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Effect of Storage Temperature and Water Activity on the Content and Profile of Isoflavones, Antioxidant Activity, and in Vitro Protein Digestibility of Soy Protein Isolates and Defatted Soy Flours

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The aim of this work was to study the effect of 1 year of storage at different temperatures and 1 month of storage at different water activities on the content and profile of isoflavones, antioxidant activity, and in vitro protein digestibility of defatted soy flours (DSF) and soy protein isolates (SPI). The storage for up to 1 year, at temperatures from −18 to 42 °C, had no effect on the total content of isoflavones, but the profile changed drastically at 42 °C, with a significant decrease of the percentage of malonylglucosides with a proportional increase of β-glucosides. A similar effect was observed for SPI stored at $a_w = 0.87$ for 1 month. For DSF, however, there was observed a great increase in aglycons (from 10 to 79%), probably due to the action of endogenous β-glucosidases. The antioxidant activity decreased after storage at 42 °C, but the in vitro protein digestibility did not change.

KEYWORDS: Isoflavones; soy protein products; storage; in vitro protein digestibility; antioxidant activity

INTRODUCTION

Isoflavones are a subclass of flavonoids and have an extremely limited distribution in nature, mainly found in soybeans and soy foods. These compounds are usually known as phytoestrogens because of their ability to interact with cellular receptors for estrogens, due to their structural similarity (*1*). They seem to decrease the risk of cardiovascular diseases by reducing the level of total cholesterol, low-density lipoprotein (LDL) cholesterol, and LDL oxidation, due to the reactive oxygen species scavenging properties (*2*−*4*). Other health benefit claims include reductions in postmenopausal symptoms, incidence of cancer, and risk of osteoporosis in women (*5*).

According to Naim et al. (*6*), the antioxidant capacity is related to the number of hydroxyl groups present in isoflavone nucleus. Glycosylation or substitution of hydroxyl groups for methoxyl groups decreases the antioxidant activity.

The main isoflavones found in soybeans are genistein, daidzein, and glycitein, each of which exists in four chemical forms: an aglycon form (genistein, daidzein, and glycitein), a β-glucoside form (genistin, daidzin, and glycitin), a malonylglucoside form (6″-*O*-malonyl-β-daidzin, 6″-*O*-malonyl-β-genistin, and 6″-*O*-malonyl-β-glycitin), and an acetylglucoside form (6″-*O*-acetyl-β-daidzin, 6″-*O*-acetyl-β-genistin, and 6″-*O*-acetyl-β-glycitin) (*7*).

The isoflavone content in soybean is influenced by many factors, including genotypes, crop years, crop locations, storage period, and genotype × environmental interactions (*8*−*10*).

Heating causes a change in the conjugation profile of isoflavones present in soy products. Coward et al. (*11*) reported that baking and frying of isolated soy protein and textured vegetable proteins did not alter the total isoflavone contents but changed the profiles of individual isoflavones, due to conversion of the heat-labile malonylglucosides into other forms. Similar results were reported by Hayes et al. (*12*) for soy beverages containing isolated soy protein. Wang and Murphy (*13*) reported that cooking did not influence the isoflavones retention during tofu making, but altered the distribution of isoflavones by dramatically decreasing the malonylglucosides and increasing the aglycon and β-glucoside forms. Grun et al. (*14*) also reported de-esterification of malonyl and acetylglucosides to the β-glucosides during thermal processing of tofu. In addition, the endogenous β-glucosidases can hydrolyze glucosides of isoflavones to their aglycons (*15*). The activities of β-glucosidases can be favored during storage, affecting sensorial attributes of soybeans by leading to the formation of the more bitter aglycons.

Water activity (a_w) is known to be a fundamental parameter for the stability of foods. For instance, values below $a_w = 0.7$ facilitate, from the microbial stability point of view, long-term shelf life at room temperatures (*16*). Values of water activities are well-known for cereals, oilseeds, and many foods (*17*, *18*) but are scarcely widespread for protein isolates and soy protein in particular. Knowledge of safe moisture values for protein isolates would be useful as a contribution for shelf-life information, whereas the measurement of isotherms, the meaningful relationship between the water or moisture content and the water activity at constant temperature, may assist in identifying the

state of the water associated with the proteins and the more suitable models for sorptional equilibrium (*19*).

The objectives of this work were to determine the effect of storage temperature on the content and profile of isoflavones, antioxidant activity, and in vitro protein digestibility of soy protein isolates and defatted soy flours and to assess the effect of storage water activity on the content and profile of isoflavones. This information would be useful for the improvement of soy products as a source of isoflavones for functional foods. Also, this is the first time that conversion of malonylglucosides to β-glucosides during storage of soy products at high temperatures was reported.

MATERIALS AND METHODS

Materials. Two samples of defatted soy flours (DSF), 1 and 2, and three samples of soy protein isolates (SPI), MP (multipurpose), LH (low hydrolysis), and NB (nutritional beverage), were supplied by Bunge Alimentos S.A. (Esteio, RS, Brazil). These isolates are obtained by alkaline extraction, followed by precipitation of the proteins by acidification to the isoelectric point, neutralization, and spray-drying. SPI LH is submitted to enzymatic hydrolysis before spray-drying. All chemicals and solvents were of reagent or HPLC grade.

Storage of Samples. The samples of DSF and SPI, 1 kg each, were stored in polyethylene bags at -18 ± 1 °C (freezer), 13 ± 1 °C (refrigerator), or room temperature ($\sim 25 \pm 3$ °C) and in a temperature-controlled chamber at 42 °C for up to a year. For the water activity experiment, the samples were placed in desiccators inside the temperature-controlled chamber, at 40 °C.

Isoflavone Extraction. Powdered samples (1 g) of the DSF and SPI were extracted with 80% aqueous methanol (20:1 v/w) under agitation for 2 h at 4 °C, according to the method of Genovese and Lajolo (*20*). The homogenate was filtered through Whatman no. 06 filter paper and concentrated until methanol elimination on a rotatory evaporator (Rotavapor RE 120, Büchi, Flawil, Sweden) at ≤40 °C. The volume of the extracts was adjusted to 5 mL with HPLC grade methanol, and aliquots were filtered through a 0.22 μm polytetrafluoroethylene (PTFE) filter unit (Millipore Ltd., Bedford, MA) and analyzed by HPLC.

HPLC Quantitation of Isoflavones. Isoflavone quantitation was performed according to the method of Song et al. (*21*) with a C18 NovaPak (30 cm × 4.6 mm i.d.) column (Waters, Milford, MA) and a Hewlett-Packard 1100 system with an autosampler and a quaternary pump coupled to a diode array detector (Palo Alto, CA), based on external calibration. Standards of daidzein and genistein were from Sigma Chemical Co. (St. Louis, MO), daidzin and genistin were from Apin Chemicals Ltd. (Abingdon, U.K.), and glycitin and glycitein were from Nacalai-Tesque Inc. (Kyoto, Japan). Concentrations of malonyl and acetylglucosides were calculated using standard curves for the respective β-glucosides, adjusting for differences in molecular weight. Total isoflavone contents were expressed as milligrams of aglycon per 100 g of sample, after normalization of individual isoflavones to account for differences in molecular weight between glucoside derivatives. The mass of each isoflavone form (β-glucoside, malonylglucoside, and acetylglucoside) was multiplied by the ratio of its aglycon molecular weight to the molecular weight of the individual form before summing.

Determination of Water Activity (a_w). The determination of water activity was performed according to the method of Labuza et al. (*22*), placing the samples of DSF 2, SPI LH, and SPI NB (1 g each) in desiccators containing saturated salt solutions of a given a_w. The following saturated salt solutions were used at 40 °C to condition the samples: H_2SO_4 ($a_w = 0$); $K(CH_3COO)$ ($a_w = 0.23$); $Mg(NO_3)_2$ ($a_w = 0.51$); NaCl ($a_w = 0.75$); $BaCl_2$ ($a_w = 0.87$) (*23*). After reaching equilibrium (48 h), the samples were weighed and the water activity approximately estimated by plotting weight variation versus the a_w values of salt solutions at 40 °C.

Storage at Controlled Water Activity. Samples of DSF 2, SPI LH, and SPI NB (1 g each) were placed in controlled-humidity chambers containing three different saturated salt solutions: $K(CH_3COO)$ ($a_w = 0.23$); $Mg(NO_3)_2$ ($a_w = 0.51$), and $BaCl_2$ ($a_w = 0.87$). After 1 month of storage at 40 °C, the samples were analyzed for isoflavones.

Determination of Nitrogen Solubility Index (NSI). The determination of NSI was performed for the samples of DSF 2, SPI LH, and SPI NB. Two grams of each sample was dispersed in 100 mL of water or 0.2 M NaCl by stirring in a water bath (model 2568; Forma Scientific, Marietta, OH) at 40 °C for 90 min. The suspension was centrifuged at 10000*g* for 20 min. The total nitrogen of the sample and the soluble nitrogen (supernatant) were determined according to the micro-Kjeldahl method (*24*), in triplicate, using the conversion factor of 6.25. The correlation between the total and soluble nitrogen of the sample allows one to calculate the solubility through the formula

$$\text{NSI (\%)} = \frac{N_S \times 100}{N_T}$$

where N_S is soluble nitrogen and N_T is total nitrogen contained in the sample.

Determination of Total Phenolics. The determination was performed according to the method of Zielinski and Kozlowska (*25*). The samples were extracted in 80% methanol (20:1 v/w) for 2 h at 4 °C. After centrifugation (10000*g*/20 min), a 0.25 mL aliquot was mixed with 0.25 mL of Folin−Ciocalteu reagent and 2 mL of distilled water. After 3 min at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added and the mixture was placed at 37 °C in a water bath for 30 min. The absorbance was measured at 750 nm using a spectrophotometer model Ultrospec 2000 UV−visible (Amersham Biosciences, Cambridge, U.K.). The results were expressed as milligrams of catechin per 100 g of sample.

Antioxidant Activity. The extracts obtained above were used to assess the antioxidant activity by the bleaching β-carotene method according to Miller (*26*), with little modifications. For the preparation of the reactive solution, aliquots of β-carotene (18 μL) in chloroform (2 mg/mL) were mixed with linoleic acid (0.4 mL), chloroform (1 mL), and Tween 40 (0.4 mL). After this, chloroform was completely evaporated under nitrogen flow, and 20 mL of distilled water saturated with oxygen was added to the mixture. The absorbance was adjusted with water to 0.6. For the oxidation reaction, an aliquot of the sample (9 μL) was mixed with 241 μL of the β-carotene solution in a microplate. The samples were submitted to autoxidation at 45 °C for 3 h. The absorbance at 470 nm was measured at zero time and at 15 min intervals using the microplate spectrophotometer (Benchmark Plus, Bio-Rad). The control consisted of 80% methanol. The antioxidant activity was expressed as the inhibition percentage compared to the control (100% oxidation).

In Vitro Protein Digestibility. The in vitro digestibility was determined by the quantification of digested and total nitrogen according to the micro-Kjeldahl method (*24*) for samples of DSF 2, SPI LH, and SPI NB. Aqueous protein solutions in 0.1 N HCl (10 mg/mL) were hydrolyzed with pepsin for 3 h at 37 °C, under mild agitation, with a ratio of enzyme to substrate of 1:25. The hydrolysis was interrupted by adding trichloroacetic acid to a final concentration of 5%. After centrifugation (10000*g*/20 min), supernatant aliquots were used for nitrogen determination according to the micro-Kjeldahl method (*24*). Two blanks were prepared, a sample blank containing the protein sample under study and an enzyme blank, containing only the protease used. Casein was used as a control in each determination. The correlation between total nitrogen and nitrogen of the hydrolysate (supernatant) allows the estimate of protein digestibility through the formula

$$\text{digestibility (\%)} = \frac{N_{\text{digested}} \times 100}{N_{\text{total}}} = \frac{N_{Ss} - (N_{SBe} + N_{SBs})}{N_T - N_{Be}} \times 100$$

where N_{Ss} is the nitrogen contained in the sample supernatant, N_{SBe} is the nitrogen contained in the enzyme blank supernatant, N_{SBs} is the nitrogen contained in the sample blank supernatant, N_T si the total nitrogen contained in the sample, and N_{Be} is the total nitrogen contained in the enzyme blank.

Table 1. Total Isoflavones Content (Milligrams per 100 g) of Defatted Soy Flours (DSF) and Soy Protein Isolates (SPI) before and after 1 Year of Storage at Different Temperatures[a]

	months						
storage conditions/soy product	1	2	3	5	6	9	12
DSF 1							
before storage	204 ± 5a						
after storage at							
–18 °C	187 ± 3a	188 ± 2a	192 ± 6a	182 ± 7a	187 ± 6a	174 ± 6a	178 ± 5a
13 °C	193 ± 1a	186 ± 4a	180 ± 5a	181 ± 8a	178 ± 5a	176 ± 6a	178 ± 3a
25 °C	200 ± 3a	200 ± 1a	182 ± 6a	177 ± 2a	183 ± 3a	177 ± 7a	181 ± 2a
42 °C	176 ± 3a	186 ± 4a	180 ± 4a	174 ± 6a	184 ± 4a	179 ± 4a	178 ± 3a
DSF 2							
before storage	101 ± 3b						
after storage at							
–18 °C			109 ± 2b			110 ± 1b	112 ± 2b
42 °C			102 ± 1b			112 ± 2b	114 ± 2b
SPI MP							
before storage	154 ± 2c						
after storage at							
–18 °C	149 ±3c	147 ± 3c	147 ± 6c	148 ± 7c	153 ± 4c	153 ± 3c	149 ± 6c
13 °C	147 ± 1c	143 ± 4c	146 ± 2c	143 ± 4c	151 ± 1c	155 ± 3c	155 ± 2c
25 °C	133±3c	141 ± 2c	142 ± 3c	144 ± 4c	148 ± 2c	144 ± 3c	145 ± 1c
42 °C	147±2c	148 ± 4c	148 ± 2c	147 ± 3c	153 ± 7c	146 ± 5c	148 ± 3c
SPI LH							
before storage	85 ± 2d						
after storage at							
–18 °C			88 ± 1d			91 ± 1d	92 ± 2d
42 °C			90 ± 3d			91 ± 3d	96 ± 2d
SPI NB							
before storage	107 ± 2b						
after storage at							
–18 °C			99 ± 1b			96 ± 2b	97 ± 3b
42 °C			107 ± 1b			101 ± 1b	99 ± 2b

[a] The controls consisted of samples before storage. Values are expressed as means ± SD for triplicates. Statistical analyses were performed by taking into account a 10% coefficient of variation for the within-laboratory monthly reproducibility. Means in the same row with common letters are not significantly different ($p < 0.05$).

Statistical Analysis. All analyses were run in triplicate. Isoflavones and protein concentrations were expressed as mean ± standard deviation (SD). Statistical analysis was done by using the Statistic software package version 5.0 (StatSoft, Inc., Tulsa, OK). Differences between means were first analyzed by ANOVA test and then least significant difference (LSD) test ($p < 0.05$).

RESULTS AND DISCUSSION

Effect of Storage Temperature on the Content and Profile of Isoflavones in Soy Products. Two samples of defatted soy flour and three kinds of soy protein isolates, destined for different food uses, were submitted to storage for up to 1 year at temperatures of −18, 13, 25, and 42 °C (DSF 1 and SPI MP) or only at the extreme temperatures, −18 and 42 °C, in the case of DSF 2, SPI LH, and SPI NB.

The total content and the profile of the isoflavones in soy products, before and after 1 year of storage, are shown in **Tables 1** and **2**, respectively. It can be observed that the total isoflavone content of soy protein products was not significantly altered by the 1 year of storage at the temperatures studied (**Table 1**). On the other hand, the isoflavone profiles changed drastically, and a significant reduction of the percentage of malonylglucosides was observed for all samples stored without refrigeration (**Figure 1**; **Table 2**). Most of these forms seem to have been converted into de-esterified β-glucosides, as their percentage increased upon storage, mainly at the higher temperatures. A significant increase in acetylglucosides was observed for the samples of DSF 1 and SPI MP stored at 42 °C, but in DSF 2 and SPI LH the contrary was observed. For aglycons, a temperature-independent increase occurred during storage of DSF 1 and SPI MP, but for the other samples this increase was more intense at 42 °C.

The main difference between the isoflavone profile of defatted soy flour and soy protein isolates was the much higher proportion of aglycons in isolates. Among these, the higher content of malonylglucosides of SPI MP in relation to SPI LH and SPI NB indicates a milder heat treatment during the production of this isolate, as minimal heat processing can convert substantial amounts of malonylglucosides to de-esterified β-glucosides (*13*).

Although isoflavones are not destroyed by heat in conventional food-processing operations, heating causes a change in the conjugation profile of the isoflavones in soy products (*11*). Wang and Murphy (*13*) also reported that cooking did not influence the isoflavone retention during tofu making, but altered the distribution of isoflavones.

It is known that malonylglucoside forms are thermally unstable when exposed to heat, but this is the first time that conversion to β-glucosides during storage at higher temperatures in soy products was reported. The differences in the chemical structure of isoflavones may influence the biological activity, bioavailability, and as a result, the physiological effects of these constituents (*27*). There is little information about the bioavailability of these different forms, and the results are conflicting. The absorption of aglycons in humans was shown to be faster and more extensive than that of the glycosides by Izumi et al. (*28*). Contrarily to this, Setchell et al. (*29*) reported a higher bioavailability of glycosides compared to aglycons, and Xu et al. (*30*) observed no differences in bioavailability associated with isoflavone form, indicating that more studies are necessary.

Effect of Storage Temperature on the Nitrogen Solubility Index (NSI) and in Vitro Protein Digestibility of Soy Products. The NSI, both in water and in 0.2 M NaCl, remained

Table 2. Isoflavone Profile (Percent) of Defatted Soy Flours (DSF) 1 and 2 and Soy Protein Isolates (SPI) MP, LH, and NB before and after 1 Year of Storage at Different Temperatures[a]

		after storage at			
isoflavone/soy product	before storage	−18 °C	13 °C	25 °C	42 °C
DSF 1					
β-glucosides	36.8 ± 0.8a	38.9± 0.8a	40 ± 1a	45.4 ± 0.9b	52 ± 1c
malonylglucosides	55 ± 1a	50.5 ± 0.9a	51 ± 1a	44.3 ± 0.8b	33.5 ± 0.6c
acetylglucosides	4.4 ± 0.1a	4.8 ± 0.2a	4.0 ± 0.1a	4.9 ± 0.1b	8.9 ± 0.3c
aglycons	3.7 ± 0.1a	5.8 ± 0.1b	5.1 ± 0.1c	5.4 ± 0.2c	5.5 ± 0.2b
DSF 2					
β-glucosides	38.1 ± 0.3a	40.0 ± 0.5a			53.8 ± 0.6b
malonylglucosides	44.1 ± 0.5a	42.1 ± 0.6a			26.7 ± 0.4b
acetylglucosides	8.3 ± 0.1a	8.1 ± 0.3a			6.6 ± 0.1b
aglycons	9.5 ± 0.2a	9.8 ± 0.1a			12.8 ± 0.3b
SPI MP					
β-glucosides	22.2 ± 0.3a	21.6 ± 0.3a	27.3 ± 0.4c	24.3 ± 0.2a	30.4 ± 0.6d
malonylglucosides	40.3 ± 0.5a	36.6 ± 0.4a	26.9 ± 0.3d	29.3 ± 0.4c	18.7 ± 0.2e
acetylglucosides	5.2 ± 0.1a	3.6 ± 0.1b	4.8 ± 0.2a	6.4 ± 0.2c	10 ± 1d
aglycons	32.3 ± 0.4a	38.2 ± 0.5b	41.4 ± 0.4c	40.0 ± 0.5b	40.7 ± 0.5c
SPI LH					
β-glucosides	20.6 ± 0.2a	21.8 ± 0.1a			27.4 ± 0.7b
malonylglucosides	26.7 ± 0.9a	25.5 ± 0.3a			15.8 ± 0.6b
acetylglucosides	11.3 ± 0.1a	9.5 ± 0.4b			8.8 ± 0.1c
aglycons	41.5 ± 0.8a	43.2 ± 0.9a			48.0 ± 0.2b
SPI NB					
β-glucosides	22.9 ± 0.2a	18.0 ± 0.1b			24.3 ± 0.5a
malonylglucosides	25.1 ± 0.3a	25.2 ± 0.5a			16.2 ± 0.6b
acetylglucosides	3.69 ± 0.03a	6.6 ± 0.3b			6.4 ± 0.2b
aglycons	48.3 ± 0.5a	50.2 ± 0.6a			53.1 ± 0.1a

[a] The controls consisted of samples before storage. Values are expressed as means ± SD for triplicates. Means in the same row with common letters are not significantly different ($p < 0.05$).

almost unchanged after 1 year of storage of soy protein products at −18 °C (**Table 3**). However, storage at 42 °C led to a significant decrease of the nitrogen solubility for all of the products, but in a higher degree for soy protein isolates (**Table 3**). The nitrogen solubility in 0.2 M NaCl was more affected than that in water except for SPI NB, for which the 0.2 M NaCl NSI was already very low before storage. Defatted soy flour presented decreases of 9 and 20% for the NSI values in water and 0.2 M NaCl, respectively, whereas SPI had a 35−37% decrease of NSI in water and SPI LH a decrease of 55% in 0.2 M NaCl.

According to Wagner and Añón (*31*), the lower the solubility in 0.2 M NaCl, the higher the denaturation degree of soy protein isolates. These authors reported values of NSI in water between 24 and 84% and of NSI in 0.2 M NaCl between 10 and 49% for 13 commercial soy protein isolates analyzed.

These results indicated that prolonged storage at higher temperatures may cause protein denaturation and, as a consequence, protein insolubilization, which can limit product utilization.

However, as can be seen in **Table 4**, despite the differences in protein solubility among the samples stored at −18 and 42 °C, they presented similar values of in vitro digestibility by pepsin, indicating that the nutritional value may not be affected. The defatted soy flour and the two protein isolates showed a high in vitro digestibility, similar to that of casein.

Effect of Storage Temperature on the Antioxidant Activity of Soy Products. To assess the influence of storage temperature on the antioxidant activity of soy protein products, samples stored for 1 year, at temperatures of −18 and 42 °C, were analyzed through the β-carotene bleaching method and compared. The β-carotene bleaching is caused by free radicals generated during peroxidation of linoleic acid; in this way, this method evaluates the ability of a sample to protect a lipidic substrate from oxidation.

The results are presented in **Table 5** and show a decrease of 14−40% of the antioxidant activity for the samples stored at 42 °C when compared to those stored at −18 °C. This decrease was accompanied by a decrease of the total phenolic content (**Table 5**), from −9 to −22%. A high correlation between antioxidant activity and total phenolic content has been previously reported for fruits (*32*, *33*) and grape juices (*34*). However, there was no correlation with total isoflavone content (**Table 1**), which was not affected by storage. A lack of correlation between antioxidant activity and isoflavone content has already been reported for Brazilian and American soybean varieties (*35*, *36*), indicating that these compounds are not those mainly responsible for this property.

Effect of Water Activity (a_w) on the Content and Profile of Isoflavones in Soy Products. Soy quality decreases gradually during long periods of storage, in an intensity dependent on the conditions, mainly temperature and relative humidity. Protein solubility is one of the main physicochemical properties negatively affected (*37*). To assess the effect of a_w on the content and profile of isoflavones, samples of DSF 2, SPI LH, and SPI NB were stored for a month at three different values of water activity (**Table 6**), at 40 °C. The results showed that there was no significant difference in the total contents of isoflavones for the samples stored at water activities of 0.23, 0.51, and 0.87 except for SPI NB stored at $a_w = 0.87$, which presented a 20% decrease. This behavior was different from that observed during 1 year of storage at 42 °C, when despite protein insolubilization no alteration of isoflavone content was detected. This could be explained by the fact that soy protein products presented low values of water activity (0.16 for SPI LH, 0.11 for SPI NB, and 0.46 for DSF 2) and, in this way, despite the high temperatures of storage, a lower extent of modifications would occur.

Table 7 shows the effect of water activity on the isoflavone profiles of DSF, SPI LH, and SPI NB after 1 month of storage.

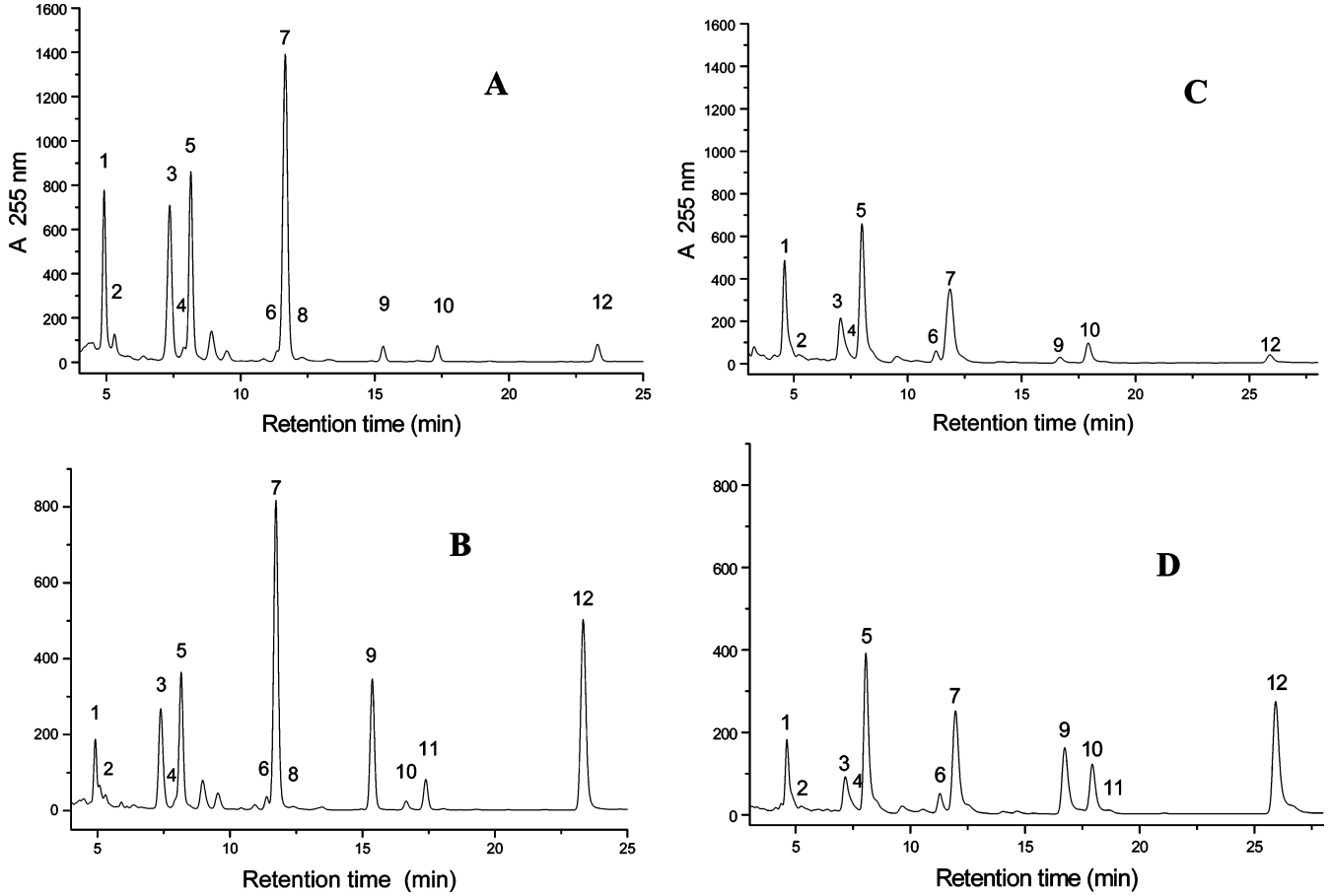

Figure 1. HPLC chromatograms of soy products obtained of the samples before and after 1 year of storage: (**A**) defatted soy flour 1 and (**B**) soy protein isolate MP before storage; (**C**) defatted soy flour 1 and (**D**) soy protein isolate MP after 1 year of storage at 42 °C; (1) daidzin; (2) glycitin; (3) malonyldaidzin; (4) malonylglycitin; (5) genistin; (6) acetyldaidzin; (7) malonylgenistin; (8) acetylglycitin; (9) daidzein; (10) acetylgenistin; (11) glycitein; (12) genistein.

Table 3. Nitrogen Solubility Index (Percent) in Water and in 0.2 M NaCl of Defatted Soy Flour (DSF) 2 and Soy Protein Isolates (SPI) LH and NB before and after 1 Year of Storage at Different Temperatures[a]

			after storage at			
	before storage		–18 °C		42 °C	
soy product	water	0.2 M NaCl	water	0.2 M NaCl	water	0.2 M NaCl
DSF 2	69.9 ± 0.2a	52.3 ± 0.3b	70.47 ± 0.04a	54 ± 1b	63.6 ± 0.1c	41.9 ± 0.5d
SPI LH	60.0 ± 0.4a	30.5 ± 0.5b	66.3 ± 0.9c	31.7 ± 0.1b	38.9 ± 0.5d	13.7 ± 0.8e
SPI NB	45.5 ± 0.2a	10.3 ± 0.4b	48.7 ± 0.1c	11.7 ± 0.2b	16.7 ± 0.7d	10.1 ± 0.5b

[a] The controls consisted of samples before storage. Values are expressed as means ± SD for triplicates. Means in the same row with common letters are not significantly different ($p < 0.05$).

Table 4. Effect of Storage Temperature on the in Vitro Protein Digestibility (Percent) by Pepsin of Defatted Soy Flour (DSF) 2 and Soy Protein Isolates (SPI) LH and NB after 1 Year of Storage[a]

soy product	–18 °C	42 °C
DSF 2	79.6 ± 0.3a	84.3 ± 0.3b
SPI LH	77.5 ± 0.8a	76 ± 2a
SPI NB	90 ± 2a	93 ± 1a
casein	86 ± 1a	

[a] Values are expressed as means ± SD for triplicates. Means in the same row with common letters are not significantly different ($p < 0.05$).

As can be observed, no alterations resulted from the storage at $a_w = 0.23$, and after storage at $a_w = 0.51$, almost no changes were observed in the isoflavone profile, apart from a slight decrease of malonylglucosides with a proportional increase of β-glucosides.

The effect of storage at the water activity of 0.87 was different for samples of DSF and SPI. On DSF 2 a great decrease of malonylglucosides and β-glucosides with a respective increase of aglycons was detected, which changed from 8 to 80% of the total. For both SPI stored at $a_w = 0.87$, a significant decrease of malonylglucosides and a proportional increase of β-glucosides were observed, but no change in the percentage of aglycons.

Similar to the results obtained for the defatted flour, Hou and Chang (*38*) reported that soybeans stored in high relative humidity and high temperature (84% relative humidity at 30 °C) presented a decrease in the percentage of β-glucosides and malonylglucosides, from 99 to 3% in 9 months, and the aglycons increased from 1 to 97%. It is known that endogenous β-glucosidases can hydrolyze glucosides of isoflavones to their aglycons, as occurs during the soaking of soybeans (*15*). Storage at high water activity must have favored the β-glucosidase activity that is unable to act in lower water activities. In general,

Table 5. Effect of Storage Temperature on the Antioxidant Activity (Percent Inhibition of β-Carotene Bleaching) and Total Phenolics Content (Milligrams of Catechin per 100 g) of Defatted Soy Flour (DSF) 2 and Soy Protein Isolates (SPI) LH and NB after 1 Year of Storage[a]

	total phenolics (mg of catechin /100 g)			antioxidant activity[b] (% inhibition)		
soy product	–18 °C	42 °C	variation[c] (%)	–18 °C	42 °C	variation[c] (%)
DSF 2	171.6 ± 0.3a	133 ± 5b	–22	48 ± 2a	37 ± 2b	–23
SPI LH	141 ± 2a	128 ± 4b	–9	36 ± 2a	31 ± 2b	–14
SPI NB	94 ± 1a	76 ± 2b	–19	33 ± 2a	20 ± 1b	–40

[a] Values are expressed as means ± SD for triplicates. Means in the same row with common letters are not significantly different ($p < 0.05$). [b] A methanolic solution of BHT (25 μg/mL) was used as a control and resulted in a 52 ± 2% inhibition. [c] Values were obtained by comparing samples stored at 42 °C with samples stored at –18 °C.

Table 6. Total Content of Isoflavones (Milligrams per 100 g) of Defatted Soy Flour (DSF) 2 and Soy Protein Isolates (SPI) LH and NB after 1 Month of Storage at Different Values of Water Activity (a_w)[a]

		a_w		
soy product	control	0.23	0.51	0.87
DSF 2	114 ± 1a	117 ± 2a	117 ± 2a	110 ± 3a
SPI LH	88 ± 2b	86 ± 1b	87 ± 1b	82 ± 4b
SPI NB	96 ± 2c	95 ± 1c	94 ± 1c	76 ± 1d

[a] The controls consisted of samples before storage. Values are expressed as means ± SD for triplicates. Means in the same row with common letters are not significantly different ($p < 0.05$).

Table 7. Effect of Water Activity (a_w) on Isoflavone Profile (Percent) of Defatted Soy Flour (DSF) and Soy Protein Isolates (SPI) LH and NB before and after 1 Month of Storage at Different Values of Water Activity[a]

		a_w		
isoflavone/soy product	control	0.23	0.51	0.87
DSF 2				
β-glucosides	39.9 ± 0.8a	39.3 ± 0.2a	45.6 ± 0.5b	7.05 ± 0.02 c
malonylglucosides	46 ± 0.9a	47.4 ± 0.2a	43.0 ± 0.1b	13.3 ± 0.5c
acetylglucosides	5.8 ± 0.2a	5.3 ± 0.1 a	3.5 ± 0.1b	1.9 ± 0.1c
aglycons	8.3 ± 0.1a	8.0 ± 0.2a	7.9 ± 0.5a	77.7 ± 0.3b
SPI LH				
β-glucosides	20.2 ± 0.3a	23.4 ± 0.9a	26.8 ± 0.5b	41 ± 2c
malonylglucosides	27.4 ± 0.4a	26.7 ± 0.4a	24.4 ± 0.6a	14.0 ± 0.7b
acetylglucosides	8.9 ± 0.1a	8.4 ± 0.02a	8.3 ± 0.1a	5.7 ± 0.3b
aglycons	43.5 ± 0.5a	41.5 ± 0.5a	40.5 ± 0.2a	39.4 ± 0.7a
SPI NB				
β-glucosides	18.7 ± 0.1a	22.3 ± 0.4ab	23.8 ± 0.3b	37.6 ± 0.4c
malonylglucosides	27.8 ± 0.5a	27.2 ± 0.1a	25.2 ± 0.7b	12.5 ± 0.4c
acetylglucosides	4.4 ± 0.3a	3.4 ± 0.1b	3.25 ± 0.04b	3.2 ± 0.1b
aglycons	49.1 ± 0.6a	47.1 ± 0.5a	47.7 ± 0.4a	46.6 ± 0.1a

[a] The controls consisted of samples before storage. Values are expressed as means ± SD for triplicates. Means in the same row with common letters are not significantly different ($p < 0.05$).

no enzymatic activity is observed in water activities below 0.3, and in water activities below 0.6 the reaction rates generally are very low (*39*).

In stored SPI no increase of aglycons was observed in any water activity studied. This is probably because the β-glucosidases present in DSF are lost during the SPI preparation, probably during the isoeletric precipitation step.

In conclusion, the effect of storage on isoflavones present in soy protein products depends on the conditions of storage (temperature and a_w) and product composition. In general, no degradation of isoflavones is observed, and the main changes are related to the isoflavones composition. High values of temperature and relative humidity tend to decrease the amount of malonylglucosides, forming β-glucosides or aglycons, depending on the presence of endogenous β-glucosidases.

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