FOOD SCIENCE AND TECHNOLOGY

Mirzaolimov Mirzohid Mirzavalievich
Karimov Muxammadali Abduxolikovich
Abduraximov Adxamjon To'xtasinovich
Namangan State University, Namangan
Pop medical collej, Namangan
mirzohid_0421@mail.ru
https://doi.org/10.5281/zenodo.7825203

Abstract. Soy products produced in Ecuador, with a local developed and cultivated soybean variety (INIAP 306), were analysed for isoflavone content and profile. The products presented high total isoflavone contents, varying from 53 to 106 mg/100 g (wet basis, expressed as aglycones), the lowest content being for okara and the highest for the low fat soybean flour obtained by extrusion cooking of the seeds at the field moisture. Soy nuts showed the same content of isoflavones than the seeds, but with lower amounts of malonylglycosides and higher of the deesterified b-glycosides. The malonylglycosides were the predominant form of the isoflavones in the flours, and the b-glycosides in soymilk and textured soy protein. Genistein derivatives were the compounds present in the highest proportions in all the products analysed.
Key words: Ecuadorian soybean products, isoflavone content, isoflavone profile.
Resumo. Produtos derivados de soja produzidos no Equador, com uma variedade de soja (INIAP 306) desenvolvida e cultivada localmente, tiveram o teor e perfil de isoflavonas determinados através de cromatografia líquida de alta eficiência. Os produtos apresentaram altos conteúdos de isoflavonas, variando de 53 a 106 mg/100 g (base úmida, expresso como agliconas), sendo o menor conteúdo encontrado em okara e o maior na farinha parcialmente desengordurada obtida através de extrusão das sementes. Os snacks de soja apresentaram o mesmo conteúdo de isoflavonas que as sementes, mas com quantidades menores de malonilglicosídeos e maiores de b-glicosídeos desesterificados. Os malonilglicosídeos foram as formas predominantes encontradas nas farinhas e os b-glicosídeos no leite e na proteína texturizada de soja. Os derivados de genisteína foram os compostos presentes nas maiores proporções em todos os produtos analisados.

Introduction. The consumption of soybeans has been associated to reduced risks of several chronic diseases such as coronary heart disease, osteoporosis, breast and prostate cancer. The main soy components related to these effects are the protein and the isoflavones (Adlercreutz and Mazur, 1997). Soybean contains three types of isoflavones, in four chemical forms: the aglycones daidzein, genistein, and glycitein; the b-glycosides daidzin, genistin, and glycitin; the acetyl-b-glycosides 6''-O-acetyl-b-daidzin, 6''-O-acetyl-b-genistin, 6''-O-acetyl-b-glycitin; and the malonyl-b-glycosides 6''-O-malonyl-b-daidzin, 6''-O-malonyl-b-genistin, and 6''-O-malonyl-b-glycitin (Friedman and Brandon, 2001).

The importance of soy products is that they represent a way of incorporating isoflavones in the diet of populations that are not used to the consumption of the grains, such as the Ecuadorian. Soy flours, as an example, can be used in the preparation of bread high in phytoestrogens, whose daily consumption has been recently shown to favourably influence PSA (prostate-specific antigen) levels in men diagnosed with prostate cancer after just one

month (Dalais et al., 2004). Soy protein products may be used as ingredients in meat products, breads, beverages, soups and other foods due to the physicochemical properties of soy proteins which help conferring texture and other important properties such as water retention and gelification. Soy milk can be used to elaborate flavored soymilk, yogurt, ice cream and tofu, and soy-nuts can be consumed as snacks and granola ingredients.

The objectives of this work were to determine the content and profile of isoflavones present in soy products produced in Ecuador, using a local developed and cultivated soybean variety (INIAP 306), in order to identify potential isoflavone sources.

Materials and methods.

Materials. Soybeans used in the production of the derivatives were from the variety INIAP 306 developed and cultivated in Ecuador. All the products were obtained at the Escuela Politécnica Nacional (Quito, Ecuador), according to the following procedures:

Raw whole and dehulled soybean flours

The seeds were first fractured in a Rietz disintegrator model RP-8-K115 (Rietz Manufacturing Co., Santa Rosa, USA) and after that milled in an Alpine mill, type 160 UPZ, Nº 3644.1 (Alpine Aktiengesellschaft and Co., Augsburg, Germany) at 18000 rpm for the obtention of the raw whole soybean flour. The raw dehulled soybean flour was obtained in the same way, except for the previous dehulling of the seeds in a dehuller-classifier Bowermeister serial BR (Muehlenbau GmbH, Hamburg, Germany).

Low fat soybean flour.

This flour was obtained based on extrusion cooking of soybeans at field moisture content, followed immediately by continuous expelling. Extrusion was carried out in a Bonnot Model 2 single-screw extruder (Bonnot Co., Kent, USA). The average residence time of material within the extruder barrel was approximately 30 seconds, with a screw speed of 143 rpm and discharge temperature of 124 ºC. The material was cooled and milled in a Schutte-Buffalo hammer mill Model 38 (Schutte Pulverizer Co., Inc., New York, USA).

Soy-nuts. The production of soy-nuts was made after blanching soy cotyledons by dropping them directly into boiling water (100 g of soybeans/500 ml water) containing 0.05 % sodium bicarbonate, for 10 minutes. After drainage of the water, cotyledons were spread on paper towel to remove excess water and shallow fried in soybean oil until brown and crispy. After removal of the excess of oil, powdered salt was sprinkled to taste.

Soymilk. For the production of soy milk, dehulled soybeans were first blanched for 5 min in water containing 0.05 % sodium bicarbonate (1:5 w/v). After discarding blanching water, soybeans were rinsed with hot water, drained, and ground with hot water with a Rietz disintegrator. The slurry was then filtered through a fine mesh sack to separate soymilk from the insoluble residue (okara), and the soymilk was cooked for 20 min at 85-88 ºC. Pasteurised soymilk was homogenised and freeze-dried for posterior analysis.

Textured soy protein. Textured soy protein was prepared after hydrating the low fat soybean flour in alkaline water for one hour. The material obtained (20-24% moisture) was extruded using a Brabender Do-Corder extruder (Duisburg, Germany), with screw speed of 110 rpm, and temperature profile: T1 = 80 ºC, T2 = 120 ºC and T3 = 200 ºC. Extruded samples were dried in a forced circulation air dryer (Proctor and Schwartz, Inc. , Philadelphia, USA).

Methods. Proximate composition

Proximate composition was determined according to the AOAC (1995) methods.

Isoflavone. Extraction. Soy samples, when necessary, were previously ground in a mill Janke and Kunkel A-10 (Wilmington, U.S.A.) under refrigeration until the sample passed through a 0.25 mm sieve. Powdered samples (1 g) were extracted with 80% aqueous methanol (1:20 w/v) under agitation for 2 h at 4 ºC (Genovese and Lajolo, 2001). The extracts were filtered through Whatman nº 6 filter paper and concentrated until methanol elimination on a rotatory evaporator (Rotavapor® RE 120 - Büchi, Flawil, Sweden) at <40 °C. The volume (~2 ml) was then adjusted to 5 ml with HPLC grade methanol (final methanol concentration of 80%). Aliquots of the samples were filtered through a 0.22-µm PTFE filter unit [poly (tetrafluoroethylene), Millipore Ltd., Bedford, U.S.A.] and analysed by HPLC. The extractions were made in triplicate.

HPLC Quantitation of Isoflavones.

Isoflavone separation and quantitation was performed according to Song et al. (1998) with a C18 NovaPak (30 cm x 4.6 mm id) column (Waters, Milford, U.S.A.) and a Hewlett Packard 1100 system equipped with a diode array detector and the software ChemStation (Palo Alto, U.S.A.). Identification was made based on the spectra and retention time, and quantitation based on external calibration. Concentrations of malonyl and acetylisoflavones were calculated using standard curves for the respective b-glycosides, adjusting for differences in molecular weight. Total isoflavone contents were expressed as mg of aglycone/100 g of sample fresh weight (FW), after normalisation of individual isoflavones to account for differences in molecular weight between glycoside derivatives. The mass of each isoflavone form (b-glycoside, malonylglycoside and acetylglycoside) was multiplied by the ratio of its aglycone molecular weight to the molecular weight of the individual form before summing.

Results and discussion. Presents the proximate composition of both the INIAP 306 soybean variety and the low fat soybean flour obtained from it. The partial elimination of oil through the extrusion increased the protein content of the flour and eliminated more than 90% of the trypsin inhibitory activity (data not shown). The proximate composition of soy seeds was in accordance with previous results from the literature (Souci et al., 1994).

Isoflavone contents of soy products are presented in Table 2 and representative HPLC chromatograms in Figure 1 (except for that of soy seeds presenting the same content of the raw whole soybean flour). Total isoflavone content varied among different soy varieties and environmental growing conditions (Wang and Murphy, 1994; Lee et al., 2003) and the Ecuadorian variety (~ 68 mg per 100 g) was found in the same range of Brazilian varieties, from 57 to 188 mg per 100 g of soybeans (Genovese et al., 2005) and could be considered low when compared to American (116 to 274 mg/100 g) and Korean varieties (188 to 949 mg/100 g) (Wang et al., 2000; Lee et al., 2003).

The soy products represent a way of incorporating isoflavones in the diet of populations that do not consume the grains. Almost all the products analysed presented similar or higher isoflavone contents when compared to the seeds (Table 2). Dehulling was shown to be a simple way of increasing the isoflavone content of the flour prepared from raw seeds. This was in accordance with a previous report showing that the hulls, which represented almost 8% of the seed, had the lowest isoflavone content (10-20 mg/100 g) in relation to the cotyledons (160-320 mg/100 g) and hypocotyls (~1500 mg/100 g) (Eldridge and Kwolek, 1983). When the oil content was reduced from 24 to 15% through extrusion, a significant concentration of isoflavones occurred in the low fat flour as the oil was free from isoflavones.

Texturization of this flour, however, led to a slight decrease (~9%) of the isoflavone content in the textured soy protein (Table 2).

Frying was shown not to alter the isoflavone content of the seeds, similar to the results previously reported by Coward et al. (1998). Okara, the water insoluble residue obtained in the production of soy milk, which was normally discarded, also presented a significant isoflavone content, 70% that of the dehulled flour. Considering that soymilk presented a 94% moisture content before freeze-drying, the beverage in its form of consumption would have an isoflavone content of 67 mg/L, similar to previous results of Genovese and Lajolo (2002) for commercial soy beverages. The profile of the isoflavones contained in the different soy products can be seen in Table 3 and Figure 2 and showed a predominance of glycosides over aglycones in all products.

Malonylglycosides were the main form present in the three flours, indicating that extrusion cooking for the obtention of the partially defatted flour was not drastic enough to affect these thermally unstable forms. Frying and texturization, on the other side, led to a significant decrease of malonylglycosides accompanied by an increase of the deesterified b-glycosides. Textured soy protein also presented an increase in the proportion of acetylglycosides (Table 3). According to Coward et al. (1998), these compounds were more commonly formed during processing involving dry heat, such as toasting of soy flour or extrusion to produce texturized soy protein. Comparing the isoflavone profile of okara and soymilk a decrease was observed in both in the proportion of malonylglycosides in relation to the raw flours, but while in soymilk this was accompanied by an increase in b-glycosides, in okara the proportion of aglycones was higher. This could be explained by differences in water solubility between these forms.

The ratios of malonyldaidzin to daidzin (MD/D) present in the raw flours and malonylgenistin to genistin (MG/G) (1.9 and 1.8, for whole and dehulled seeds, respectively) were superior to the values previously reported for Brazilian soybeans [0.4 to 0.7 (MD/D) and 0.6 to 0.9 (MG/G)] (Genovese et al., 2005), confirming that these values would be characteristic for the different genotypes.

Shows the distribution of isoflavones in terms of total genistein, daidzein and glycitein, which represent the sum of all the forms of each isoflavone (aglycones naturally present, acetyl, malonyl and b-glycosides) expressed as total aglycones. The products presented similar compositions, with a predominance of genistein followed by daidzein. When comparing soymilk to okara, a preferential solubilization of daidzein derivatives seemed to have occurred. An explanation would be the more hydrophilic nature of daidzein compared to genistein (Coward et al., 1993).

Conclusions. Soy products prepared from the INIAP 306 variety preserved the isoflavone content of the seed and represented significant isoflavone sources for direct incorporation into the diet and/or preparation of derived products such as bread, flavored soymilks, yogurts, and ice creams. Removal of the seed coat and oil increased isoflavone content of soy products. Processing altered isoflavone profile mainly in relation to a decrease of malonylglycosides and increase of b-glycosides. In textured soy protein, an increase of acetylglycosides was also observed. Soy products, in general, presented isoflavone composition similar to the seeds with prevalence of genistein followed by daidzein derivatives.

Acknowledgements. The authors acknowledge CYTED (Programa Iberoamericano de Ciencia y Tecnologia para el Desarrollo - CYTED XI. 19. Aplicación de los nuevos ingredientes funcionales em alimentación infantil y para adultos) and FAPESP, for the financial support, and the student Márcia da Silva Pinto (Bolsa de Iniciação Científica FAPESP) for the collaboration with isoflavone analysis.

References:

1.Сулейменова Н.Ш., Мазиров М.А., Райымбекова И.К. Экологически безопасный прием стабилизации фитосани тарной устойчивости агрофитоценозов в технологии возделывания сои.//Ж. «Вестник Алтайского государствен ного аграрного университета», – № 3 (89). – 2012.

2.Уклеин А.И. В сб.: Соя. – М., 1963. 5. Терентьев Ю.В. Масличные культуры. – 1981. – № 1. 6. Щербаков В.Г. Биохимия и товароведение масличного сырья. – М., 1979

3.Kaprelyants, L., Yegorova, A., Trufkati, L., Pozhitkova, L. (2019). Functional foods: prospects in Ukraine. Food Science and Technology, 13 (2), 15–23. doi: https://doi.org/10.15673/fst.v13i2.1382

4.Kaprelyants, L. (2016). Functional foods and nutraceuticals – modern approach to food science. Visnyk of the Lviv University. Series Biology, 73, 441–441. Available at: http://nbuv.gov.ua/UJRN/VLNU_biol_2016_73_122

5.Kim, Y., Yoon, S., Lee, S. B., Han, H. W., Oh, H., Lee, W. J., Lee, S.-M. (2014). Soy milk fermentation via Lactobacillus plantarum Improves Dysregulated Lipid Metabolism in Rats on a High Cholesterol Diet. PLoS ONE, 9 (2), e88231. doi: https://doi.org/10.1371/journal.pone.0088231

6.Božanić, R., Lovković, S., Jeličić, I. (2011). Optimising fermentation of soymilk with probiotic bacteria. Czech Journal of Food Sciences, 29 (1), 51–56. doi: https://doi.org/10.17221/97/2010-cjfs

7.Niyibituronsa, M., Onyango, A. N., Gaidashova, S., Imathiu, S., Boevre, M. D., Leenknecht, D. et. al. (2019). The Growth of Different Probiotic Microorganisms in Soymilk from Different Soybean Varieties and their Effects on Anti-oxidant Activity and Oligosaccharide Content. Journal of Food Research, 8 (1), 41. doi: https://doi.org/10.5539/jfr.v8n1p41

8.Myagmardorj, B., Purev, M.-E., Batdorj, B. (2018). Functional properties of fermented soymilk by Lactobacillus fermentum BM-325. Mongolian Journal of Chemistry, 19 (45), 32–37. doi: https://doi.org/10.5564/mjc.v19i45.1087