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Review

Sample preparation for the analysis of isoflavones from soybeans and soy foods

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ABSTRACT

This manuscript provides a review of the actual state and the most recent advances as well as current trends and future prospects in sample preparation and analysis for the quantification of isoflavones from soybeans and soy foods. Individual steps of the procedures used in sample preparation, including sample conservation, extraction techniques and methods, and post-extraction treatment procedures are discussed. The most commonly used methods for extraction of isoflavones with both conventional and "modern" techniques are examined in detail. These modern techniques include ultrasound-assisted extraction, pressurized liquid extraction, supercritical fluid extraction and microwave-assisted extraction. Other aspects such as stability during extraction and analysis by high performance liquid chromatography are also covered.

Contents

Abbreviations: ε, Dielectric constant; ACE, Acetone; ADi, Acetyl daidzin; AGi, Acetyl genistin; AGly, Acetyl glycitin; ASE, Accelerated solvent extraction; CE, Capillary electromigration techniques; De, Daidzein; Di, Daidzin; DMSO, Dimethylsulfoxide; DSM, Defatted soybean meal; EtOH, Ethanol; Ge, Genistein; Gi, Genistin; Gle, Glycitein; Gly, Glycitin; MAE, Microwave-assisted extraction; MeCN, Acetonitrile; MeOH, Methanol; MGi, Malonyl genistin; MDi, Malonyl Daidzin; MGly, Malonyl glycitin; PLE, Pressurized liquid extraction; PSE, Pressurized solvent extraction; SC-CO_2, Supercritical CO_2; SFE, Supercritical fluid extraction; SPE, Solid phase extraction; SPI, Soy protein isolate; SPME, Solid phase microextraction; SWE, Superheated water extraction; UAE, Ultrasound-assisted extraction.

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1. Introduction

Functional foods are one of the most promising fields concerning nutritional sciences. These food-stuffs are interesting from the consumer point of view with the prospect of maintaining health and preventing diseases by using natural foods as part of the habitual diet, and also from the industry point of view, for the added value of the products. There are several raw materials that can be used for healthy purposes and soybeans are among those with the greatest potential. Soybeans are one of most produced and commercialized commodities worldwide. Actually, there are several foods derived or based on soybeans such as soy milk, tofu and tempeh, and the consumption and use of soybeans (texturized soy protein, concentrated soy protein and soy protein isolate) as additives by the food industry is increasing every year [1–4].

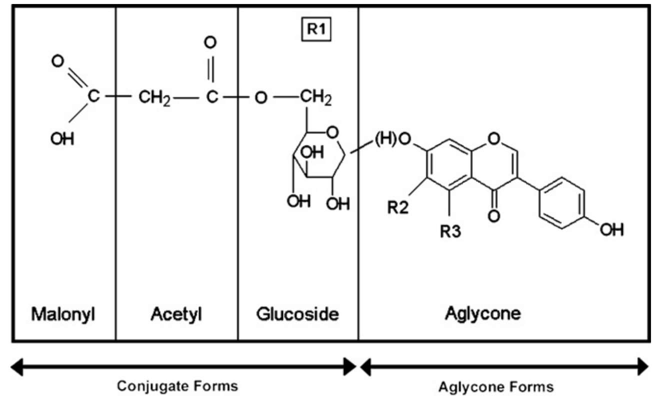
The potential of soybeans as a functional food is being currently explored by the food industry. Indeed, soybeans and soy foods, like soymilk, tofu, miso and tofu, are widely promoted and eaten based on assumed relationships between its consumption and beneficial health effects in humans including chemoprevention of breast and prostate cancer, osteoporosis, cardiovascular disease as well as relieving menopausal symptoms. Evidence provided not only by epidemiological studies showing a lower incidence of these health conditions in Asian countries like Japan and China, which have high soy consumption, but also from intervention studies, is the basis of this relationship [5–12].

During the last decades our knowledge about the dietary impact on health and well-being has been highly increased and often related to specific food components. Several classes of phytochemicals have been identified in soybeans, including protease inhibitors, phytosterols, saponins, phenolic acids, phytic acid and isoflavones [13–16]. Of these, isoflavones are particularly noteworthy because soybeans are the only significant dietary source of these compounds. Isoflavone content in soybeans can range from 0.4 mg to 9.5 mg of total isoflavones per gram, which can be influenced by genetics, crop year and growth location [17–19]. More importantly, these compounds have shown several in vitro and in vivo beneficial properties consistent with the potential soybean effects on health.

There are several possible mechanisms of action by which isoflavones may act on disease prevention, including estrogenic/anti-estrogenic activity, cell anti-proliferation, induction of cell-cycle arrest and apoptosis, prevention of oxidation, anti-inflammatory, regulation of the host immune system, and changes in cellular signaling [7,20–28]. The actual mechanisms in the human organism have not been fully established and metabolism may play an important role. Furthermore, besides of evidence of available epidemiological or intervention studies and "in vitro" observations, there are several reports indicating that several of the specific potential soybean health benefits are linked to isoflavone intake [8,29–32].

However, there is still controversy and an unanimous position about if isoflavones, other soy phytochemicals or components are responsible for the health benefits of soy consumption is still far from being reached. Because the data in humans are not conclusive for any of these possible benefits, it is important to conduct more studies investigating isoflavones and soy foods in the diet to health outcomes. An accurate food composition database is crucial for such studies. That is the reason why there is an increasing interest of scientists focused in developing newer extraction and analysis methods for the characterization of soybean functional components, especially isoflavones, and about the relationships between their consumption and beneficial health effects in humans.

Isoflavones are a subclass of flavonoids and are also described as phytoestrogen compounds, since they exhibit estrogenic activity (similar effects to estradiol hormones). The basic characteristic isoflavone structure is a flavone nucleus, composed by two benzene rings (A and B) linked to a heterocyclic ring C (Fig. 1). The benzene ring B position is the basis for the categorization of the flavanoid class (position 2) and the isoflavonoid class (position 3). The main isoflavones found in soybeans are genistein (4′,5,7-trihidroxyisoflavone), daidzein (4′,7-dihidroxyisoflavone), glycitein (4′,7-dihidroxy-6-metoxi-isoflavone) and their respective acetyl, malonyl and aglycone forms (Fig. 1) [33–39]. Biochanin A and formononetin (which are derivatives of genistein and daidzein) are generally less abundant in soy than the 12 main forms and which are found mostly in clover and alfalfa sprouts [40].



Isoflavone	Symbol	R1	R2	R3
Genistein	Ge	H	H	OH
Daidzein	De	H	H	H
Glycitein	Gle	H	OCH_3	H
Genistin	Gi	$C_6O_5H_{11}$	H	OH
Daidzin	Di	$C_6O_5H_{11}$	H	H
Glycitin	Gly	$C_6O_5H_{11}$	OCH_3	H
Acetyl-genistin	AGi	$C_6O_5H_{11}+COCH_3$	H	OH
Acetyl-daidzin	ADi	$C_6O_5H_{11}+COCH_3$	H	H
Acetyl-glycitin	AGly	$C_6O_5H_{11}+COCH_3$	OCH_3	H
Malonyl-genistin	MGi	$C_6O_5H_{11}+COCH_2COOH$	H	OH
Malonyl-daidzin	MDi	$C_6O_5H_{11}+COCH_2COOH$	H	H
Malonyl-glycitin	MGly	$C_6O_5H_{11}+COCH_2COOH$	OCH_3	H

Fig. 1. Chemical structures of soybean isoflavones and abbreviations.

Isoflavone content of available soy foods in several countries is been intensively investigated. Quantification of isoflavones in the soybeans and soy foods consumed in the USA [40–44], Japan [45,46], Italy [47], UK [48,49], Singapore [43,50], Australia [51], Indonesia [50,51], Brazil [52], and Canada [53] have been published in the last decade.

Besides of individual reports, there are food composition databases and compilations from these values specifically focusing on isoflavone distribution [54–62]. These reports supply useful information to investigators determining the intake of phytoestrogens in order to relate intakes to potential biological activities. Also, they can be used by health professionals and consumers to estimate individual phytoestrogens intake and design personalized diets in order to achieve biologically active concentrations of these functional compounds.