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Ultrasound-assisted extraction and bioaccessibility of saponins from edible seeds: quinoa, lentil, fenugreek, soybean and lupin

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ABSTRACT

The efficient production of saponin-rich extracts is of increasing interest due to the bioactive properties that have being demonstrated for these compounds. However, saponins have a poor bioavailability. In this respect, the knowledge about the bioaccessibility of saponins as a first step before bioavailability has been scarcely explored. In this study, the production of ultrasound-assisted extracts of saponins from edible seeds (quinoa, soybean, red lentil, fenugreek and lupin) was carried out with ethanol, ethanol:water or water. Extraction yield, total saponin (TSC), fat and total phenolics content (TPC) were determined. Then, the bioaccessibility of saponins after the *in vitro* gastrointestinal digestion of the extracts was determined and the effect of TPC and fat in the extracts on bioaccessibility was evaluated.

The highest saponin-rich extracts were obtained by ethanol, being fenugreek and red lentil the richest extracts (12% and 10%, respectively). Saponins from ethanol:water extracts displayed variable bioaccessibility (from 13% for fenugreek to 83% for lentil), but a bioaccessibility closer to 100% was reached for all ethanol extracts. Correlation studies showed that TPC of the extracts negatively affected the bioaccessibility of saponins, whereas fat of the extracts enhanced this parameter.

As summary, ultrasound-assisted extraction is shown as an efficient method for obtaining saponin-rich extracts from edible seeds, being ethanol the most advantageous solvent due to the richness of saponins and the successful bioaccessibility from these extracts, likely caused by the co-extracted fat with ethanol. Regardless of the extracts, phenolic compounds or fat may hinder or enhance the bioaccessibility of saponins, respectively. Additionally, an adequate balance between saponins to lipids has shown to be relevant on such an effect.

1. Introduction

Saponins constitute a wide group of structurally related compounds consisting of a triterpenoid or steroid non-polar aglycone (also known as sapogenin) attached to one or more hydrophilic oligosaccharide moieties through an ether or ester glycosidic linkage. Such combination of polar and non-polar structural elements confers them foaming and emulsifying properties. Saponins are largely distributed in the plant kingdom and are mainly found in the seeds, leaves, roots, fruits and stems. Triterpenoid saponins have been identified in legumes (soybean, lentils, alfalfa and chickpeas, among many others), quinoa seeds, ginseng roots, quillaja bark or liquorice roots, whereas steroid saponins have been found in fenugreek seeds, yucca, ginseng roots, asparagus or oats (Güçlü-Üstündağ & Mazza, 2007; Makkar, Siddhuraju, & Becker, 2007).

Although saponins have traditionally been recognized as anti-nutrients due to their hemolytic activity, their inhibitory activity of digestive enzymes, or their effect on the permeability of the small intestinal mucosal cells, current research is being focused on saponins and sapogenins as bioactive compounds in view of an increasing evidence on their hypocholesterolemic, anti-inflammatory, antitumor, immunomodulatory, antibacterial, antiviral, antifungal and antiparasitic activities (Singh, Singh, Singh, & Kaur, 2017). Taking into consideration the potential of these molecules as bioactive agents, great efforts

Abbreviations: EY, Extraction yield; E, Ethanol extract; E:W, Ethanol:Water extract; FC, Fat content; GAE, Gallic acid equivalents; TPC, Total phenolic content; TSC, Total saponin content; UAE, Ultrasound-assisted extraction

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are being made to obtain saponin-rich extracts from non-conventional extraction methods, as conventional technologies (maceration, Soxhlet extraction, serial exhaustive extraction or hydrodestillation) are time-consuming, require high purity solvents and present low extraction selectivity and efficiency (Nguyen, Pham, Bowyer, Altena, & Scarlett, 2016). Among the most studied non-conventional technologies for the extraction of bioactive compounds from plant materials, ultrasound-assisted extraction (UAE) has been successfully developed for such purpose thanks to significantly reduced extraction times, energy consumption and higher extraction efficiency, although it has not been sufficiently explored in saponin extraction (Cheok, Salman, & Sulaiman, 2014). Both direct and indirect sonication has been applied on the extraction of saponins from different varieties of ginseng roots in order to evaluate the effect of water, methanol and buthanol on the yield of total saponins and ginsenosides (Wu, Lin, & Chau, 2001). Nonetheless, the influence of other green solvents, such as ethanol or water, and their combinations on the total saponin content of the final UAE extracts has been scarcely evaluated, being the studies rather recent (Champa, Whangchai, Jaturonglumlert, Nakao, & Whangchai, 2016; Ha et al., 2006). In the specific case of saponin extraction from edible seeds, the UAE is also novel and scarce (Wani, Bishnoi, & Kumar, 2016).

However, the major challenge in developing saponin-rich extracts for their use as functional ingredients, is their limited gastrointestinal absorption and, consequently, their poor bioavailability (Navarro del Hierro, Herrera, Fornari, Reglero, & Martin, 2018). In any oral-taken compound, its aqueous solubility in the intestinal lumen is one of the key properties that modulates its bioavailability. Generally, saponins are hydrosoluble thanks to the hydrophilic sugar chain(s) in their structures. Moreover, the amphiphilic nature of these molecules grants them the capacity to self-micellate, which increases their dispersion in the aqueous media for further absorption by enterocytes (Böttcher & Drusch, 2017). However, the good solubility of saponins should not be generalized for all saponins, since variable results of water solubility depending on the type of saponin have been described (Güçlü-Üstündağ & Mazza, 2007; Navarro del Hierro et al., 2018). On the other hand, due to the lack of sugar moieties in sapogenins, the aglycones have shown improved chemical properties compared to their precursor saponin that enhance their permeability and bioactivity, such as a lower molecular weight, higher lipophilicity or lower molecular flexibility (Gao, Basu, Yang, Deb, & Hu, 2012). However, due to these properties, the solubility of sapogenins in water is considerably lower than its corresponding glycoside. Therefore, the importance of the solubility of saponins and sapogenins on their final bioavailability seems to be relevant, and it could be determined through a preliminary study of bioaccessibility. This term refers to the amount of a compound that is released during digestion to a potentially absorbable form (Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009; Kamiloglu et al., 2015). In the specific case of saponins, bioaccessibility might be defined as the fraction of total ingested saponins that remains solubilized and stable before cell absorption (Navarro del Hierro et al., 2018). Nonetheless, studies regarding the bioaccessibility of saponins are quite limited. Serventi et al. (2013) reported values of bioaccessibility in the range of 30–91% for different types of soybean and chickpea saponins incorporated in bread formulations, concluding that bile salts modulated their solubility either positively or negatively depending on the type of saponin. Other authors have evaluated the effect of cooking time on the bioaccessibility of soyasaponins from lentils, reporting values of 9–10% as well as a correlation between these two factors (Sagratini et al., 2013). The variable results in the described studies might be related to certain factors (type of saponin, temperature, salt concentration and pH of the aqueous phase) that condition the size and structure of micelles, as stated by diverse authors (Mitra & Dungan, 2001; Oakenfull, 1986). In addition to that, Martin et al. (2016) have recently demonstrated that the co-digestion of a marigold extract with olive oil enhanced the bioaccessibility of triterpenoid compounds present in such extract, which are molecules chemically analogous to the typical triterpenoid sapogenins. Thus, not only physicochemical factors during the digestion might influence the bioaccessibility of saponins, but also other components found in the extracts would affect either positively or negatively the potential absorption of saponins.

The present study aims to evaluate the effect of solvent (ethanol, aqueous ethanol or water) during UAE on the total saponin content of extracts from edible seeds (quinoa, lentil, fenugreek, soybean and lupin), as well as on the extraction of other compounds of interest, such as fat and total phenolic content. The subsequent *in vitro* gastrointestinal digestion of the extracts was performed, in order to assess the bioaccessibility of saponins of the extracts, and the effect of other components in the extracts on such parameter.

2. Materials and methods

2.1. Reagents and materials

Seeds of red quinoa (*Chenopodium quinoa*), soybean (*Glycine* max), peeled red lentil (*Lens culinaris*) and lupin (*Lupinus albus*), as sources of triterpenoid saponins, were purchased from Hijo de Macario Marcos (Salamanca, Spain). Fenugreek (*Trigonella foenum-graecum*), as source of steroidal saponins, were from Murciana de Herboristeria (Murcia, Spain).

Oleanolic acid, gallic acid, quillaja bark saponins, diosgenin and vanillin were from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Folin-Ciocalteu's reagent and sodium carbonate salt were from Panreac (Barcelona, Spain).

Trizma, maleic acid, Amano lipase A from *Aspergillus niger*, pepsin, pancreatin from porcine pancreas, bile salts, phosphatidyl choline from egg yolk were from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

2.2. Ultrasound-assisted extraction (UAE)

Seeds were ground in a knife mill (Grindomix GM200 RETSCH) at 10000 rpm for 1 min. The resulting powder was sieved in a vertical sieve (CISA Cedacería Industrial, España) in order to obtain fractions with a particle size ≤ 250 μm. Extraction was carried out by direct sonication (Branson SFX250 Digital Sonifier, Branson Ultrasonics, USA) with an ultrasonic probe (1/2″ diameter). Samples were extracted with either ethanol, water or ethanol:water (1,1, v/v) at a ratio of sample to solvent of 1:10 (w/v) for 15 min, with a sonication output amplitude of 60%. The temperature during the extraction process was kept under 75 °C and extractions were performed at least in duplicate. The mixture was then centrifuged at 3400 $\times g$ for 10 min. Supernatant of samples extracted with ethanol and ethanol:water was dried under vacuum using a rotary evaporator, whilst the aqueous fraction was lyophilized. Extraction yield (EY) was estimated and expressed as g of UAE extract per 100 g of seed.

For the subsequent characterization of the extracts, the following treatment was performed. Hexane and methanol were added to the UAE extracts at a ratio 1:1:0.03 (v/v/w). Samples were vortexed and centrifuged at 14129 $\times g$ for 3 min. The upper hexane fraction was collected and the extraction of the sample with hexane was repeated. The two collected fractions of hexane were dried in order to assess the total fat content gravimetrically (expressed as g of fat per 100 g of UAE extract). The lower methanol fraction was dried and the total saponin content was determined.

2.3. Total saponin content

Total saponin content (TSC) of the UAE extracts was determined using a spectrophotometric method as described by Ncube, Ngunge, Finnie, and Van Staden (2011) with minor modifications. Briefly, the dried methanolic extracts previously obtained were prepared at 2 mg/mL in methanol. Aliquots of 125 μL were transferred to vials, followed