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Micellar systems of aliphatic alcohol ethoxylates as a sustainable alternative to extract soybean isoflavones

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ABSTRACT

Ethoxylated aliphatic surfactants belonging to the Genapol and Tergitol series were assessed as extraction systems of isoflavones. They showed good extraction properties when compared with different solvents, the Genapol X-080 exhibiting the best performance. Available commercial isoflavone pills were used, as a starting simple matrix, to determine the parameters that affect the extraction procedure. The temperature and the surfactant concentration showed to be factors that favored significantly the extraction performance. The application of optimized variables (Genapol X-080 11% m/m, pH 4.5; extraction temperature of 54 °C and extraction time of 60 min) on soybean flour (natural) allowed extracting 3.237 ± 0.173 mg of isoflavone per gram of treated flour. This result was three times what it was for methanol under identical conditions. Extraction with these micellar systems represents a sustainable alternative methodology for industrial purposes due to its low cost, biodegradability, non-toxicity and easy scaling up.

1. Introduction

For centuries, soybean has supplied most of the protein requirements in the Asiatic diet and since its industrialization, in the 1940s, it has also become a significant part of the Western human and animal diet (Fernandez-Lopez, Lamothe, Delample, Denayrolles, & Bennetau-Pelissero, 2016). Soybean is mainly used to produce oil and protein derivatives, however, it is increasingly considered as an important source of bioactive phytochemicals such as isoflavones, saponins, phenolic acids and protease inhibitors (Luthria, Biswas, & Natarajan, 2007). Isoflavones (IF) are present in soybean products under two chemical forms (Fig. S1, Supplementary Material): aglycones (i.e., daidzein, glycitein and genistein) and their β-glycosides (i.e., daidzin, glycitin and genistin). In addition, β-glycosides may be conjugated as malonyl/acetylglycosides (i.e., 6′-O-malonyldaidzin, 6′-O-acetyldaidzin, 6′-O-malonylglycitin, etc.) (Murphy et al., 1999). Among these phytochemicals, daidzein and genistein, have showed protective properties such as reducing the risk of cardiovascular disease, lowering rates of some types of cancer and preventing menopause symptoms (Dong, Xu, Sikes, & Wu, 2013; Filiberto et al., 2013; Park, Ju, Park, & Han, 2013). However, they have also been considered to be potent endocrine disruptors, due to their estrogen-mimetic activity, thus becoming potential triggers of reduced fertility (Fernandez-Lopez et al., 2016; Omoruyi, Kabiersch, & Pohjanvirta, 2013). In addition, high consumption of soy isoflavones in Asian-American children has been associated with an increased risk of Kawasaki disease (Portman, Navarro, Bruce, & Lampe, 2016). These controversial effects need of further investigations to be clarified. In the meantime, removing IFs from the general population diet and reserving them for specific applications is a proper conduct to be adopted (Bennetau-Pelissero, 2017). In this way, developing scalable processes for recovering IFs from soybean byproducts will allow both to obtain an IF-enriched extract, consumable by those people susceptive of its beneficial properties, and to render safer IF-free soybean derivatives for population sensitive to their adverse effects.

Solvent extraction has been widely used for IF recovering due to its high efficiency, simplicity and easy scaling up (Murphy, Barua, & Hauck, 2002; Xu & He, 2007). Aqueous methanol, ethanol, and acetone solutions are typical solvents used for this purpose (Rostagno, Palma, & Barroso, 2003). Recently, a wide range of new solvents non-toxic, non-inflammable and biodegradable are been evaluated in order to develop sustainable and green extraction methods (Bajkacz & Adamek, 2017). Among them, certain surfactants fulfill the mentioned properties thus representing an economical alternative to hazardous, expensive organic solvents. Surfactants are amphiphilic molecules with the ability to form aggregates, namely micelles, above a critical micelle concentration (CMC) (Yazdi, 2011). These assemblies can interact with either hydrophilic or lipophilic molecules through hydrophobic and dipolar

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interactions and hydrogen bonding, these features being useful for separation purposes (Sharma, Kori, & Parmar, 2015). Several surfactants such as sodium dodecyl sulfate, Triton X-100, PEG 2000 and Brij 35 were evaluated to extract polyphenols from fruit juices (Hosseinzadeh, Khorsandi, & Hemmaty, 2013; Sharma et al., 2015). In a previous work (Cordisco, Haidar, Coscueta, Nerli, & Malpiedi, 2016), our research group demonstrated that aqueous micellar two-phase systems of Triton X-114 were capable of recovering the 93% of IFs from soybean flour with a purification factor of almost 10 under adequate conditions of time, concentration and temperature. Despite this good performance, the UV absorbance signals of Triton X-114, which overlap those of IFs, make the analytical evaluation of process progress difficult, this being a disadvantage at industrial scale. Other surfactants with similar extraction and eco-friendly properties but transparent at 240–280 nm spectral range would be desirable. Ethoxylated primary and secondary aliphatic alcohols such as those belonging to the Genapol and Tergitol series (Fig. S2, Supplementary Material) possess these characteristics. Particularly, aqueous micellar two-phase systems of Genapol X-080 (GX080) has been successfully applied to recover and quantify vitamins A and E from human serum (Sirimanne, Patterson, Ma, & Justice, 1998) and those formed by Tergitol 15-S-7 (Tg7) and Tergitol 15-S-9 (Tg9) have been used to extract polycyclic aromatic hydrocarbons from aqueous solutions (Alibrahim, 2014).

Regard to IF recovery, dissimilar results were reported. According to He et al. (2005) the micellar systems of GX080 showed to be successful at extracting daidzein from Puerariae radix, however, Luthria et al. (2007) found these systems to exhibit a poor performance at recovering total IFs from soybean under their experimental conditions. A complete study of these micellar systems, aided by rigorous statistical tools, would be required to understand these discrepant reports and to define whether the GX080 systems are adequate to extract isoflavones.

In this context, the goal of this work is to compare rigorously the efficiency of IF extraction by different solvents, focusing the attention in Genapol (GX080) and Tergitols (Tg7 and Tg9) as potential micellar extractants for industrial purposes. In addition, factors that affect significantly the extraction performance will be determined to optimize the process.

2. Experimental

2.1. Materials

The surfactants Genapol X-080 (GX080); Tergitol 15-S-7 (Tg7); Tergitol 15-S-9 (Tg9); Triton X-114 (TX114) and sodium dodecyl sulfate (SDS) were supplied from Sigma-Aldrich (St. Louis, MO, USA). Polyethylene glycols of molecular mass 8000 (PEG8000) and 600 (PEG600) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (MetOH) and ethanol (EtOH) absolute were of HPLC grade. All the reagents were used as received without further purification. Isoflavone standards (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in pure methanol until obtaining the following concentrations: daidzin 1.5–80.0 μg/mL; daidzein 0.3–17.0 μg/mL; genistin 0.7–40.0 μg/mL and genistein 0.2–13.0 μg/mL. All the other reagents were of analytical grade and used without further purification. Deionized water was used to prepare all the solutions.

Soy isoflavone tablets containing 79 mg IF/g were purchased at a local pharmacy.

Soybean flour was supplied by the food processing company Molinos Río de la Plata SA (San Lorenzo, Argentina).

2.2. Extraction procedures

The extraction efficiency of several solvents was determined by selecting commercial isoflavone tablets as solid matrix to minimize interferences. The pills were placed in a glass mortar and crushed with the pestle until obtaining homogenous fine particles. This powder was divided into fractions of 0.14 g which were introduced into the bottom of glass tubes. Then, 2 mL of the following solvents were added on each sample: water, ethanol (99 and 60% m/V), methanol, GX080 (1, 5 and 10% m/m), Tg7 (1, 5 and 10% m/m), Tg9 (1, 5 and 10% m/m), TX114 (5% m/m), SDS (5% m/m), PEG8000 (5% m/m), PEG600 (5% m/m) and NaOH 10 mM. The heterogeneous matrix-solvent systems were mixed vigorously for 30 min at a constant temperature (25.0 °C ± 0.1 °C) and then centrifuged in Eppendorf tubes at 12000 rpm for 10 min. The supernatants (extracts) were separated for analytical evaluation.

The performance of GX080 at recovering IFs from tablets (screening experiments and optimization, Section 2.4) was evaluated through the mentioned extraction procedure, carried out at several conditions of surfactant concentration (5–15% m/m), temperature (25.0–55.0 °C), pH (4.5–8.0) and incubation time (10–110 min).

Extraction of IFs from their natural matrix, the soybean flour, was also assessed. Different amounts of soybean flour were weighed and placed in glass tubes containing approximately 10 g of GX080 solution (10% m/m pH 4.5), thus leading to systems of flour/extractant ratios within the range 1–16% mass of soybean flour/mass of aqueous micellar system (F/AMS). After mixing vigorously for 60 min at a constant temperature (54.0 °C ± 0.1 °C), and centrifuging, the supernatants were separated for determination of total phenolic content and chromatographic analysis.

2.3. Analytical determinations

The total phenolic content (TPC) of extracts, was determined by the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965) modified. The procedure consisted in mixing 30 μL of each supernatant (or its dilution if necessary) with 200 μL of the Folin-Ciocalteu reagent (diluted 1:10) and 100 μL of anhydrous sodium carbonate solution (7.4% m/V). After shaking thoroughly and incubating for 30 min at 25 °C, the absorbance of the resulting blue mixtures was measured at 765 nm. A calibration curve of gallic acid (0.025–0.200 mg/mL) was prepared in order to express the results as milligrams of gallic acid equivalents per milliliter of sample (mg GAE/mL). Incubation and absorbance measurements were performed on the Multiskan GO spectrophotometer (Thermo Fisher Scientific Corporation) operated using the SkanIt 3.2 software (Thermo Fisher Scientific Corporation).

Extracts with high TPC were also analyzed by thin layer chromatography (TLC). TLC was carried out by the one-way ascending technique using pre-coated plates with silica gel (Merck & Co.). The plates were developed in a solvent system of ethyl acetate/methanol/water (100:13.5:10), and then were dried and visualized under ultraviolet light (254 nm). The retention factor (Rf) of each spot was calculated and compared with those of standard daidzein, daidzin, genistein, and genistin. The densitometric chromatogram was obtained by acquiring a UV image of the plate with the UVP-Chromato-Vue C-75 cabinet and analyzing the spot intensity pattern with the aid of the software Fiji/ImageJ (Schindelin et al., 2012).

Optimized GX080 extract and methanol extract (as reference) were conveniently diluted (1:200) and analyzed by high-performance liquid chromatography (HPLC). The procedure was performed in a reverse phase column coupled with a guard column containing the same stationary phase (COSMOSIL 5C18-AR-II Packed Column – 4.6 mm I.D. × 250 mm). Separation of isoflavones was carried out by applying the operating conditions described previously (Cordisco et al., 2016). The separation analysis was performed using a Waters e2695 separation module system interfaced with a photodiode array UV/Vis detector (PDA 190–600 nm). The identification and quantification of daidzein, daidzin, genistein and genistin in the GX080 extract were performed by comparison of the retention times and the absorption spectra (peak area) with those corresponding to the standard of each isoflavone.