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Analytical Methods

Isoflavone extraction from okara using water as extractant

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ABSTRACT

We here report on the use of water as a 'green' extraction solvent for the isolation of isoflavones from okara, a by-product of soymilk production. At a low liquid-to-solid ratio of 20 to 1 and 20 °C, 47% of the isoflavones that can be extracted with 70% aqueous ethanol were extracted. The malonyl-glucosides were fully recovered with a ratio of 20 to 1, while β-glucosides were recovered with an increased liquid-to-solid ratio of 40 to 1. The extraction of aglycones was better at higher ratios, but leveled off before reaching a 100% yield. Temperature hardly affected the total amount of isoflavones. At a 20 to 1 ratio, 20 °C, and pH 10, there was no significant difference ($p > 0.05$) between isoflavone extraction in water and in 70% aqueous ethanol. The results suggest that water may be used as a green alternative for separation of isoflavones from okara.

1. Introduction

Okara is the by-product of soymilk and tofu production. Okara can be used in traditional dishes immediately after production, but when produced in large quantities on industrial scale, the by-product becomes a waste stream, usually distributed to farmers or incinerated (O'Toole, 1999; Rinaldi, Ng, & Bennink, 2000). One peculiar characteristic of okara is its high moisture content of about 80%, which makes it very susceptible to spoilage, and seems to be the largest challenge for economically attractive utilisation of this by-product. One route is therefore to dry the okara to allow storage and transportation to a site at which it can be further processed. However, okara is known to be difficult to dry (Choicharoen, Devahastin, & Soponronnarit, 2010), and research has increased in recent years to improve the efficiency of its drying without damaging the okara and its components (Perussello, Mariani, & do Amarante, 2012; Wachiraphansakul & Devahastin, 2007). Drying is not only energy intensive; it also degrades the okara due to the heat load, which reduces its economic value. Another approach is therefore to bypass the drying and immediately process okara in the wet state for the recovery of valuable components (Jankowiak, Trifunovic, Boom, & van der Goot, 2014). The use of okara without pre-processing would preserve isoflavones, which are sensitive, heat-labile bioactive components. Isoflavones belong to a group of polyphenols believed to be partially responsible for the health benefits of soy (Cederroth & Nef, 2009; Messina, Persky, Setchell, & Barnes, 1994), and the recovery of bioactive components from by-products has become a research area of great interest (Galanakis, 2012). Twelve isoflavones have been isolated from soybean, which can be classified in four main groups namely the aglycones (daidzein, genistein, and glycitein), the β-glucosides (daidzin, genistin, and glycitin), the malonyl-glucosides (malonyl-daidzin, malonyl-genistin, and malonyl-glycitin), and the acetyl-glucosides (acetyl-daidzin, acetyl-genistin, and acetyl-glycitin) (Wang & Murphy, 1994).

The total isoflavone concentration and the different isoflavone forms present in the soybeans and their products (including okara) depend on the soy variety, its cultivation, the process and the storage conditions (Jackson et al., 2002; Jung, Murphy, & Sala, 2008; Kao, Lu, Hsieh, & Chen, 2004; Rickert, Meyer, Hu, & Murphy, 2004). The most common chemical changes in the isoflavone form include the decarboxylation of the malonyl to acetyl-glucosides and the ester hydrolysis of the acetyl or malonyl-glucosides to β-glucosides. Furthermore, cleavage of the glucosidic bond leads to an increased amount of aglycones (Coward, Barnes, Setchell, & Barnes, 1993; Kao et al., 2004). Soaking times and temperatures applied during processing lead to increased amounts of aglycones and glucosides in the according soy system (Kao et al., 2004; Wang & Murphy, 1996). Cold-grinding instead of hot-grinding of the slurry during soymilk production also leads to an increased amount of aglycones due to prolonged β-glucosidase activity (Prabhakaran & Perera, 2006).

The isoflavone production process usually comprises at least the two steps of extraction and purification. The extraction step often uses a large amount of organic solvents, while the purification step involves multiple chromatography columns. Therefore, there is a clear demand for alternative, more efficient and

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environmentally friendly ways to produce isoflavones. Aqueous ethanol is in general considered a good solvent for extraction of isoflavones from various sources (Achouri, Boye, & Belanger, 2005; Chang & Chang, 2007; Rostagno, Villares, Guillamon, Garcia-Lafuente, & Martinez, 2009). Also for okara, a wide range of aqueous ethanol was tested with the result of 50–70% ethanol being superior amongst the solvents tested regarding yield and purity of isoflavones in the extract (Jankowiak et al., 2014). The use of high ethanol concentrations in the solvent may require either drying as a pretreatment of okara to avoid dilution of the ethanol, especially if an industrially interesting low liquid-to-solid ratio is desired, or an extremely large amount of ethanol. Drying as well as distillation are both undesired operations as they are energy intensive and expensive. That is why water was tested as an alternative solvent for isoflavone extraction. In the scope of green separation technologies in modern food processing, aqueous extractions are considered in many cases more favourable in terms of energy and cost than any organic solvent extraction. A further reason to test the potential of water as solvent was the existence of isoflavones in okara in glucosylated forms, whose nature suggests good solubility in water. However, the 12 different structures of isoflavones in soy may generate difficulties since some are relatively apolar. Nevertheless, it can be a good solvent to recover the naturally present forms from the okara, leaving the altered forms in the okara. Previous studies show the potential of using hot, pressurised water or alkaline extraction for the extraction of flavonoids, but those results cannot be directly translated to soy, because the optimal extraction method and solvent largely depend on the target compounds and the surrounding matrix. Polyphenols include more than 4000 flavonoids with their solubility depending on their natural structure (Ignat, Volf, & Popa, 2011; Ko, Cheigh, & Chung, 2014). In case of isoflavones from soy, it is known that some of the soy isoflavones are rather instable in their natural state, and will be affected by high temperatures, high pressures and long extraction procedures. Matrix components of okara such as proteins complicate the targeted extraction of isoflavones, especially when using water as solvent (Jankowiak et al., 2014). The fact that other solvents, such as methanol and ethanol allow good extraction (Rostagno et al., 2009) could explain why only very few studies were conducted using water as solvent for soy isoflavones (Chang & Chang, 2007; Li-Hsun, Ya-Chuan, & Chieh-Ming, 2004). Therefore, the systematic investigation of different processing parameters on the isoflavones and their isolation in a water environment is indispensable for a basis of new environmentally friendlier ways to process isoflavones.

The selection of the solvent is part of the overall process design, which can be done based on the partitioning of the isoflavones over the okara and the extraction phase, which can be estimated experimentally, by matching the octanol–water partition coefficient ($\log P$) or by estimating the solubility parameters, based on models such as the UNIFAC/UNIQUAC model (Galanakis, Goulas, Tsakona, Manganaris, & Gekas, 2013). The extraction does not only depend on the equilibrium solubility between the matrix and the solvent, but also depends on the nature of the binding in and accessibility of the matrix. Therefore we combine the solubility criteria with experimental extraction trials to find alternative process routes for the recovery of isoflavones from the by-product okara.

The solubility of isoflavones in a solvent depends on many different factors. There is the structure of the solute itself, which determines its polar/non-polar nature, hydrophobicity and tendency to form hydrogen bonds, but also parameters such as temperature, pH, and liquid-to-solid ratios influence the solubility of isoflavones in a solvent. Furthermore, the behaviour of isoflavones towards solvents can vary depending on the matrix they have to be extracted from (Malaypally & Ismail, 2010; Murphy, Barua, & Hauck, 2002).

Up to date, there is no data available on the behaviour of isoflavones in the matrix of okara, which after processing occurs with rather high water content. Elucidating the specific reactions and mechanisms occurring in this system will help to retain the most natural profile of the isoflavones during processing or to obtain the isoflavone profile that is wanted in the product, and support the development of a milder and more environmentally friendly process to recover isoflavones from okara. The objective of this study is therefore to investigate the potential of water as extraction solvent for the recovery of isoflavones from okara. Results were compared to an extraction with 70% ethanol, and different parameters tested in order to discuss their effect on the yield and profile of an isoflavone extract.

2. Materials and methods

2.1. Materials

Ethanol (Sigma Aldrich Co., Schnelldorf, Germany) and Milli-Q water (Q-Gard 2 Purification Pack, Millipore, France) were used for extraction. Methanol and formic acid for HPLC analysis were purchased from Sigma Aldrich Co. (Schnelldorf, Germany) and acetonitrile from Biosolve B.V. (Valkenswaard, The Netherlands). Isoflavone standards: daidzin, glycitin, genistin, daidzein, glycitein, genistein, acetyl-daidzin, acetyl-glycitin, acetyl-genistin, malonyl-daidzin, malonyl-glycitin, and malonyl-genistin were purchased from Nacalai USA Inc. (San Diego, USA). The standards were dissolved in analytical grade DMSO (Sigma Aldrich Co., Schnelldorf, Germany). Solutions containing all 12 isoflavones standards with concentrations of 0.05–100 μg/g were prepared using methanol and stored at −20 °C. All solvents were HPLC grade.

2.2. Okara production

The okara was produced with an ASC50 soymilk system (ProSoya Inc.; Ottawa, Canada). Soybeans were soaked for 2 h to soften the beans before adding the mixture into a cooker grinder vessel in a ratio 7:1 (14 l water:2 kg soybeans). After grinding, the slurry was heated up by steam injection and cooked for 3 min at 105 °C. Before the separation of okara from the soymilk, the slurry passed through a deodorizer vessel to remove undesired volatiles responsible for the beany flavour of the soymilk. At the outlet, the soy slurry had a temperature around 80 °C. A filter centrifuge separated the fibrous by-product okara from the soymilk. The mass balance of this process is described by Eq. (1). The okara was stored at −20 °C in sealed containers until analysed.

$$\text{Soybeans (2 kg)} + \text{Water (14 kg)} + \text{Steam (7.2 kg)} = \text{Soymilk (20.2 kg)} + \text{Okara (3 kg)} \quad (1)$$

2.3. Moisture content

The moisture content of okara was analysed by heating the samples for 60 min at 130 °C. The dry matter was determined in order to calculate the concentrations of isoflavones on dry weight basis. Nevertheless, the isoflavones were extracted from the wet okara. The water present in the okara was taken into account when the total amount of the extraction solvent was identified.

2.4. Ethanol extraction

For the ethanol based extraction, pure ethanol was added in a tube containing wet okara until a ratio of 20 to 1 on dry basis was obtained. A final ethanol concentration of 70% was achieved taking the water content of okara into account. The samples were