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An ecofriendly procedure to extract isoflavones from soybean seeds

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ABSTRACT

A novel procedure for isoflavones extraction from soybean seeds using the "green solvent" water was set-up for its future industrial up-scaling. The optimized processing flow-scheme proposed here consists of a 1:5 flour weight-to-water volume ratio mixed by vertical stirrers for 6 min at 40 °C, with a final 12000-rpm centrifugation, and subsequent lyophilization to ensure stability and easy dosage of the extracts. Results showed that preliminary UV-C light flour irradiation for at least 1 h can improve isoflavones extraction and reduce anti-nutritional factors. Principal component analysis (PCA) and discriminant factorial analysis (DFA) highlighted specific operational conditions, such as extraction volume and time, UV time exposure, and centrifugation speed, for preferential extraction of specific isoflavones. The extraction efficiency varied according to variety choice within a range of 40–73%, possibly due to the relative abundance of the most water-soluble malonyl forms of isoflavones. As the extracts showed promising high stability under freezing and lyophilization conditions, it is concluded that, this eco-friendly extraction approach with can be successfully scalable, helping to save time and energy and obviating at disposal problems in the soy nutraceutical chain.

1. Introduction

Soybean currently plays an important role in food and feed at a global level as the main source of vegetal proteins and essential amino acids, while production has a favorable environmental impact (Tessari et al., 2016; Cassini et al., 2010). Health claims concerning soybean are, however, largely attributed to isoflavones, which commonly bond with proteins in the seeds (Liu et al., 2013).

Isoflavones are recognized as having chemopreventive potential, antioxidant and anti-inflammatory properties, as well as the capacity to modulate steroid hormone levels and receptors (Zhang et al., 2007; Villares et al., 2011). Recently, some authors suggested that isoflavones supplements are also associated with a reduced risk of postmenopausal breast cancer, while some others highlighted their anti-aging activity in dermatology. Interest in soybean isoflavones is increasing in the field of nutrition and preventive medicine, along with the use of botanical or herbal ingredients, to prevent or improve a number of health problems (Boucher et al., 2013; Polito et al., 2012; Gao et al., 2015). Soybean seeds contain four groups of isoflavones and twelve distinct compounds: i.e., aglycones (daidzein, genistein and glycitein), and their acetyl-, malonyl-, and β-glycoside forms. The two latter classes are commonly the highest isoflavones fractions in intact seeds and other soy products (Niamnuy et al., 2011). The biological effects of isoflavones seem to depend mainly on aglycones abundance (Cao et al., 2012), although some author consider it unlikely that aglycones enter the systemic circulation, and report that their percentage in plasma is generally low (Islam et al., 2014). This suggests the need to quantify all types of isoflavones in both seeds and derived foods as their biological activities may differ, and to identify crucial steps in isoflavones degradation during food processing.

Several factors, like genotype-phenotype (Barion et al., 2016; Barion at al., 2010), cultivation environment (Vamerali et al., 2012; Barion et al., 2008a, 2008b), location and post-harvest storage (Lee et al., 2003), play a major role in seed isoflavones accumulation and preservation. It was suggested that high- and low-isoflavones cultivars maintain the same ranking across locations, and that in favorable conditions the same cultivar could present up to a 4-fold increase in isoflavones concentration in the cotyledons,

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the specific class - genistein or diadzein - depending on variety (Berger et al., 2008).

It is possible to identify varieties with stable responses to agricultural management, but there are few suitable genotypes available and they have low isoflavones concentrations (Barion et al., 2010). Concerning soybean processing, the distribution of major isoflavones compounds in soy-based foods depends on the production process (Villares et al., 2011).

Against this background, it is desirable that isoflavones from soybean extracts used as food ingredients have the widest spectrum and highest concentrations as possible, although extraction of all the isoflavones, which vary greatly in polarity, is still a challenging task. Until now, several techniques have been proposed, from classical heat refluxing extraction and Soxhlet and pressurized solvent extraction (Vacek et al., 2008) to modern techniques, such as ultrasonic-assisted (Kaufmann and Christen, 2002) microwave-assisted (Wu et al., 2012) and supercritical-fluid extractions (Pyo et al., 2009).

Pressurized solvent extraction (PSE) has advantages in terms of extraction time, isoflavones yield and reproducibility, as the high pressure and temperature used facilitate solvent penetration into the matrix pores, although the energy costs are high. Given the large particle sizes in plant matrices and the difficulties in applying continuous traditional or PSE methods, a solution may be to introduce ultrasonic techniques into the extraction procedure (Lee and Lin, 2007). The use of ultrasonics for improving extraction yield has greatly advanced since the 1950s, with improvements attributed to a phenomenon called cavitation, produced in the solvent by the passage of an ultrasonic wave (Vinatoru, 2001) usually generated by a transducer, which converts mechanical or electrical energy into high frequency vibrations (Romdhane and Gourdon, 2002). Cavitation bubbles are produced and compressed during the application of ultrasonic waves, allowing greater solvent penetration into the raw material and greater intracellular compound release through disruption of the cell walls, thereby considerably reducing the extraction time (Albu et al., 2004; Rostagno et al., 2003). Continuous microwave-assisted extraction is a viable tool for collecting soybean isoflavones, It can be set an 8 min residence time and 73 °C extraction temperature as optimal parameters (Terigar et al., 2010).

Evaluating supercritical carbon dioxide fluid extraction (SFE) for extracting soybean isoflavones and comparing it with Soxhlet and ultra-sonication methods it was evidenced that, although SFE was the most selective method, maximum isoflavones extraction was achieved with ultra-sonication (Rostagno et al., 2002).

Conventional extraction methods primarily focus on solvent choice - methanol, ethanol and acetonitrile being the main options - with varying proportions of acidified and non-acidified water. The second crucial issue is the use of heat and the procedure for mixing the solvent and raw material in order to increase the solubility and transfer of the compounds sought. In this regard, the literature offers contrasting results. Comparing various ethanol and methanol water mixtures (30–80% v/v) in a pressurized liquid extractor it was reported that the optimal isoflavones yield can be achieved with a 70% ethanol solution (Rostagno et al., 2004) however, a 90% v/v methanol solution is the best for isoflavones extraction from soybean samples (Klejdus et al., 2004). Comparing the extraction efficiency of various aqueous (acidified and non-acidified) mixtures of methanol and acetonitrile and it was reported that 58% v/v non-acidified acetonitrile allowed optimal extraction of isoflavones (Lin and Giusti, 2005). A single step extraction method can markedly underestimate the true isoflavones content. Specifically, protein concentration may influence isoflavones extractability due to stronger protein-polyphenol interactions (Achouri et al., 2005). Whey wastewater, generated by precipitation of soy protein isolates, contains high levels of isoflavones, particularly aglycone forms, which readily bond with proteins; in order to overcome this problem, it was suggested foam fractionation and acidic hydrolysis to collect isoflavones using simple equipment and with low energy consumption and good environmental compatibility (Zhang et al., 2007).

There is, therefore, great scope for improving the methodology of isoflavones extraction from soybean. This study is an attempt to establish an ecofriendly method of extraction from milled seeds using simply water as solvent, and to compare different extraction volumes, temperatures, mixing types and times, sonication times and centrifugation speeds. Extraction parameters were tested on 4 soybean varieties to assess whether isoflavones composition affects the extraction yield.

2. Materials and methods

2.1. Plant materials

Seeds of four soybean varieties, *Luna, Bahia*, *Demetra* and *Hilario*, were collected from field trials at the University of Padua's experimental farm (Legnaro, Padua, Italy) and stored at room temperature until analysis. The varieties belonged to different maturity classes (from 0 to 1+) and had varying chemical compositions (Table 1 SI - Supplementary Information).

2.2. Preparation of soybean flour

The milling conditions were selected on the basis of preliminary trials comparing four types of mill with the seeds of the variety Luna: a) large hammer mill (500 Universal, Peruzzo s.r.l., Curtarolo, Padua, Italy); b) electronic disk mill (ZM 200, Retsch GmbH & Co. KG, Haan, Germany); c) disk rotary type mill (SM100 Confort, Retsch GmbH & Co. KG, Haan, Germany); and d) cooled blade mill (M20, IKA-Werke GmbH & Co. KG, Staufen, Germany).

Non-defatted flours were obtained by grinding (mean value 60 mesh) intact seeds with the four mills following traditional extraction with 7 mL 80% v/v methanol solution (Sigma-Aldrich, Chemie GmbH, Steinheim, Germany) on 0.1-g samples.[12]

2.3. UV light irradiation

For UV light irradiation, the soybean flour was placed in a laminar flow hood (Polaris48, Steril s.p.a., Italy) in a 1-cm thick layer. The apparatus was equipped with a UV-C lamp tube (Germicidal 3′T8 Tube) emitting at a 253.7 nm wavelength with a nominal power of 30 W. The irradiation process was carried out for 30, 60, 120 and 180 min at a constant temperature of 25 °C.

2.4. Experimental conditions of isoflavones extraction

To establish the best solid-to-liquid extraction ratio, several dilutions were tested: 1:5, 1:7, 1:20, 1:50, and 1:70 weight of flour-to-solvent volume (g/mL) ratios using deionized water (obtained with a Millipore-Q purification system, Millipore Corp., New Bedford, MA, USA). The best performing mixture was treated by heating at temperatures of 25 °C or 40 °C for different times (6 min, 2, 5, 18 and 24 h) while constantly stirring the samples. For the purposes of comparison, the solutions were shaken with magnetic, horizontal or vertical stirrers at 70 rpm. We then assessed whether a sonication procedure, applied to the flour-water solution for 2, 4, 8, 10 and 15 min, resulted in comparable isoflavones extraction to non-sonicated samples. Lastly, different centrifugation speeds were compared: 6000, 8000 and 12000 rpm for 12 min in order to separate the extracts from the insoluble fraction (pellet). Three