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The germination of soybeans increases the water-soluble components and could generate innovations in soy-based foods

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ABSTRACT

Changes in the aqueous solubility of proteins, amino acids, sugars, and other bioactive compounds of the soybean (BRS 257) were studied after different germination times (0, 8, 32, 56, 80, 104 and 176 h). The germination process clearly changed the solubilization of the components, and even antioxidant activities, in aqueous extracts. Significantly ($p < 0.05$) the highest protein extraction yield occurred after 80 h of germination. Except for time zero, most of the protein has already been extracted in the first of the 3 extraction cycles performed. A variation in the concentration of amino acid residues was observed throughout germination. A significant increase in digestibility occurred initially at 32 h reaching its maximum with 176 h. After 32 h there was a significant increase in sugar solubility, and the total phenolic and flavonoids concentrations it was 4 and 2.5 times higher than the nongerminated grain extract, respectively. The maximum antioxidant capacity was also reached with 32 h of germination. In general, the germination time of 32 h presented outstanding results but the first changes were already detected after the first 8 h. The data demonstrate the usefulness of germination for innovations in soy-based food production with better nutritional and nutraceutical values.

1. Introduction

Newer food market segments offered a number of products considered healthier than traditional foods with specific health claims (Marrubini, Papetti, Genorini, & Ulrici, 2017; Sethi, Tyagi, & Anurag, 2016). Among these foods, plant-based milks have been highlighted, represented by beverages obtained mainly through processes of aqueous extraction from nuts, cereals, pseudocereals, vegetables or seeds (Sethi et al., 2016). The preparation of these products generally includes "disintegration" of the plant material in the presence of water as the solvent as the main step. Thus, although particles of varying sizes may be present, depending on the grain and grinding/separation process, the presence of water-soluble components ultimately gives the product its main characteristics (Sethi et al., 2016). The original solubility or processes that alter the solubilization of plant material components lead to differences in the sensorial, nutritional, bio- and technofunctional characteristics of the final product. Processes that increase the solubility of the proteins, phenolic compounds or other components in water can provide an aqueous extract with better protein extraction yields and differentiated nutritional and nutraceutical potentials (Sethi et al., 2016).

In this sense, the process of grain germination can be considered to improve the profile of the plant material destined for the production of new foods, especially vegetable aqueous extracts (Oyedeji, Mellem, & Ijabadeniyi, 2018). This improvement occurs because the germination process can generate changes in the profiles of the sprout components that include the mobilization of protein or carbohydrate materials and an intensification of metabolic activities leading to the release and/or production of many soluble compounds. As highlighted by Gan et al. (2017), the germination process can be seen as a potential molecular mechanism of accumulating bioactive compounds in edible seeds, sprouts and in their derivatives. This composition change contributes to

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the process of technological innovation and expanding the options to consumer preferences. In addition, it is important to note that during germination, minerals are released from phytates and also proteins and carbohydrates can be hydrolysed, releasing amino acids and glucose, which could increase their bioavailability (Lemmens et al., 2018; López-Martínez, Leyva-López, Gutiérrez-Grijalva, & Heredia, 2017; Portari, Tavano, Silva, & Neves, 2005).

Generally, the germination time used ranges between 3 and 5 days (Lemmens et al., 2018). The use of short germination times can facilitate the application of the process, making it more economical and relatively secure to the risk of contamination during incubation. The terms "germinated" or "sprouted" by itself are flawed and confusing. As highlighted by Lemmens et al. (2018), a "sprouted grain" usually designates a seed with a visible radicle, but physiological changes of the grains begin in the first hours of hydration (Kim, Choi, Ryu, Lee, & Kwon, 2011). Here, the term "germinated grain" will be used to refer the seeds from the end of the soaking until 176 h of the process.

The soybean BRS 257 used in this study (developed by EMBRAPA - Brazilian Agricultural Research Corporation) is a non-transgenic cultivar and particularly interesting because it has no lipoxygenases, dispensing heat treatments to inactivate these enzymes and making this cultivar with high technological potential for human development (Rigo, Dahmer, Steffens, Steffens, & Carrão-Panizzi, 2015).

2. Materials and methods

2.1. Materials

Soybean seeds BRS 257 cultivar were purchased from Empresa Sementes e Alimentos Paraná, Mauá da Serra, Paraná, Brazil. Pepsin (from porcine gastric mucosa), pancreatin, o-phthaldialdehyde (OPA), benzoyl-DL-arginine-*p*-nitroanilide (BAPNA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and L-amino acids standards were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA), AccQ.FluorTM pre-column derivatization kit was purchased from Waters (Milford, MA, USA). The reagents were of analytical grade, except HPLC solvent acetonitrile was LC grade. Others chemicals were reagent grade.

2.2. Methods

2.2.1. Germination process

Soybean germination was prepared as reported by Yoshiara et al. (2012), with slight modifications. Briefly, seeds were washed with distilated water, immersed for 1 min in sodium hypochlorite solution at 0.07 g/100 mL and rinsed twice with sterile distilled water. Then, seeds were soaked in sterile distilled water for 8 h at room temperature. After soaking, 50 drained seeds were placed on each one of sheets of germitest paper which were rolled and maintaining in a B.O.D chamber (Biochemical Oxygen Demand incubator - SOLAB SL225, Campinas, São Paulo, Brazil) at 21 °C and 12 h light/dark cycle. Germination process was carried out for 176 hous. Samples of germinated grains were collected each 24-h. Only distilled water was sprayed daily during germination period. The freshly harvested soybean sprout was immediately weighed and freezed at −18 °C till use.

2.2.2. Moisture and total solids

Moisture content was estimated gravimetrically after drying at 105 °C until constant weight. Residue was considered total solid and loss weight as moisture.

2.2.3. Extracts preparations

Aqueous extracts were prepared grinding 1 g of seeds with 20 mL of distilled water for 20 s, using Ultraturrax (HomoMix D-500, Biosystems, Curitiba, Paraná, Brazil). The samples were centrifuged at 7000 *g* for 15 min/5 °C using a microprocessed refrigered centrifuge (FANEN Excelsa 4 – MOD 280R – São Paulo - Brazil). Extraction was repeated twice using 10 mL of distilled water and supernatants were pooled.

2.2.4. Nitrogen/protein determination

Nitrogen was determined according to the Kjeldahl method (AOAC, 1995). Crude protein was calculated as nitrogen × 5.71.

2.2.5. Alpha-amino groups determination

Alpha-amino groups were determined spectrophotometrically (UV-VIS spectrophotometer BEL photonic UV-M51 - made in PRC), using OPA (o-phthaldialdehyde) reagent, as describe by Church, Swaisgood, Porter, and Catignani (1983). An analytical reference curve was constructed using L-Leucine as standard.

2.2.6. Free amino acids determination

Aqueous soybean extract were mixed with AccQ.Fluor® borate buffer, allowed to rest for 1 min and then it was heated in a water bath at 55 °C/10 min and filtered using a PTFE 0.22 μm pore size membrane (Minisart SRP 4®, Sartorius, Goettingen, Germany) to be analyze by Waters AcquityTM Ultra Performance LC (UPLC) system (Waters, Milford, MA, USA), as described by Moreira et al. (2017). UPLC system was equipped with an AcquityTM tunable ultraviolet detector for 249 nm detection and C18 column (50 × 2.1 mm i.d., 1.7 μm, Acquity UPLC). Amino acids identification was performed by comparison of the retention time of the peaks of the analytes in the sample with those of the standard solution and calculated by interpolation in the respective analytical curves and the recovery via internal standard.

2.2.7. SDS-PAGE

SDS-PAGE was carried out as described by Laemmli, 1970, using a 12.0 g/100 mL polyacrylamide gel as separating gel and a 4.0 g/100 mL stacking gel. The samples were pre-mixed, at a 1:1 ratio, with sample buffer containing 0.5 mol/L Tris–HCl buffer pH 6.8, 1 g/100 mL bromophenol blue, 10 g/100 mL glycerol, and 2 g/100 mL SDS. The gels were stained with Coomassie brilliant blue G-250 and destained using a methanol-acetic acid solution.

2.2.8. Amylase and protease activity

Amylase activity in the aqueous extracts was determined using starch as substrate (1 g/100 mL in 100 mmol/L of citrate-phosphate buffer at pH 6.0). Starch hydrolysis was monitored at 37 °C by determination of reducing sugar using Miller (1959) dinitrosalicylic acid method at 540 nm. Protease activities were verified using 1 g/100 mL casein as substrate at 37 °C in phosphate buffer pH 8.0. The reaction was stopped by boiling for 10 min and the casein hydrolysis was measured by the increase of free alpha-amino groups released, as described in 2.2.5. section. Additionally, the proteolytic activity was performed using benzoyl-DL-arginine-p-nitroanilide (BAPNA) as substrate. Enzyme activities were expressed in katal.

2.2.9. 9. In vitro protein digestibility

In vitro protein digestibility of 50 mg proteins of sample were determined as described by Akeson and Stahman (1964) using a pepsin-pancreatin incubation sequence (0.75 mg pepsin and 2.0 mg pancreatin), at 37 °C, for 3 h and 24 h, respectively. The enzymatic reaction was then interrupted by adding trichloroacetic acid (TCA) until 10 g/100 mL final concentration, followed by centrifugation at 7000*g* for 15 min, or by boiling for 10 min. Hydrolysis degree was determined using OPA reagent, as described in 2.2.5. section. Results were expressed as a perceptual of disrupted peptide bonds, considering that each alpha-amino group released represents one hydrolysed bond. The 100% of hydrolysis degree was estimated by the total protein mass in the sample and the average molecular weight of amino acids (MW = 113), whereas each amino acid contains an alpha-amino group and each one represents a potential bond to be disrupted.

2.2.10. Trypsin inhibitors

Trypsin inhibitors were measured as described by Kakade, Rackis, Mcghee, and Puski (1974), using BAPNA as substrate. Results are expressed as the number of trypsin units inhibited (TIU).

2.2.11. Reducing sugar and free glucose concentration

Reducing sugar was determined as described in 2.2.8. section. Free glucose was determined using a colorimetric peroxidase-glucose oxidase kit (Glucose Monoreagent- BioClin- K082). Glucose curve references were used for both assays.

2.2.12. Total phenolic and flavonoid content

Total phenolic was determined spectrophotometrically (UV-VIS spectrophotometer BEL photonic UV-M51 - made in PRC), using the Folin-Ciocalteu reagent, according to the Singleton and Rossi (1965) and some modifications as described by Boateng, Verghese, Walker, and Ogutu (2008). A standard curve of gallic acid was constructed (0–16 μg/mL), and the results expressed as gallic acid equivalent per gram of samples. Flavonoid contents were determined using aluminum chloride spectrophotometric assay as described by Boateng et al. (2008). A catechin curve was used as standard (0–50 μg/mL) and the results expressed as mg catechin equivalent per gram of samples.

2.2.13. Potential antioxidant activity

$ABTS^{+}$ radical was generated by incubation of 7 mmol/L ABTS with 2.4 mmol/L potassium persulfate for 16 h in the dark, as described by Shalaby and Shanab (2013). The ABTS solution was diluted with water until an absorbance of 0.700 at 734 nm. To 250 μl of sample was added 750 μl of ABTS solution. Absorbance was read after 60 min in the dark. DPPH Radical-Scavenging activity according to Brand-Williams, Cuvelier, and Berset (1995), with some modifications. Briefly, 0.1 mL of sample extract was added to 3.9 mL of an 80 mL/100 mL ethanol DPPH solution. The absorbance was determined at 517 nm. A standard curve was prepared using TROLOX as reference and results expressed as μmol of TROLOX equivalent/g of sample for both assays.

2.2.14. Statistical analyses

All assays were performed in triplicate and are expressed as means ± SD. Analysis of variance (ANOVA) and Tukey test was used to compare results ($p \leq 0.05$). Statistical analysis was conducted using Statsoft STATISTICA 8.0 (2007).

3. Results and discussion

3.1. Nitrogenous compounds solubility changes

As mentioned, the germination process can be considered initiated from the hydration of the grains (Kim et al., 2011). In this work, after 8 h of soaking it was possible to observe the change in grain shape, but the first radicle visualizations occurred after the first 24 h of germination on germitest paper, i.e., after 32 total hours of processing (Fig. 1). Although little visual alterations were noticed in the grains, their metabolic activities already showed alterations soon after the first 8 h of hydration. In Fig. 2 A, it is observed that the content of water-soluble nitrogen already increased after soaking, rising significantly from 19.3 to 22.2 mg of nitrogen extracted per gram of solids of the grains. Fig. 2A also shows the results expressed by grams of grain (wet basis), which

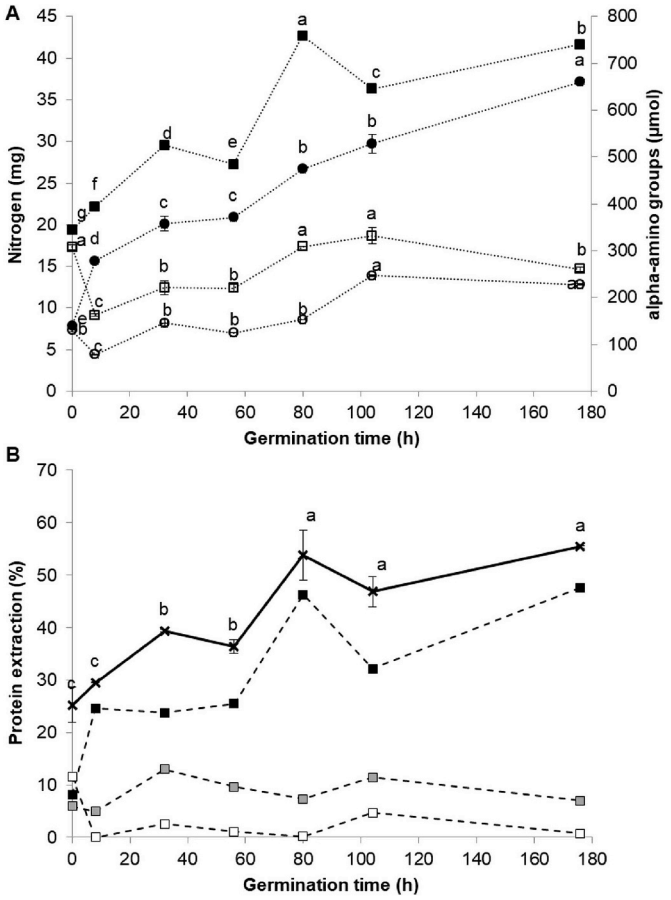

Fig. 2. Changes in aqueous solubility of nitrogenous materials during the germination of soybeans (BRS 257) at different times (0 h, 8 h, 32 h, 56 h, 80 h, 104 h and 176 h) considering (A) mg of water soluble nitrogen (squares) and μmol of free α-amino groups (circles) per 1 g of seeds in wet basis (open symbol) or in dry basis (closed symbol). The total protein extraction yield (-x-) was expressed in B, also considering the results in each extraction cycle: 1^{a} extraction (■), 2^{a} re-extraction (□) and 3^{a} re-extraction (□). All results are expressed as mean of three determinations. Different lowercase letters in results of the same sample express significant differences ($p < 0.05$) and error bars express their standard deviation.

Table 1
Moisture of soybean (BRS 257) at different germination times.

Germination time (hours)	Moisture (g/100 g)	Total solids (g/100 g)
0	$10.4^{e} \pm 0.4$	89.6
8	$58.8^{bc} \pm 1.1$	41.2
32	$57.9^{bc} \pm 0.6$	42.1
56	$54.5^{c} \pm 0.7$	45.5
80	$59.3^{bc} \pm 0.6$	40.7
104	$48.5^{d} \pm 0.6$	51.5
176	$64.7^{ab} \pm 1.5$	35.3

Results are expressed as mean ± standard deviation of three determinations. Different superscripts letters in results express significant differences ($p < 0.05$).

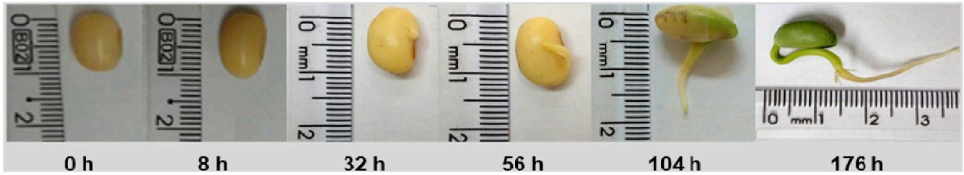

Fig. 1. Soybean (BRS 257) grains development during germination process.

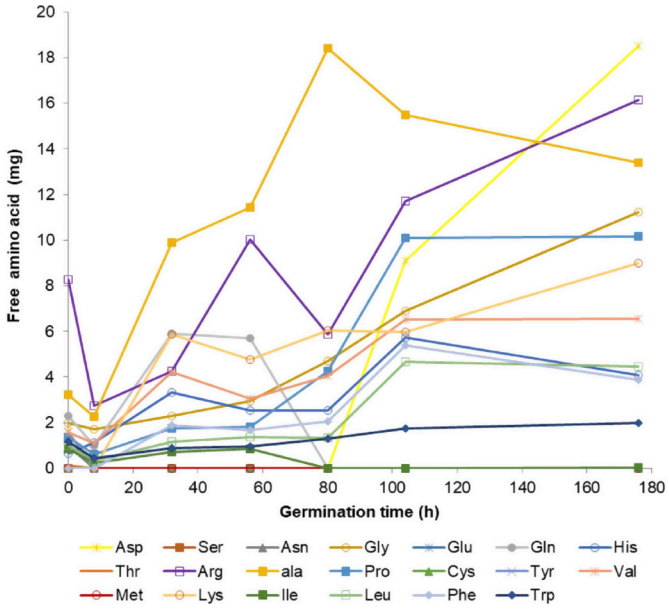

Fig. 3. Free amino acids in aqueous extract from germinated soybean (BRS 257) at different times (0 h, 8 h, 32 h, 56 h, 80 h, 104 h and 176 h). Results are expressed as total mg of each amino acid solubilized in water per 1 g of wet basis grains.

could indicate at first sight a reduction in these contents; however, in fact, these data would be reflecting a higher moisture content was incorporated by the grains, as observed by the data of Table 1. The pronounced increase in the moisture content by approximately six times in relation to the grains at the zero germination time was verified. Considering this fact, to avoid misinterpretation by the dilution of the components, the majority of the data are expressed as a dry basis.

The variation in nitrogen solubility is probably due to the mobilization of protein material for the synthesis of enzymes and new tissues. It was reported that 18 h of germination it is sufficient to soy storage protein hydrolysis and consequent liberation of amino acids and peptides with biofunctional characteristics (Huang, Cai, & Xu, 2017; González-Montoya, Hernández-Ledesma, Silván, Mora-Escobedo, & Martínez-Villaluenga, 2018; Oyedeji et al., 2018). This intense metabolic activity alters the protein profile of the material, increasing the protein solubility, which is one of the main factors that confer stability to plant-based-milks (Sethi et al., 2016). The detection of amino acid residues in the aqueous extracts confirms these changes, with a frequent variation in these contents (Fig. 3). This process indicates the constant amino acid release and the new protein synthesis which can promote increase in protein digestibility (Kim et al., 2011). These natural hydrolytic processes make this material more promising in terms of protein yield for the final product and can improve the nutritional value. The synthesis of the new proteins can be results of the changes in the enzyme activities detected (Fig. 4 A), suggesting protease synthesis as indicated by both activity increase by trypsin type assay and general proteolytic assay, detected by BAPNA or casein substrate, respectively.

Although intense metabolic activity was detected in the germinated grains, the electrophoretic profiles did not change before 80 h of germination (Fig. 5). At this point, bands of high molecular weight (in the 200 kDa range) disappeared, followed by the disappearance of a band at approximately 12.4 kDa at 104 h. These facts are consistent with the intense hydrolytic activity and the production of low molecular weight peptide chains that cannot be detected under the conditions of a 12 g/100 mL acrylamide gel.

Oyedeji et al. (2018) also observed an increased in soluble protein contents and breakdown of soy storage proteins in sprouted soy-based foods.

Another interesting change associated with the germination process is that both the total soluble proteinaceous material tended to increase as well as the ease of its extraction along the germination time (Fig. 2 B). All germinated grains, even those with only 8 h of soaking, had most of the solubilized nitrogen in the first extraction cycle, unlike non-germinated grains. It is worth mentioning that in this work, the objective was not to simulate an industrial production process of soybean milk, which uses proportions between grains and extractor medium different from those used here. Here, the extraction process was planned to solubilize the protein components as much as possible, thus using optimized conditions. Additionally, the centrifugation of the extracts before analysis had the objective of eliminating the interference of the particle suspension in the results, which are constituents commonly found in plant-based milks (Sethi et al., 2016). However, the extraction data obtained by this research may contribute to new product designs and protocols for extracting soy components.

As shown in Fig. 2B, from the material of the first day of germination on germinated paper (32 h of process), a significantly higher protein solubilization was obtained in relation to time zero, representing 35.9% more (total extraction 39.38% against 25.25% respectively). At germination times of 80 and 176 h, a total protein solubilization of 53.8% and 55.4%, respectively, occurred. At both times, approximately 86% of the solubilized proteins were extracted in the first extraction cycle (at time zero, only 32.5% of the total solubilized protein was extracted in the first cycle. Even when only the first extraction cycle is considered, the positive effect of germination on protein extraction capacity in water was notable. This effect was already evident after the processing of the material after soaking (8 h), when about 82% of the soluble protein had already been extracted in the first extraction cycle.

Soybean, as it usually occurs in legumes, presents globulins as its main fraction (Ciabotti et al., 2016) and these proteins are dependent on the presence of salts in solution to present solubility; that is, these proteins would not be normally present in products generated from aqueous extracts. On average, soybean albumins and globulins are expected to account for approximately 20% and between 40 and 60% of the total proteins, respectively (Ciabotti et al., 2016). It is notable that the percentage of protein solubilization achieved, especially considering the last three germination times (Fig. 2B), exceeds what would be due to albumins, a water-soluble fraction, according to the classification proposed by Osborne (1924). This result indicated that other originally water-insoluble protein fractions of the grains, such as globulins, may be becoming soluble through hydrolytic processes. These

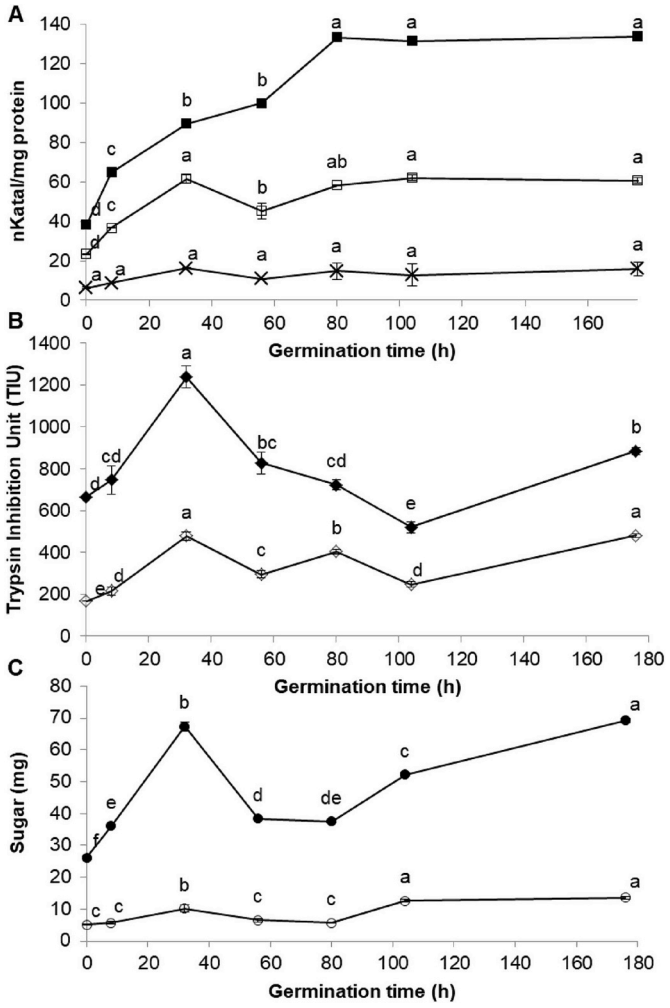

Fig. 4. Effect of germination process of soybean (BRS 257) at different times (0 h, 8 h, 32 h, 56 h, 80 h, 104 h and 176 h) on the (A) amilolytic (-x-) and proteolytic (-□- trypsin-like activity; -■- casein hydrolysis) activities detected in aqueous extracts; in trypsin inhibition activity (B) expressed as TIU/total nitrogen in the grains (-♦-) and TIU/mg of nitrogen solubilized in the aqueous extract (-◊-); and on the solubilized (C) reducing sugar (-●-) and free glucose in the aqueous extract per 1 g of dry basis grains. Results are expressed as mean ± standard deviation (error bars) of three determinations. Different lowercase letters in results for the same parameters express significant differences ($p < 0.05$).

processes that can make soluble not only albumins, but also globulins and other naturally insoluble protein fractions will improve extraction yield in the final product. However, it is also important to consider that, if these fractions originally have different solubilities due to their distinct amino acid profiles and sequences, they may have different nutritional qualities. Thus, the quantitative increase in the final protein content on the product does not necessarily imply a direct qualitative gain, and this aspect must be considered. Factors such as amino acid profile, amino acid balance and protein digestibility should be taken into account (Boye,Wijesinha-Bettoni, & Burlingame, 2012). Following are presented and discussed data on *in vitro* digestibility of protein extracts obtained in this study.

3.2. Protein digestibility of aqueous extracts

For a better discussion of digestibility data the alpha-amino groups already present in the solution and those released after the enzymatic assay are presented separately (Fig. 6). The increase in the degree of hydrolysis of the sample during germination and consequent

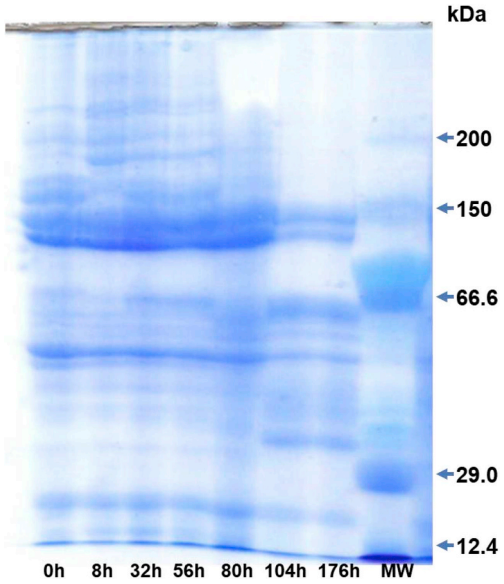

Fig. 5. Protein profiles on SDS-PAGE gel of aqueous extract of soybeans (BRS 257) after different germination times (0 h, 8 h, 32 h, 56 h, 80 h, 104 h and 176 h of germination, MW = molecular weight markers), using a concentration of acrylamide of 12 g/100 mL in the presence of 0.1 g/100 g SDS and protein staining using Coomassie Blue G-250.

production of low molecular weight material (free amino acids or small peptides) should be understood as a contribution to the availability of these proteins for uptake by enterocytes and consequently to increase the protein digestibility. The data in Fig. 6 A indicate that the germination process alone increases protein digestibility from 56 h. This higher presence of prehydrolyzed protein material may mean not only better nutritional value but also nutraceutical improvement since fragments that present biofunctional activities that are beneficial for human health may be present among the released peptides. González-Montoya et al. (2018) demonstrated the potential of germinated soybean proteins as a source of peptides with anticancer and anti-inflammatory activities.

Fig. 6 B, depicts the results after the digestion process had been stopped by boiling, i.e., all fragments resulting from the digestion were maintained in the solution, from amino acid residues to larger peptides. The detection of amino groups by OPA, although considered specific for the alpha-amino group, did not inform what kind of materials this terminal amino group comes from. The amino group may originate from an amino acid residue or a peptide of more than 50 amino acids, but the absorption processes of these materials will be different (Tavano, Neves, & Silva, 2016). Fig. 6 A shows the results after stopping the hydrolysis with the addition of TCA followed by centrifugation. Under these conditions, only small fragments and free amino acids are kept in solution, which would better represent the absorption potential of the digested material. Since the data of Fig. 6 A and 6 B are very similar, it is suggested that the detections come primarily from low molecular weight materials.

According to the data in Fig. 6 A, a significant increase in protein digestibility is observed soon after the first 32 h of germination. Huang et al. (2017) also report the increased detection of free amino acids in germinated grains of black and yellow soybeans, reflecting increased hydrolysis of grain proteins.

Still regarding protein digestibility, although some authors indicate that the germination process may reduce protease inhibitor activity in soybeans (Joshi and Varma (2016), this was not observed by the results of this research (Fig. 4 B). However, this activity was not associated with loss of digestibility (Fig. 6). Maetens et al. (2017) also observed a reduction on trypsin inhibitors content in soybean germinated for 3 or

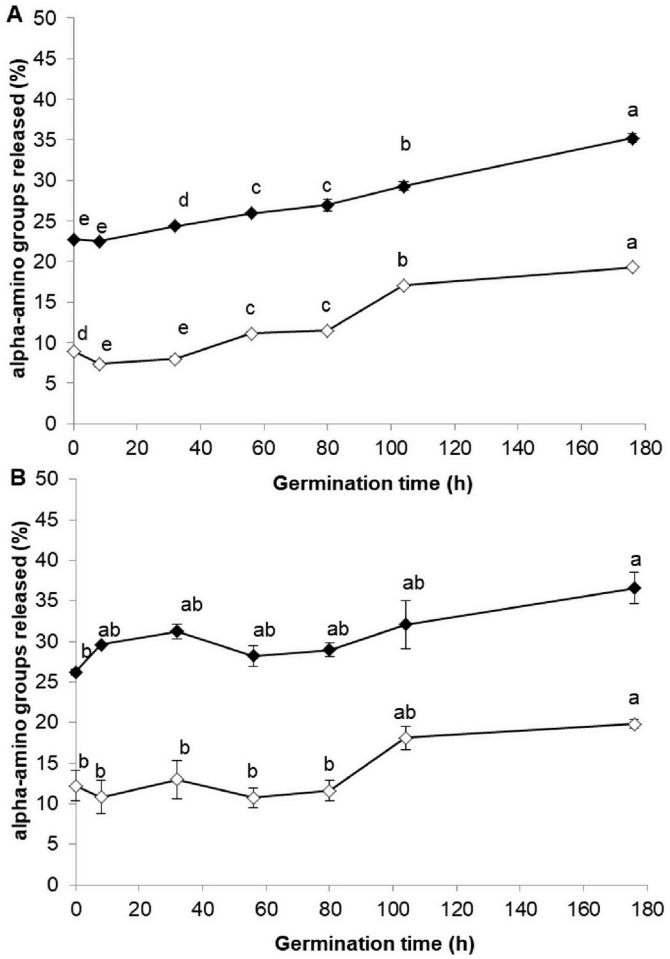

Fig. 6. *In vitro* protein digestibility of aqueous extracts from soybean (BRS 257) germinated at different times (0 h, 8 h, 32 h, 56 h, 80 h, 104 h and 176 h). The results were presented as percentage of alpha-amino groups released before (open symbol) and after (dark symbol) pepsin-pancreatin digestion. Reaction was stopped with trichloroacetic acid addiction and centrifugation (A) and by boiling (B). Results are expressed as mean ± standard deviation (error bar) of three determinations. Different overwritten letters in the same parameters express significant differences ($p < 0.05$).

5- days.

3.3. Carbohydrate and phenolic compounds solubility changes

Considering the possibility of developing soy-based beverages from germinated grains, it is very relevant to increase the solubility of components that increase the nutritional density and biofunctional content of the product. Huang et al. (2017) also detected increased levels of isoflavones, GABA, vitamin B2 and beta-carotene in germinated grains of black and yellow soybeans. In this sense, it is important to note that the germination process also caused changes in the solubilization of carbohydrates and phenolics (Figs. 4 C and 7). In the first germination times, an increase of solubilized sugars was observed in the aqueous extract (Fig. 4 C), coinciding with the increase in amylase activity (Fig. 4 A), indicating intense hydrolysis activity, including release of free glucose. After 8 h germination there was a significant increase in soluble reducing sugar content and after 32 h a significant increase in soluble glucose content compared to time zero. Regarding the phenolics (Fig. 7 A), the data points to a significant increase in the solubilization of compounds at the beginning of the germination process (after 8 h). The extract produced after 32 h of germination presented a total phenolic concentration approximately 4 times greater and an increase of flavonoids approximately 2.5 times greater than the nongerminated grain extract.

In soybean flour or soyfoods, it is possible to find a large variety of antioxidant compounds, such as isoflavones, tocopherols, amino acids and peptides, and other phenolic acids, such as chlorogenic, caffeic and ferulic acids, which may be soluble in water (Lemmens et al., 2018). In the same way as it was observed for nitrogen compounds, the germination process also contributed not only to the increase of their absolute contents but also to the ease of extraction of these components. Fig. 7 B to 7 D show that it was possible to obtain a higher percentage of those phenolics and antioxidant components at the first extraction.

The antioxidant activity detected in material with 32 h of germination was the highest among the extracts tested (Fig. 7 A); however, in general, the germination was favorable to improve the antioxidant capacity. Albeit the extrapolation of this capacity measured *in vitro* to *in vivo* systems can to be questionable, these data are important to compare foods with each other and if considered the gastrointestinal tract some benefits can be supposed (Sies, 2007).

It is important to note that between the germination time of 80 and 106 h the amount of total phenolic and flavonoid concentration decreases drastically while increasing antioxidant capacity. These results indicate the presence of other components with antioxidant capacity besides the phenolic ones, such as peptides without aromatic amino acids, as indicated by the data of Fig. 7 A.

In this work, the DPPH reagent assay was also used, but this assay was much less sensitive and unable to be detected the antioxidant activities in the aqueous extracts (data not shown), although maximum aliquots were used.

It is important to emphasize that the parameters considered here may have other results depending on environmental conditions during germination, such as light, humidity and temperature (Maria John, Natarajan, & Luthria, 2016). Even culture medium variables can be explored, but the trend of the changes exposed here should be expected. In addition, in the development of new soyfoods, especially soymilks, a shelf-life approach should be taken to verify possible alterations related to antioxidant capacity and phenolic content and ensure the stability of the biofunctional activity of the product.

Considering that the germination process requires care and can be laborious, it is important to note that results concerning 8 h of soaking already qualify the material obtained for the production of an aqueous extract with superior nutritional value and biofunctional potential. These benefits go beyond the rationale often used to explain the nutritional improvement obtained with soaking that would be the hydration and softening of the tissues or removing antinutritional compounds by solubilization (Amistá & Tavano, 2013). However, combining interests in the improvements of nutritional and biofunctional qualities with the shortest germination time the time of 32 h or after soaking for one day stand out.

4. Conclusions

The data presented here indicated that the germination process is promising because it increased the content of solubilized components in the aqueous extract as nitrogenous material, free amino acids, sugars and phenolic components. For most germination times an increase in the antioxidant potential of the aqueous extracts associated. After 80 h of germination, a significantly ($p < 0.05$) increase of protein extraction yield occurred. The large oscillation in the concentration of amino acid residues observed throughout germination, indicate strong metabolic activities, as well as the variation in hydrolytic activity detected. In general, the germination time of 32 h presented remarkable results, such as significant ($p < 0.05$) increase in sugar, and the total phenolic and flavonoids concentrations, and also a maximum antioxidant capacity reached. But the first changes were already detected after the first 8 h of process. The data demonstrate the usefulness of germination for innovations in soy milk production with better nutritional and

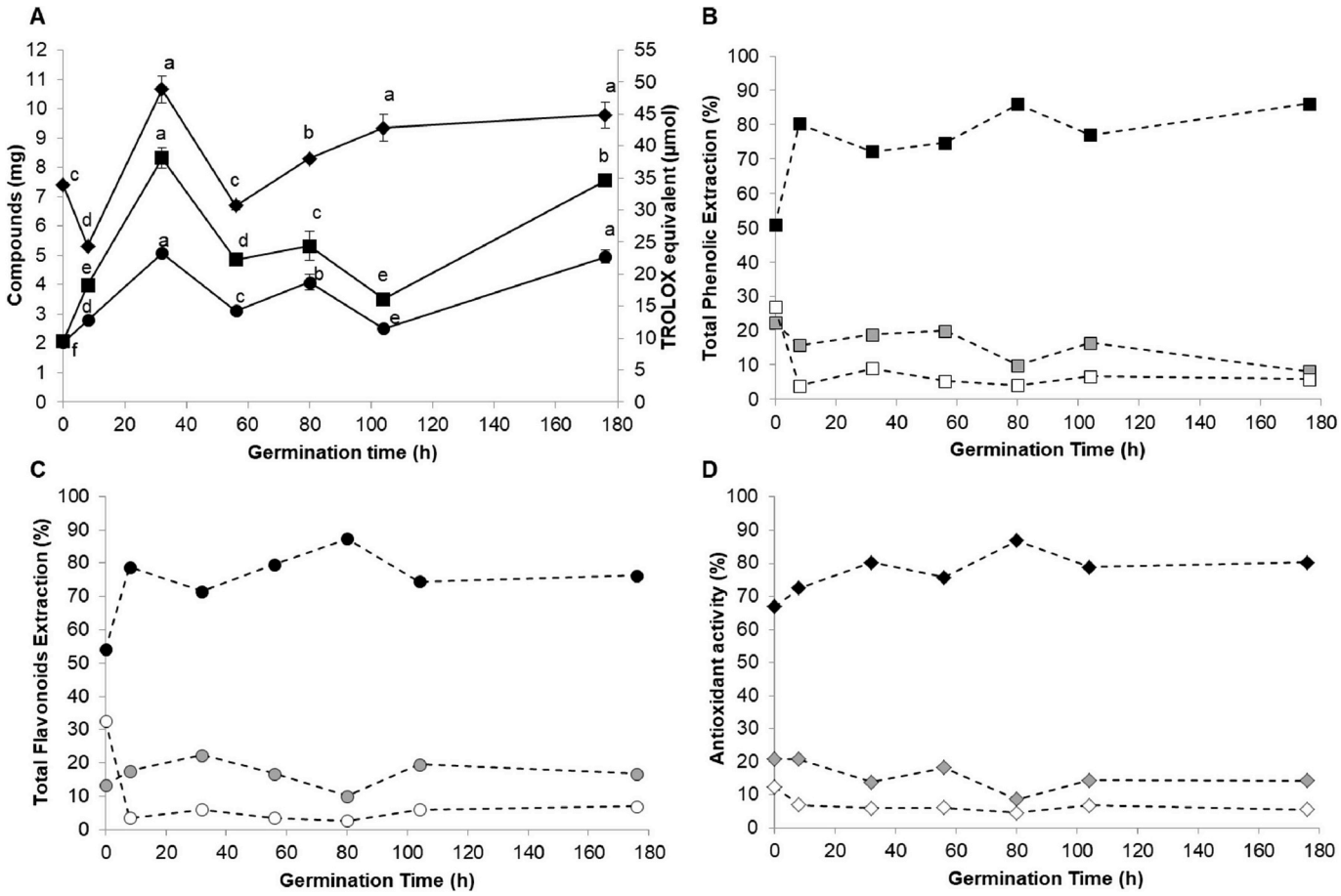

Fig. 7. Effect of germination at different times (0 h, 8 h, 32 h, 56 h, 80 h, 104 h and 176 h) of soybean grains (BRS 257) on the (A) total phenolics (-■-) as mg of gallic acid equivalent, total flavonoids (-●-) as mg of catechin equivalent, and antioxidante activity (-◆-) as µmol of TROLOX equivalent, detected in aqueous extracts as obtained from germinated soybeans per 1 g of dry basis grains. Results are expressed as mean ± standard deviation (error bars) of three determinations. Different lowercase letters in results of the same sample express significant differences ($p < 0.05$). The percentage of total phenolics (B), flavonoids (C) and ABTS antioxidant activity (D) extraction in aqueous extracts at different germination times were considered for each aqueous extraction cycle: 1[a] extraction (dark symbol), 2[a] re-extraction (gray symbol) and 3[a] re-extraction (white symbol).

nutraceutical values. This gives some characteristics to the material that may be useful for the development of innovative soy foods, such as new soy milks, which should be targeted for sensory evaluation.

Notes

The authors declare no conflict of interest.

Conflicts of interest

The authors declare no conflict of interest.

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