



Profile of the contents of different forms of soybean isoflavones and the effect of germination time on these compounds and the physical parameters in soybean sprouts



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ABSTRACT

The aim of this study was to evaluate the profile of the contents of different forms of soybean isoflavones and the effect of germination time on these compounds and the physical parameters in BRS 284 soybean sprouts. Soybean seeds were germinated for 168 h, and the sprouts were collected every 24 h. The physical parameters and contents of different forms of isoflavones of the seeds and soybean sprouts were evaluated, and the data were subjected to regression analysis. The soybean seeds contained 26.0% β -glucosides, 72.9% malonylglucosides and 1.2% aglycones. The yield of soybean sprouts was 632.4%. The effect of germination time was quadratic on the length, moisture and on the daidzin, genistin and genistein content; linear on the fresh weight and on the malonyldaidzin content. The dry matter and malonylglucitin content was constant, and glycitin and glycitein were not detected in the soybean sprouts.

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1. Introduction

During the course of soybean domestication, the Chinese gradually discovered that it was possible to transform soybeans into various forms of soy foods, such as tofu, soy sauce, soy paste and soy sprouts, making soy-based foods more versatile, more flavourful and more digestible (Liu, 2008). Soybean sprouts, often known as moyashi, are considered healthy and natural foods in many parts of the world (Mwikya, Camp, Rodriguez, & Huyghebaert, 2001). Soybean sprouts are an important vegetable source that are rich in nutrients and available all year, and they have been consumed for thousands of years in countries such as Korea, China and Japan (Lee, Hwang, Cho, Kim, & Chung, 2002). Recently, there has been an increase in demand for these products due to a renewed interest in healthy food (Kim, Lee, Won, & Yoo, 2005).

Seed germination occurs as a naturally complex biological process that involves catabolic reactions, such as the hydrolysis of reserve substances, and anabolic reactions, such as enzyme synthesis and the repair of cellular components to produce new cells of the embryo (Abdul-Baki, 1980). This process can be used to improve the sensorial quality (Liu, 2004) and nutritional value (Kim et al., 2013; Paucar-Menacho, Berhow, Mandarino, Chang, & Mejia, 2010) of soybean seeds because it reduces the amount of

undesirable substances, such as phytic acid (Ramadan, 2012; Ribeiro, Ida, & Oliveira, 1999), oligosaccharides (Kim et al., 2005; Martín-Cabrejas et al., 2008), trypsin inhibitors, and compounds with lipoxygenase activity (Paucar-Menacho, Berhow, Mandarino, Chang et al., 2010). Germination can also promote a significant increase in the content of vitamins, such as ascorbic acid, riboflavin and thiamine (Ahmad & Pathak, 2000); phytosterols and tocopherols (Shi, Nam, & Ma, 2010); isoflavones (Lee et al., 2007; Phommalth, Jeong, Kim, & Hwang, 2008b); and isoflavone aglycones (Kim et al. 2005; Paucar-Menacho, Berhow, Mandarino, Chang et al., 2010; Shi et al., 2010; Yuan, Liu, Peng, Wang, & Liu, 2009).

Among edible vegetables, soy is the only one that contains a high content of isoflavones, which are divided into four groups and 12 distinct forms: aglycones (daidzein, genistein and glycitein), β -glucosides (daidzin, genistin and glycitin), malonylglucosides (6''-O-malonyldaidzin, 6''-O-malonylgenistin and 6''-O-malonylglycitin) and acetylglucosides (6''-O-acetyldaidzin, 6''-O-acetylgenistin and 6''-O-acetylglycitin) (Liu, 2004). During the seed development of soybeans, aglycones are synthesised by the phenylpropanoid metabolic pathway and stored in vacuoles as β -glucosides and malonylglucoside isoflavones (Graham, 1991; Kudou et al., 1991). In soybean seeds, malonylglucoside represents 68.0–93.0% of the total isoflavones, while β -glucosides represent 7.0–33.0%. However, aglycones and acetylglucosides are absent or present in low concentrations (Paucar-Menacho, Berhow,

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Mandarino, Chang et al., 2010; Tsukamoto et al., 1995). In addition, the isoflavone content in soybean seeds may vary according to the cultivar and harvest year (Berger, Rasolohery, Cazalis, & Daydé, 2008; Wang & Murphy, 1994), the sowing date and growing location (Tsukamoto et al., 1995) and the pathogen load in the soybean growing season (Wegulo, Yang, Martinson, & Murphy, 2005).

The isoflavones are associated with human health benefits, such as protective effects against bone loss in postmenopausal women (Atteritano et al., 2009), the prevention of cardiovascular diseases (Chan et al., 2007), the relief of postmenopausal symptoms (Williamson-Hughes, Flickinger, Messina, & Empie, 2006) and a protective effect against breast cancer (Wada et al., 2013) and prostate cancer (Dong, Xu, Sikes, & Wu, 2013).

The objective of this study was to evaluate the profile of the contents of different forms of soybean isoflavones and the effect of germination times of up to 168 h at 35 °C on the contents of these compounds and physical parameters in BRS 284 soybean sprouts.

2. Materials and methods

2.1. Materials and reagents

BRS 284 soybean seeds, a conventional cultivar, grown in the 2011/2012 season, were provided by a specialist company in the region and used for this study. According to the Empresa Brasileira de Pesquisa Agropecuária (Embrapa, 2010), this cultivar is designated for industrial processing, has an average size of 14.60 g per 100 seeds and contains 38.7% protein and 20.4% lipids. All reagents used were of analytical grade and from different sources.

2.2. Experimental design of the germination process

Prior to germination, soybean seeds were carefully selected to eliminate any damaged and stained seeds or foreign material. Fifty soybean seeds were placed on two germination papers (dimensions of 37.5 cm × 28.2 cm, Germitest) previously wetted with distilled water with the support of a perforated plate, then the seeds were covered with another paper, the bottom edge was folded, and the papers were curled longitudinally to form rolls.

To evaluate the effect of soybean germination time on the physical parameters and content of different forms of isoflavones in soybean sprouts, a randomised complete block design with three replications was used. A total of 15 paper rolls were used for each block and each germination time. Germination was carried out in a germination chamber (model Mangelsdorf, J. Prolab – São José dos Pinhais, PR, BR) with natural light at 35 °C (± 1 °C) and a relative humidity of 100% for 24 h, 48 h, 72 h, 96 h, 120 h, 144 h and 168 h, as described by Yoshiara et al. (2012).

The effect of germination time on the physical parameters and contents of different forms of isoflavone was considered only after 48 h of germination, when it was possible to observe the initial formation of soybean sprouts. For each germination time, soybean sprouts were randomly collected (Fig. 1) and the physical parameters were evaluated. Thereafter, the soybean sprouts were freeze-dried to a constant weight, ground (model A-11, Ika), held in polyethylene bags and stored at –22 °C until isoflavone quantification. Ungerminated seeds were used for comparison.

2.3. Analytical procedures

For the measurements of the physical parameters, twenty soybean sprouts per block and per germination time were used. To measure the length, the soybean sprouts were photographed and measured using Foxit Reader version 6.0 software. The total length



Fig. 1. BRS 284 soybean development.

was determined by measuring from the tip of the root to the tip of the epicotyl and was expressed in centimetres. An analytical balance was used to determine the fresh weight, and the results were expressed in grams per 100 sprouts. To determine the moisture and dry weight, the shoots were freeze-dried to a constant weight, ground (model A-11, Ika) and the moisture content of the lyophilised sample was determined by the oven method at 105 °C. The yield of soybean sprouts was calculated by the ratio between the mass of 100 sprouts and the weight of 100 seeds, multiplied by 100. All physical measurements were performed in triplicate.

The quantification of isoflavones was performed in triplicate using an ultra-performance liquid chromatography (UPLC) system, as described by Handa, Couto, Vicensoti, Georgetti, and Ida (2014). The lyophilised and milled samples were defatted with hexane in a 1:10 ratio (w/v) for 1 h at room temperature by continuous and rotary agitation followed by vacuum filtration. The isoflavone extraction was performed in triplicate with 0.1 g of defatted sample using 2 ml of extraction solution containing ultra-pure water, acetone and ethanol (1:1:1, v/v/v), as described by Yoshiara, Madeira, Delarozza, Silva and Ida (2012). The different forms of isoflavones and the sum of all forms of isoflavones were expressed in μmol of the respective isoflavone per gram of sample on a dry and defatted basis.

2.4. Statistical analysis

The results of the physical parameters (moisture, sprout length, fresh weight and dry weight) and different forms of isoflavones in the soybean sprouts were subjected to regression analysis to construct mathematical models using all experimental data with the software Statistica 10.0.

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