubilised in 10 mL of ultra-pure water and filtered (Millex-GV, PVDF hydrophilic membrane, 0.22 µm, Millipore, Billerica, MA, USA) prior to injection into the high-performance anion exchange chromatography (HPAEC) instrument. Aliquots of 10 µL of filtered extract were automatically injected into an ICS 5000 (Dionex Canada Ltd., Oakville, Canada) chromatograph equipped with a CarboPac® PA1 column (250 mm × 4 mm, 10 µm; Dionex/Thermo Fisher Scientific), preceded by a CarboPac® PA1 guard column (50 mm × 4 mm, 10 µm), and a pulsed amperometric detector (PAD; Dionex/Thermo Fisher Scientific). Sugars were separated using 20 mmol of NaOH/L of ultra-pure water, which was comprised of 90% solvent A (ultra-pure water) and 10% solvent B (200 mmol of NaOH/L of ultra-pure water) with isocratic elution for 52 min at 1 mL/min at 25 °C. At the end chromatographic run, a column washing step was performed with 200 mmol of NaOH/L of ultra-pure water for 10 min at 25 ºC followed by column stabilisation with 20 mmol of NaOH/L of ultra-pure water for 15 min. For the detection of sugars, a working gold electrode connected to a pH-Ag/AgCl reference electrode (Dionex/Thermo Scientific) was used to promote the oxidation of the sugars by means of a waveform (E = potential, t = duration): E_1 = +0.1 V, t_1 = 400 ms; E_2 = -2.0 V, t_2 = 20 ms; E_3 = +0.6, t_3 = 10 ms; and E_4 = -0.10, t_4 = 70 ms. For the quantification of individual sugars, external calibration curves were constructed from standard aqueous solutions with ultra-pure water using 0.5-25 µg of galactose/mL, 0.5-25 µg of glucose/mL, 0.5-30 µg of fructose/mL, 0.5-60 µg of sucrose/mL, 0.5-60 µg of raffinose/mL and 0.5-60 µg of stachyose/mL. Chromeleon software 6.8 (Dionex/Thermo Scientific) was used for data acquisition. The sugar content was expressed as g of an individual sugar per 100 g of raw soybeans or soaked soybean flour on a dry and full fat basis.

2.4 Determination of the α-galactosidase activity

The α-galactosidases enzymes were extracted from 0.2 g of soybeans and soaked soybean flour with 6 mL of 0.05 mol/L citrate buffer solution (pH = 4.5) containing 0.1 mol of NaCl/L. This mixture was vortexed at 15 min intervals for 1 h at 25 °C and centrifuged at 8200 × *g* for 15 min at 4 ºC (Centrifuge 5804R-Eppendorf, Hamburg, Germany). These extracts were diluted 20- to 40-fold with the aforementioned buffer. Test tubes containing

0.8 mL of 1 mmol of p-nitrophenyl-α-D-galactopyranoside substrate/L in 0.1 mol/L acetate buffer (pH = 4.7) were pre-incubated for 10 min at 30 °C. Aliquots of 0.2 mL of the enzyme extracts were added to these test tubes, vortexed and kept in water bath for 30 min at 30 °C. The reaction was quenched with 1 mL of 0.5 mol of Na2CO3/L, and the absorbances were measured in a spectrophotometer (Biochrom Libra S22, Cambridge, England) at 400 nm. The quantity of *p*-nitrophenol (*p*-NP) released by the enzymatic reaction was determined from the calibration curve plotted for the *p*-NP solutions from 0.04 to 0.32 µmol in a total volume of 2 mL of the reaction mixture. One activity unit (AU) was defined as the quantity of enzyme necessary to release 1 µmol of *p*-NP per min under the assay conditions. The activity of α-galactosidases was expressed as AU per g of raw soybeans or soaked soybean flour on a dry and full fat basis.

2.5 Statistical analysis

For evaluation of the effects of the time and temperature of the soybean soaking ($n = 2$) on the oligosaccharide contents ($n = 4$) and the activity (n = 4) of α-galactosidases, the results were subjected to analysis of variance (ANOVA) followed by least-square regression analysis and mathematical models with the results properly adjusted to the models. Relative minima and maxima of second order polynomial models were estimated from the first derivative test. Results not adjusted to the mathematical models were subjected to one-way analysis of variance followed by Tukey's multiple comparisons test ($\alpha = 0.05$). All data were treated using the Statistica 10.0 software (StatSoft, Tulsa, OK, USA).

3 RESULTS AND DISCUSSION

3.1 Profile of sugars in raw soybeans

Soybean BRS 257 presents, on a dry basis, 5.22 g of total sugars/100 g of sample comprised of 10.34% raffinose, 30.27% stachyose and 59.39% sucrose. It is noteworthy that the sugars galactose, glucose and fructose were not detected. Moreover, it exhibited an α-galactosidase activity of 1.36 AU/g dry sample. Sucrose and stachyose are soluble sugars predominant in mature seeds of conventional or food-type soybeans and represent

58-60% and 31-36% of the total soluble carbohydrate content, respectively (Fan et al., 2015; Oliveira et al., 2010). The different contents of sucrose (1.70-4.07 g/100 g), raffinose (0.33-1.28 g/100 g) and stachyose (1.39-4.73 g/ 100 g) were described in cultivated soybeans in India by Kumar et al. (2010), which described that the contents of these sugars are influenced by genotypes and the planting location. The profiles and contents of soybean sugars can also be affected by other factors, such as the cultivar, maturity group, crop year, growing region, climate, storage conditions and others (Hagely et al., 2013; Saldivar et al., 2011).

3.2 Effects of the soybean soaking time and temperature on the sugar profile and activity of α-galactosidases

Soybeans soaked at 25 or 40 °C did not show significant changes ($p > 0.05$) in their raffinose and stachyose contents (**Fig. 1** and **Fig. 2**).

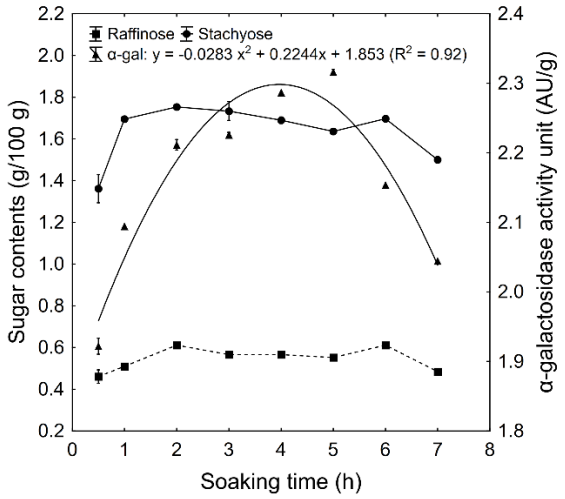

Fig. 1 - Changes in the contents (n = 4) of oligosaccharides and activity (n = 4) of α-galactosidases throughout the soybean soaking (n = 2) period at 25 °C. The solid lines without regression lines are provided as a visual guide only.

In contrast, the activity of α-galactosidases was adjusted to the second order polynomial model (R^2 = 0.92 for 25 ºC and R^2 = 0.81 for 40 ºC, $p < 0.05$) and had a significant increase for up to 5 h of soaking, followed by a reduction for up to 7 h of soaking (**Fig. 1** and **Fig. 2**).

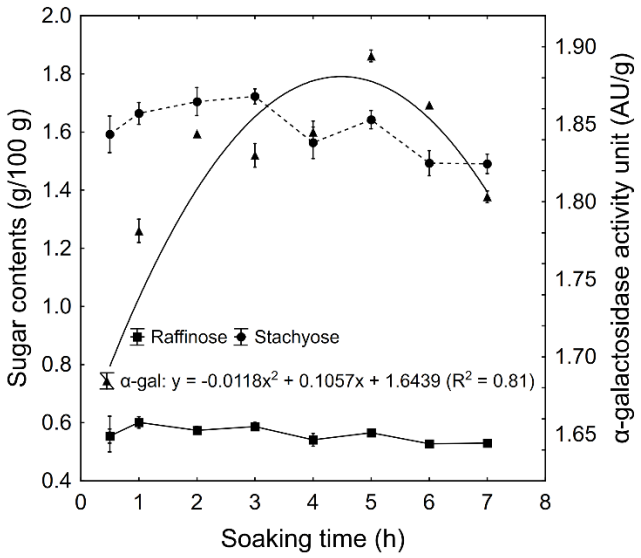

Fig. 2 - Changes in the contents (n = 4) of oligosaccharides and activity (n = 4) of α-galactosidases throughout the soybean soaking (n = 2) period at 40 °C. The solid lines without regression lines are provided as a visual guide only.

It is noteworthy that in this study, the soybean:deionised water ratio (1:1.5, g:g) in the soaking was low, and therefore, there was low leaching. However, Mulimani et al. (1997) reported that when the soybeans were soaked for 16 h at 25 ºC in a 1:10 (g:g, soybean:distilled water) ratio, mean decreases of 44.8% for stachyose and 80.3% for raffinose in relation to the raw soybeans occurred. Soaking the beans for 12–14 h reduced the oligosaccharide and sucrose contents by 20–35% (Egounlety & Aworh, 2003). In the soybean soaking process, the diffusion of water and their compounds occurs, which are dependent mainly on the temperature and concentration gradient between the soaked soybeans and the soaking medium (Lima et al., 2014). The activity of α-galactosidases (**Fig. 1** and **Fig. 2**) of soybeans soaked at 25 or 40 ºC increased possibly due to the efficiency of the extraction resulting from the increase of the permeability of the grain tissue during soaking.

Regression analysis for the soaking time of the soybeans at 55 °C indicated a model with quadratic effects on changes in raffinose ($R^2 = 0.81$, $p < 0.05$) and galactose ($R^2 = 0.98$, $p < 0.05$), whose data were adequately fitted to the proposed model. However, the stachyose content of the soybeans soaked at 55 ºC for 7 h was not significantly altered

(R^2 = 0.63, p > 0.05) (**Fig. 3**) by the soaking time, and consequently, only 63% of the data were fitted to the proposed model.

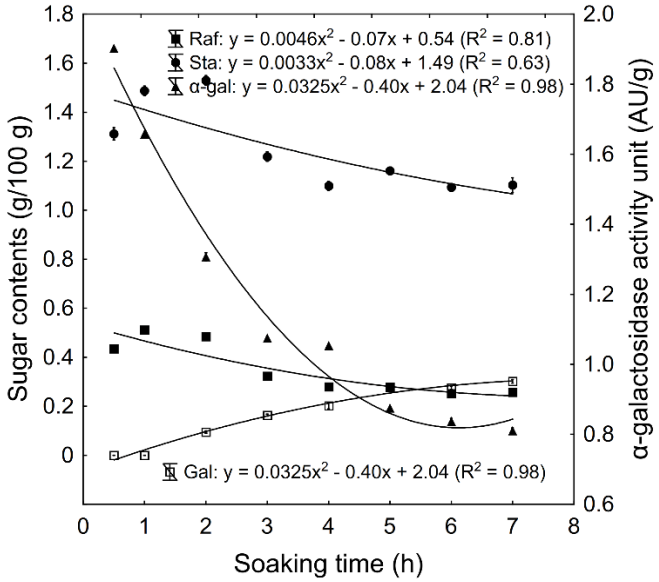

Fig. 3 - Changes in the contents (n = 4) of oligosaccharides and galactose as well as the activity (n = 4) of α-galactosidases throughout the soybean soaking (n = 2) period at 55 °C. Raf = raffinose, Sta = stachyose and Gal = galactose. The solid lines without regression lines are provided as a visual guide only.

The changes in raffinose, galactose and stachyose contents can be explained by the higher specificity of α-galactosidases toward raffinose than stachyose (Gao & Schaffer, 1999). Since the stachyose hydrolysis yields raffinose, endogenous soybean α-galactosidases simultaneously hydrolyse these two substrates. According to Porter et al. (1990), stachyose hydrolysis gives a nearly constant level of raffinose shortly after hydrolysis begins. Soybeans soaked at 55 °C after 3 h showed mean reductions of 45% for raffinose and 25% for stachyose (**Fig. 3**). However, at 55 °C and for up to 7 h of soybean soaking (**Fig. 3**), the activity of α-galactosidases decreased, and in these conditions, the raffinose and stachyose contents decreased simultaneously while the galactose content increased. These results indicate that endogenous α-galactosidases hydrolysed these oligosaccharides since these compounds contain one and two molecules of galactose bound to sucrose by means of α-(1→6) linkages. The soybean soaking possibly activated the activity of endogenous α-galactosidases hydrolysing the oligosaccharides to galactose and sucrose, which according to Herman and Shannon (1985) are used as energy sources in the germination process. According to Viana et al. (2005), the optimum temperature

for the activity of α-galactosidases is 50 °C. However, the reduction in the activity of α-galactosidases (**Fig. 3**) can be attributed mainly to inhibition by the reaction products galactose and sucrose (Porter et al., 1990) or the loss of their stability, as reported by Lima et al. (2014) for β-glucosidases from soybeans soaked at 55 ºC.

In the soybeans soaked at 70 ºC, the raffinose and stachyose contents did not significantly vary ($p > 0.05$) throughout soaking, and therefore, the data of both sugars did not fit properly to the proposed models (**Fig. 4**).

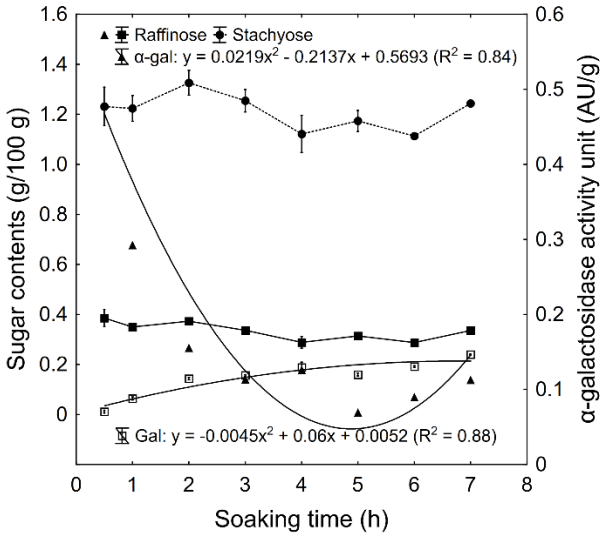

Fig. 4 - Changes in the contents (n = 4) of oligosaccharides and galactose as well as the activity (n = 4) of α-galactosidases throughout the soybean soaking (n = 2) period at 70 °C. The solid lines without regression lines are provided as a visual guide only.

In relation to the changes in the galactose content of soybeans soaked at 70 ºC, it was observed that the model (**Fig. 4**) quadratic effect and good fit ($R^2 = 0.88$, $p < 0.05$) to the experimental data. It is noteworthy that the maximum content of galactose (0.21 g/100 g of dry soybean) was estimated at 6.8 h for the first derivative of the proposed model. This increase in galactose content during the soybean soaking at 70 ºC was possibly due to activity of α-galactosidases at the start of soaking and the improved permeability of the soaked grains, which favoured the subsequent extraction of galactose. In relation to the activity of α-galactosidases of soybeans soaked at 70 ºC (**Fig. 4**), it was observed that the model showed a quadratic effect for the soaking time of the soybeans with a good fit ($R^2 = 0.85$, $p < 0.05$) to the experimental data. It is verified that the minimum activity of α-galactosidases (0.05 AU/g) was estimated at 4.88 h from the first derivative of the

proposed model. This model indicated that the activity of α-galactosidases was highest at the start of soybean soaking at 70 ºC with a significant reduction until 5 h. Porter et al. (1992) described that the activity of α-galactosidases purified from *Glycine max* showed a substantial loss starting at 60 ºC. Additionally, Oliveira et al. (2005) also observed a marked reduction in α-galactosidase activity in *Platymiscium pubescens* Micheli seeds germinated at temperatures from 60 to 70 °C, with enzyme activity being null at 70°C, demonstrating its inactivation. Therefore, to promote a significant hydrolysis of raffinose and stachyose during soybean soaking, temperatures of up to 55 °C should be chosen.

Soybeans soaked at 25, 40, 55 or 70 ºC did not show significant changes ($p > 0.05$) in their sucrose content (**Fig. 5**).

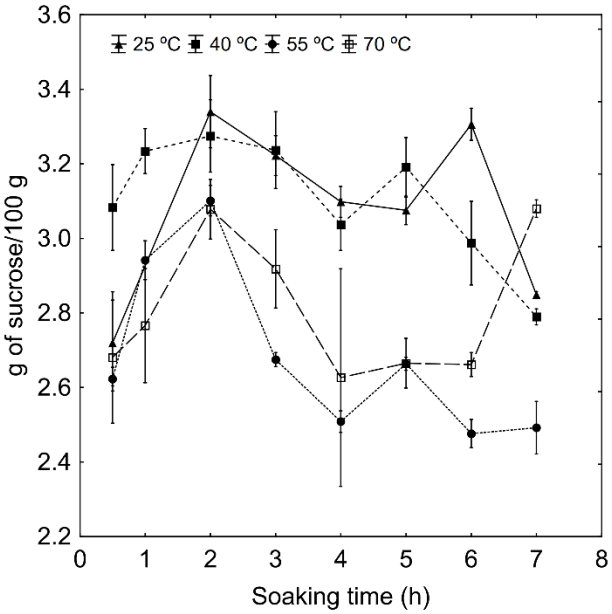

Fig. 5 - Changes in sucrose content (n = 4) throughout the soybean soaking (n = 2) period at 25, 40, 55 and 70 °C. The solid lines without regression lines are provided as a visual guide only.

Although partial leaching of sucrose has occurred throughout the soybean soaking, its content was close to that of the unsoaked soybeans, possibly due to the hydrolysis of raffinose and stachyose to sucrose. Moreover, the higher permeability of the soaked grains should have improved the subsequent extraction of sucrose. Soybeans with a high sucrose content are desired because it is a sweetness-imparting component and thus helps in wider acceptance of soy-derived food products (Kumar et al., 2010).

4 CONCLUSION

Soybean soaking at 25 or 40 °C did not show significant changes ($p > 0.05$) in the raffinose and stachyose contents. However, the α-galactosidases activity in soybeans soaked at 25, 40, 55 or 70 ºC was influenced by the soaking time. These enzymes catalysed the hydrolysis of raffinose and stachyose oligosaccharides to galactose in soybeans soaked at 55 or 70 °C for 3 h. After 3 h of soybean soaking at 55 °C, there were reductions of 45% for raffinose and 25% for stachyose. However, at temperatures of 25, 40, 55 or 70 ºC, there were no significant ($p > 0.05$) changes of the sucrose content. Therefore, soybean soaking at 40 or 55 ºC can be used to promote the partial hydrolysis of raffinose and stachyose to galactose and sucrose.

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