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The impact of high-power ultrasound and microwave on the phenolic acid profile and antioxidant activity of the extract from yellow soybean seeds

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ABSTRACT

The aim of the study was to investigate and compare several extraction protocols like 1) high-power ultrasound probe assisted solvent extraction; 2) microwave assisted solvent extraction; 3) direct acid hydrolysis; 4) direct alkali hydrolysis, and 5) two step extraction consisting of ultrasound or microwave assisted solvent extraction followed by alkaline and acid hydrolysis in terms of efficiency of the extraction of phenolic acids from the yellow soybean seed variety Laura. These extracts were screened for their total phenol content (TPC), and for their antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) assay, as well as on content of some individual phenolic acids. It appeared that the acetone containing mixtures gave much higher TPC than methanol containing ones, but the presence of acid in the methanol solvent significantly improved the extraction of phenolic compounds. To further improve the extraction, an ultrasound lab-scale probe at 20 kHz was used, with 15 and 30% of the maximum amplitude, and the extraction time was varied from 2 to 15 min. Microwave assisted extraction was performed varying the temperature from 55 to 85 °C, microwave power from 25 to 100 W and extraction time from 2 to 10 min. Changes in the content of six phenolic acids were examined: gallic, trans-cinnamic, chlorogenic, caffeic, *p*-coumaric and ferulic acid. The separation and quantification of phenolic acids was accomplished by high-performance liquid chromatography-diode array detection (DAD) procedure. The results suggested that microwaves contributed to more efficient extraction of phenolic acids from the seed of yellow soybean. The amount of phenolic acids varied from 65.52 μg/g of dry matter (d.m.) for caffeic acid, to 581.84 μg/g d.m. for *p*-coumaric acid. Both, ultrasound and microwaves contributed to more efficient extraction of total phenol compounds and enhanced antioxidant activity of soybean seed extracts. TPC varied from 12.48 to 18.77 mg GAE/g d.m. and antioxidant activity varied from 244.58 to 345.21 μmol TROLOX eq/g d.m.

1. Introduction

Phenolic acids are naturally occurring antioxidant compounds found in plants and foods of plant origin, receiving tremendous attention among nutritionists, food scientists and consumers due to their roles in human health (D'Archivio et al., 2007; Dai and Mumper, 2010). They have different functions in plants including assimilation of nutrients, protein synthesis, enzyme activity, photosynthesis, cell signaling and protection against adverse environmental conditions (Ho et al., 1992). Besides being oil and protein source, soybean (*Glycine max* L.) is increasingly being recognized as a source of polyphenolic compounds, among which phenolic acids are particularly interesting because they have roles as diverse as bestowing color and flavor characteristics and protection role against cancer and heart diseases (Kajdzanoska et al., 2011; Kroon and Williamson, 1999; Kroll et al., 2003).

Phenolic acids are non-flavonoid phenolic compounds which can be divided by their chemical structure into two classes: derivatives of benzoic acid such as gallic acid, vanillic acid or syringic acid, and derivatives of cinnamic acid like coumaric, caffeic, ferulic, chlorogenic acids and others. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall (D'Archivio et al., 2007). The qualitative and quantitative composition of phenolic

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acids varies between different soybean varieties and stages of plant development as well as between different parts of the plant (Romani et al., 2003; Ho et al., 2002). As they are present in all plant organs, they are inevitable in human nutrition. Their potential benefits in reducing certain human health related disorders are becoming increasingly apparent such as: lowering blood sugar, regulating body weight, anti-cancerous, anti-inflammatory, antithrombotic and rejuvenating effects (Cardoso Nemitz et al., 2017; Leopoldini et al., 2011).

Extraction of bioactive compounds from plant material is an important step in the production of phytochemicals as additives for formulations of dietary supplements, food ingredients, pharmaceutical and cosmetic products. The yield of the extraction depends on the type of solvents (polarity), extraction time and temperature, solvent to sample ratio, as well as the chemical composition and physical characteristics of the samples. The plant material may contain a range of phenolic compounds ranging from simple (e.g., phenolic acids, anthocyanins) to highly polymerized substances (e.g., tannins) in varying amounts. Therefore, there is no uniform or completely satisfactory procedure that is suitable for extraction of all phenolics or a specific class of phenolic substances in plant materials (Antolovich et al., 2000). Although there are several studies on soy isoflavones and other phenolic compounds (Pananun et al., 2012; Lin and Giusti, 2005), there is no comprehensive study regarding the effect of different methods and conditions on the extraction of phenolic acids from soybean.

Natural phenolic compounds can be divided into free, esterified and insoluble-bound forms, depending on whether they occur in the free form or are covalently bound to other molecules such as fatty acids (soluble esters) or insoluble macromolecules (insoluble-bound phenolics). Most insoluble-bound phenolics chemically form covalent bonds with cell wall substances including pectin, cellulose, hemicellulose, arabinoxylanes and structural proteins and account for relatively large amount compared to the soluble phenolics in foods. Phenolic acids are the main bound compounds in natural sources such as cereal grains, legumes and other seeds (Shahidi and Naczk, 2004). There are several food processes that increase the release of bound polyphenols including fermentation (enzymatic, microbiological, etc.) as well as thermomechanical processes, such as extrusion cooking and alkaline hydrolysis (Wong et al., 2006). In fruit and vegetables with higher water content, about 24% of the total phenolic compounds are in bonded form (apples 6.5%, bananas 33.5%, oranges 24.3%, carrots 37.6%, red pepper 9.8%) (Sun et al., 2002). On the other hand, in cereals and legumes with a low water content, the percentage of bound phenolic compounds is significantly higher (corn 85%, wheat 75%, rice 62%) (Adom and Liu, 2002; Zhou et al., 2004).

Acid and alkaline hydrolysis are the most common chemical methods used to extract the insoluble-bound phenolics (Stalikas, 2007). The main variables of these chemical methods are the acid/base concentration, hydrolysis time and temperature. Acid hydrolysis at elevated temperatures results in the loss of some phenols since they are unstable at low pH, thus they can be degraded during the extraction process or storage (Krygier et al., 1982; Verma et al., 2009). Normally, alkaline hydrolysis is conducted at room temperature, leading to lower rate of loss of phenolic acids during the process than the acid hydrolysis method. For example, alkaline hydrolysis appears to cause a loss of 4.8% of ferulic acid in corn grains compared to 78% loss after acid hydrolysis (Krygier et al., 1982). Alkaline hydrolysis has been widely used for the release of insoluble-bound phenolic acids in many types of foods such as cereals, legumes and seeds (Alshikh et al., 2015; Chen et al., 2015; Verardo et al., 2015; Xu and Chang, 2009; Wang et al., 2016). However, the disadvantage of this method is the more complex procedure, requiring further extraction steps after sodium hydroxide hydrolysis to isolate the liberated phenolic acids from food matrices. The effect of addition of ascorbic acid (AA) and ethylenediaminetetraacetic acid (EDTA) in order to prevent the loss of phenolic acids during alkaline hydrolysis is the subject of many studies (Nardini et al., 2002; Peričin et al., 2009; Ross et al., 2009). Addition of 10 mmol EDTA and 1% AA completely prevents the degradation of phenolic acids subject to oxidation by stabilizing their structure. Acid hydrolysis is usually carried out with the addition of concentrated HCl, with or without 10 mmol EDTA and 1% AA, at an elevated temperature (85 °C) for 30 min (Ross et al., 2009).

Recent research in extraction technologies has mainly focused on efficient and innovative techniques for processing plant materials as an eco-friendly alternative to obtain and purify bioactive compounds such as supercritical fluid extraction, subcritical water treatment, enzyme-assisted subcritical water treatment, ultrasound-assisted extraction, microwave-assisted extraction and others (Chen et al., 2014; Cong-Cong et al., 2017). The use of ultrasound and micowave means for extraction purposes is considered as a “green” concept, which could provide higher yield, minimize processing time and reduce energy and solvent consumption. Ultrasound-assisted extraction (UAE) is faster than traditional methods because the contact surface between the solid and liquid phases is increased by partial destruction. The extraction time is shortened to only a few minutes in relation to 2–20 h with traditional methods (maceration/mixing) (Herrera and Luque de Castro, 2004, 2005; Albuquerque et al., 2017). Microwave-assisted extraction (MAE) allows the solvent mixture to be heated by direct interaction with the free molecules present in the system, which leads to the destruction of plant tissue and the release of the components into a solvent (Jokić et al., 2012). This technique combines high temperature and pressure for optimum release of phenolic acids while simultaneously destroying the cell wall (Chiremba et al., 2012). The use of both ultrasound and microwave techiniques for extraction purposes in raw materials seems to play a role in the destruction of the cell wall and, therefore, facilitates the extraction of phenols and minimizes the loss of phenolic compounds due to extreme (too low or too high) pH conditions during the extraction process.

The study presented herein is the first comparative evaluation of the efficiency of several extraction techniques like high power ultrasound assisted extraction, microwave assisted extraction, direct acid and alkali hydrolysis as well as combined ultrasound or microwave assisted solvent extraction with alkaline and acid hydrolysis to extract phenolic acids from the yellow soybean seed, variety Laura.

2. Materials and methods

2.1. Chemicals

Next reference standards of different reagent were used: trans-cinnamic acid (97%), *p*-coumaric acid (98%), caffeic acid (≥98%), trans-ferulic acid (99%), gallic acid (97.5–102.5%), Folin-Denis reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX, 97%), analytical grade water, acetonitrile (≥99.9%), methanol (≥99.9%), were purchased from Sigma-Aldrich (St.Louis, SAD); 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ, ≥99%), was purchased from Fluka, (Buchs, Switzerland); L (+)-ascorbic acid (99%), was purchased from Acros Organics, (New Jersey, SAD); chlorogenic acid (99.5%) was purchased from Chromadex, (SAD); Folin-Ciocalteu reagent was purchased from Reagecon, (Ireland). All other solvents and chemicals were p.a. or higher purity.

2.2. Plant material

Plant grain material of yellow soybean genotype “Laura” (variety lacking in Kunitz trypsin inhibitor protein) was used. All grain samples were milled (in laboratory mill IKA, IP-21, SAD) and sieved through a laboratory sieve of 500 μm diameter. Samples were defatted in petrol ether in a Soxhlet extractor for 4 h. The samples were stored in the freezer before extraction.