Industrial Crops and Products 67 (2015) 414–421

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

A new approach for the purification of soybean acid extract: Simultaneous production of an isoflavone aglycone-rich fraction and a furfural derivative-rich by-product

Marina Cardoso Nemitz, Helder Ferreira Teixeira, Gilsane Lino von Poser*

Programa de Pós Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul Av. Ipiranga, 2752, 90610-000 Porto Alegre, Brazil

ARTICLE INFO

Article history:
Received 7 November 2014
Accepted 31 January 2015
Available online 12 February 2015

Keywords:
Defatted soybean
Acid hydrolysis
Isoflavone aglycones
5-Hydroxymethylfurfural
5-Ethoxymethyl-2-furfural

ABSTRACT

Isoflavones are found mostly in legumes, such as soybeans. These compounds have several therapeutic potentials, largely attributed to their aglycone forms. The soybean extraction followed by acid hydrolysis is the usual process for coverting isoflavone glucosides into their bioactive compounds. However, under extreme pH, different compounds, characterized as contaminants or even having important properties, can be obtained. In this context, this paper aimed to produce an isoflavone aglycone-rich fraction (IAF) from a soybean rich cultivar, as well as isolate and identify other compounds formed during the IAF production. The extraction of defatted soybean seeds was performed with ethanol–water (80:20, v/v) in a Soxhlet apparatus, followed by acid hydrolysis with 1.3 M HCl, under reflux at 80 °C at different times (0–360 min). During the hydrolysis procedure, besides the isoflavones, two unknown compounds were produced in high amounts. These compounds were isolated by column chromatography and identified as 5-hydroxymethylfurfural (HMF) and 5-ethoxymethyl-2-furfural (EMF), both isolated for the first time from soybeans. These substances are derived from sugars, classified as furanic derivatives, possibly toxic for medicinal uses, but with significant importance in the biofuel field. In order to produce an IAF intended for a future food or pharmaceuticals use, the soybean acid extract was submitted to liquid–liquid partition, solid phase extraction, and antisolvent precipitation processes, resulting in a fraction with high isoflavone aglycones purity (92%). From this process, the removed impurities originated a furfural derivative- rich solution, which can be an attractive by-product for the biofuel field.

1. Introduction

Soy (*Glycine max* (L.) Merr) is a bushy, coarse annual crop, long established and cultivated in many countries. Its seeds are basically used as feed for livestock and aquaculture, oil production and human food. Besides, the soybean products have been largely used as functional foods due to the presence of macronutrients such as proteins, carbohydrates and lipids, and micronutrients such as sterols, saponins and isoflavones (Cederroth et al., 2012; Chen et al., 2012; Song et al., 2013).

Isoflavones are helpful in preventing certain cancers, reducing the risk of cardiovascular diseases, improving bone health and in the prevention of skin damage (Barnes 1998; Cederroth et al., 2012; Chen et al., 2012; Nemitz et al., 2014). The biologically active compounds of soy isoflavones include mainly the aglycone forms, genistein and daidzein. However, the isoflavones present in soybeans are mostly in the beta-glucoside, 6-O-malonyl-beta-glucoside or 6-O-acetyl-beta-glucoside forms (Chen et al., 2012). In order to obtain the aglycone forms, usually hydrolysis is performed during extraction, and the best known and most frequently used catalyst for this process is the diluted hydrochloric acid (HCl) (Nemitz et al., 2014).

The presence of sugars in foods under extreme pH conditions and high temperatures often induce Maillard reaction (at pH 4–7), or caramelisation process (at pH < 3). Both phenomena can result in furanic aldehydes production, as 5-hydroxymethylfurfural (HMF) and furfural (Shen and Wu, 2004). These compounds, in high amounts, are considered toxic for human nutrition or pharmaceutical use (Toker et al., 2013). For this reason, the content of furanic aldehydes should be evaluated during food processing or development of medicinal products. In this regard, several studies have reported the analysis of HMF in foods (Abraham et al., 2011; Toker et al., 2013; Yılmaz and Gokmen, 2013), as well as in pharmaceutical products (Ulbricht et al., 1984; Hewala et al., 1993) and cosmetics

* Corresponding author. Tel.: +55 51 33085529; fax: +55 51 33085437.
E-mail address: gilsane@farmacia.ufrgs.br (G.L.v. Poser).

http://dx.doi.org/10.1016/j.indcrop.2015.01.074
0926-6690/

(Rodrigues et al., 2015). In the case of soybeans, Rufián-Henares et al. (2009) reported the HMF analysis as a quality indicator during the drying-toasting step of soybean flour used by the cereal products industry.

On the other hand, C6-sugars (e.g., D-glucose, D-fructose) are interesting precursors for chemicals compounds with potential industrial application. It is a great challenge to convert biomass-derived carbohydrates by economical processes for the production platform of chemicals such as ethanol, and other types of biofuels, e.g., HMF derivatives (Balakrishnan et al., 2012). When the production of these compounds is desirable in high amounts, the conversion of food sugars into furanic compounds can be performed by thermal systems containing catalysts, such as organic or inorganic acids, organic or inorganic salts, Lewis acids, ion-exchange resins and zeolites (Hu et al., 2012). Acid catalyzed etherification and reductive etherification of HMF in alcoholic solvent provide routes to form the 5-ethoxymethyl-2-furfural (EMF), which can be proposed as a potential liquid biofuel for the future, because it has a high energy density of 8.7 kWh/L, similar to that of regular gasoline (8.8 kWh/L), nearly as good as that of diesel (9.7 kWh/L), and significantly higher than that of ethanol (6.1 kWh/L) (Gruter and Dautzenberg, 2007).

In view of the biological potential of isoflavone aglycones (IA), the goal of this study was the extraction of soybeans, followed by acid hydrolysis and purification processes, intended to obtain an isoflavone aglycone rich-fraction (IAF) for pharmaceutical and/or food applications. Additionally, based on the extensive use of soy in different industrial sectors, and the diversity of the compounds present in this plant, it was also aimed the isolation and identification of the major compounds formed during the acid hydrolysis procedure.

2. Materials and methods

2.1. Chemicals and plant material

Deuterated chloroform (CDCl3), trifluoroacetic acid (TFA), methanol and acetonitrile HPLC grade were obtained from Merck (Darmstadt, Germany) and all the other chemicals were of analytical grade. Isoflavone standards: daidzein, genistein and glycitein were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Ultrapure water was obtained from a Milli-Q apparatus (Millipore, Billerica, USA). Stock and working standards of isoflavones were prepared by dissolving these compounds in methanol, and the standard solutions stored at 4 °C.

Soybean Brazilian cultivar, EMBRAPA BRS 262 (2011/2012), was kindly donated by SEMEL Sementes (São Paulo, Brazil). The soybean seeds were ground with an analytical mill (Quimis Q2981, Brazil), sieved with a 0.71 mm screen and stored.

2.2. Extraction

Powdered soybeans were defatted in a Soxhlet extractor using *n*-hexane at the boiling point for 9 h and drug-to-solvent ratio of 1:10 (w/v) in accordance with Rostagno et al. (2002). The remaining material, the defatted soybeans (DSS), was dried at room temperature and stored. Twenty grams of DSS were weighted in filter paper and placed in a 100 mL Soxhlet glass thimble. The extraction was carried out using 200 mL of ethanol 80% in water (v/v) at the solvent boiling point (70–80 °C) for 9 h. The crude extract was reserved for posterior acid hydrolysis.

2.3. Hydrolysis profile

The extract obtained was subsequently transferred to a reflux apparatus (two neck flask reactor) where HCl was added to give a final concentration of 1.3 M, and the temperature was maintained at 80 °C. At determined kinetic points (0, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min), hydrolyzed samples were removed, diluted, filtered and injected in HPLC.

2.4. Solvent selectivity to liquid–liquid (L–L) partition

The acid extract was evaporated under reduced pressure to remove ethanol, leaving only an aqueous phase. A double amount of water was added into this phase, and the mixture was partitioned with different organic solvents to evaluate the recovery of each interesting compound. The organic solvents analyzed were diethyl ether (Et_2O) and ethyl acetate (EtOAc). The salting-out procedure was also evaluated by adding 20% potassium chloride (KCl) in the aqueous phase. For all analysis, the L–L partition was performed in a separation funnel at a ratio of 8:2 (extract/solvent), for four-fold, and washed with water for three times to neutralize the extract. To analyze the mainly compounds, the solvent-partitioned fractions (SPF) were diluted, filtered and injected in HPLC.

2.5. Purification of isoflavone aglycone-rich fraction

The EtOAc salting-out SPF was evaporated under reduced pressure, subjected to silica gel column (CC_1) and eluted successively with a gradient system of *n*-hexane: methylene chloride (CH_2Cl_2) at different volumes and proportions: 200 mL of *n*-hexane: CH_2Cl_2 (50:50, v/v), 200 mL of *n*-hexane: CH_2Cl_2 (25:75, v/v), 200 mL of CH_2Cl_2, 500 mL of CH_2Cl_2: EtOAc (75:25, v/v), 200 mL CH_2Cl_2: EtOAc (50:50, v/v) and 200 mL of EtOAc. The fractions were collected from the column, analyzed, by HPLC and evaporated to dryness. Chloroform was added to precipitate the isoflavones in the richest fraction. The precipitate (IAF) and the remaining chloroform solution (CS) were analyzed by HPLC-DAD.

2.6. Isolation of HMF and EMF

The CS was passed through another silica gel column (CC_2), and eluted with a gradient system with CH_2Cl_2: EtOAc of increasing polarity. The new fractions were further purified by a preparative thin layer chromatograph (PTLC), using silica gel 60 F_{254} with CH_2Cl_2: EtOAc (95: 05, v/v) as mobile phase. The two compounds isolated were analyzed to evaluate purity by TLC (with different mobile phase systems and detector reagents) and their structures by ultraviolet spectrum (HPLC-DAD) and NMR analysis.

HMF: yellow gum, UV (λ max 229, 282), ^{1}H NMR ($CDCl_3$, 60 MHz): δ 9.53 (s), 7.24 (d, J = 3.6 Hz, 1H), 6.52 (d, J = 3.5 Hz, 1H), 4.68 ppm (s, 2H), 4.06 (s, OH). ^{13}C NMR ($CDCl_3$, 15 MHz): δ 177.67, 161.10, 151.91, 123.32, 109.77, 57.03 ppm.

EMF: pale-yellow liquid, UV (λ max 229, 281), ^{1}H NMR ($CDCl_3$, 60 MHz): δ 9.62 (s, 1H), 7.24 (d, J = 3.6 Hz, 1H), 6.54 (d, J = 3.6 Hz, 1H), 4.54 (s, 2H), 3.60 (q, J = 7.0 Hz, 2H), 1.24 ppm (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 15 MHz): δ 177.68, 158. 77, 152.57, 122.13, 111.06, 66.54, 64.69, 15.02 ppm.

2.7. Analytical conditions

All analyses were performed in accordance with Yatsu et al. (2014). The HPLC apparatus consisted of a Shimadzu LC-20AT system (Kyoto, Japan), coupled to a photodiode array detector (DAD), controlled by LC-Solution Multi-PDA software. The stationary phase was a Synergi-Fusion-RP column (Phenomenex, 5 mm, 150 mm × 4 μm i.d.), protected with a C-18 guard column. The method consisted of a gradient system of TFA 0.1% in water (phase A) and TFA 0.01% in acetonitrile (phase B), flow rate of 1.0 mL/min, detection at 260 nm, injection volume was 10 μL and temperature