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Stability of saponins from chickpea, soy and faba beans in vegetarian, broccoli-based bars subjected to different cooking techniques

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ABSTRACT

Recently, saponins have been controversially discussed due to increasing evidence on their health promoting impacts. The present study aimed to determine the stability of saponins in vegetarian, broccoli-based bars (BBBs) incorporating chickpea (cp), soy (sb) and faba beans (fb) as protein sources after being subjected to different cooking methods. Commonly domestic ways of BBB preparation were microwaving, frying, frying and microwaving, steaming and baking. Saponins were analyzed by high-performance thin-layer chromatography (HPTLC) coupled to mass spectrometry (MS). Results indicated that HPTLC analysis with post-chromatographic derivatization and coupling to ESI-MS was capable of separating, identification and quantification of two saponin bands in chickpeas and faba beans, i.e. saponin B and 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyranone (DDMP) saponin. Defatted soy bean flour exhibited four bands (saponin B, DDMP saponin, derivatives of soyasaponins A and B). The total saponin content was 297, 4446, and 113 $\mu g \cdot g^{-1}$ dw in chickpea, defatted soy bean flour, and faba beans, respectively. Pretreatments, for instance soaking and peeling of chickpeas and faba beans reduced the total amount of saponins by 8 and 35%, respectively. Subsequently, different cooking conditions significantly reduced the saponin content by 23–32%, 18–59% and 26–36% in sb-BBBs, cp-BBBs and fb-BBB, respectively. Particularly, the DDMP saponin/saponin B ratio was affected. Apparently, conversion of unstable DDMP saponin to saponin B has been observed during the treatments. However, percentile concentration of the different saponins in the processed BBB does not vary compared to the untreated BBB. Soy beans seem not only to be an adequate source of vegetative proteins, but might be also used as a source of valuable saponins. Finally, an efficient determination method was presented providing evidence for predicting the thermal impact on saponins in innovative vegetarian BBBs. In this regard, optimization of cooking conditions considering the retained saponin amounts is recommended, especially for designing new functional foods.

1. Introduction

Saponins are widely distributed secondary metabolites in the plant kingdom. They act as a chemical barrier or shielding compounds against pathogens and herbivores in the plant defense system (Augustin, Kuzina, Andersen, & Bak, 2011). The name of these compounds derives from the ability to form stable, soap-like foams in aqueous solutions (Francis, Kerem, Makkar-Becker, & Becker, 2002; Kerem, German-Shashoua, & Yarden, 2005; Shi et al., 2004). They are divided into two major classes: triterpenoid and steroid glycosides. Structures greatly vary because of the number of attached sugar units at different positions in the molecule. One of the major sources for saponins is legumes. Soy bean and chickpea seeds comprise saponin contents of 1.0–5.6 g 100 g^{-1} dry weight (Kerem et al., 2005; Shi et al., 2004). Also, Sharma and Sehgal (1992) found saponin content in faba bean of about 1.3–1.5 $g \cdot 100\ g^{-1}$ dw. The chemical structures of soy and chickpea saponins (for both so-called "soyasaponins") have been described previously. Soyasaponins are triterpenoidal glycosides structurally divided into two groups. Based on the individual aglycones (soyasapogenol) and the amount of attached sugar moieties they are classified in group A and B soyasaponins, respectively. Group A soyasaponins are bidesmosidic (two sugar moieties at C3 and C22) and are sub-divided in two further groups known as acetylated and de-acetylated types. Whereas group B soyasaponins have only one glycosylation site at C3 (monodesmosidic) and are categorized into two sub-groups based on the conjugation with a 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyranone (DDMP) unit at the carbon atom C22. DDMP conjugated soyasaponins are named as soyasaponin αg, βa, βg, γa and γg while non-DDMP conjugated saponins are called soyasaponin I (Bb), II (Bc), III (Bb′), IV (Bc′) and V (Ba). Group E soyasaponins (Bd, Be) usually formed as artifacts during saponin extraction are also reported (Zhang & Popovich, 2009). Soy comprises four

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different saponin types: groups A, B, E and DDMP (Price, Fenwick, & Jurzysta, 1986; Yoshiki, Kudou, & Okubo, 1998). Chickpeas contain mainly soyasaponin βg and lower amounts of Bb and Be (Kerem et al., 2005; Serventi et al., 2013). According to Amarowicz, Yoshiki, Pegg, and Okubo (1997) faba bean saponins are similar to group B soyasaponins which are also previously recorded by Shiraiwa, Harada, and Okubo (1991).

In recent years, saponins are attracting considerable interest as a result of their diverse properties, both deleterious and beneficial. Obviously, some saponins were recognized as antinutrients, because of possessing strong hemolytic activity (Price, Johnson, & Fenwick, 1987). Sparg, Light, and van Staden (2004) summarized the biological activities of different saponins from various plant families. In the same context, clinical studies suggested saponins being health-promoting components, because of lowering cholesterol levels, blood lipids, and blood glucose response. Güçlü-Üstündağ and Mazza (2007) highlighted the potential reduction of cardiovascular diseases and obesity risk in humans consuming a diet rich in legume-food containing saponins. However, saponins exhibit antioxidant activity by binding to cholesterol and preventing its oxidation. High antioxidant fractions rich in phenolic compounds and saponins might be a potential active ingredient that could be applied in nutraceuticals, functional foods as well as in natural food preservation (Chan, Iqbal, Khong, Ooi, & Ismail, 2014).

A strategy involving the incorporation of legume ingredients into commonly consumed food products in developing countries represents a viable alternative for complementing the protein deficiency and increasing the health benefits as well. Recently, legume-based ingredients are being used to develop breads as a potential means of decreasing the risk of cardiovascular disease (Nilufer, Boyacioglu, & Vodovotz, 2008; Vittadini & Vodovotz, 2003). However, processing conditions such as heat, pH, matrix, and solvents can affect saponin content and their profile in foods (Güçlü-Üstündağ & Mazza, 2007). For example, during food processing the DDMP saponins are almost hydrolyzed to B saponins and maltol (Heng, Vincken, Hoppe, et al., 2006; Reim & Rohn, 2015; Shi et al., 2004). For all that, the amount of saponin retention in processed food has not been studied sufficiently and might depend on different processing parameters and techniques.

Therefore, the objective of the present study was to assess the stability of saponins from different legumes as protein sources in innovative vegetarian, broccoli-based bars (BBB) under different cooking procedures. Saponins were analyzed by high-performance thin-layer chromatography (HPTLC) and post-chromatographic derivatization in combination with mass spectrometry (MS) for substance assignment.

2. Materials and methods

2.1. Plant materials

Broccoli florets (*Brassica oleracea* var. *italica*), chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.) sweet potato (*Ipomoea batatas* L.), naked barley (*Hordeum vulgare* L. var. *nudum*), carrot (*Daucus carota* L.), onion (*Allium cepa* L.), sweet red pepper (*Capsicum annuum* L.), fresh garlic (*Allium sativum* L.), fresh coriander leaves (cilantro; *Coriandrum sativum* L.), fresh dill (*Anethum graveolens* L.), fresh parsley (*Petroselinum crispum* Mill.), and edible salt of prime fresh quality were purchased from a local supermarket in Hamburg, Germany. Otherwise, defatted soy bean flour (*Glycine max* L.) with 48% protein and 6% fat was obtained from Food Technology Research Institute (FTRI), Agricultural Research Centre (ARC), Cairo, Egypt. In addition, the traditional seasoning species were bought in Ragab El-Attar's local spices supermarket, Egypt.

2.2. Preparation of different broccoli-based bars (BBBs) ingredients

The green leaves of fresh broccoli plants were removed; the florets were cut into 1.5–2.0 cm parts prior to blanching under live steam for 3 min. Unpeeled chickpeas and faba beans were washed and soaked in water for 12 h (1:2, w/v). The excessive water was drained and the seeds were peeled and ground for 3 min using a conventional kitchen machine. Soy bean flour was rehydrated with water (1:2, w/v) to produce soy bean flour dough. Sweet potato and carrots were peeled, washed, chopped in 1 cm slices and blanched using a live steam blancher for 7 and 5 min, respectively. Subsequently, the blanched materials were immediately cooled down and homogenized to a puree. The whole naked barley kernels were milled twice to obtain homogeneous and fine barley flour. Sweet red peppers were washed and chopped in small cubes. Peeled fresh onions and garlic were added before preparing the vegetarian bars.

A green leafy mixture of herbs, i.e. fresh coriander, dill, and parsley leaves were mixed (2:1:1). Also, the spices were ground and mixed [25 g black pepper (*Piper nigrum* L.), 20 g cumin (*Cuminum cyminum* L.), 20 g relish ('Baharat'; ready-mix of specific spices), 10 g dried coriander seeds (*C. sativum* L.), 10 g ginger (*Zingiber officinale* R.), 10 g paprika (*C. annuum* L.) and 5 g hot chili (*Capsicum chinense* L.)] to prepare 100 g of traditional spices mix for immediate use.

2.3. Preparation of ready-to-use and ready-to-eat BBBs

Broccoli-based vegetarian bars were prepared from the previously described ingredients according to the recipes in Table 1. About 1 kg from each recipe was mixed using a kitchen machine. The whole experiment was done in triplicate. Initially, raw BBBs served as control samples. Appropriate amounts of each BBB mixture were cut to BB bars of 10.0 × 0.8 × 0.6 cm prior to exposing them to various domestic cooking methods. Food preparation devices applied were microwaving, frying, frying/microwaving, steaming, and baking. For microwaving, the BB bars were treated at 590 W for 5 min. Before frying, sunflower oil was preheated to 160 °C in a deep-frying skillet. The BB bars were fried at 180 °C for 5 min. For the combination of frying and microwaving, the BB bars were similarly fried for 3 min prior to microwaving for 2 min. Excessive oil was removed by kitchen papers. Steaming was conducted by wrapping the BB bars with aluminum foil and cooking over a live steam for 5 min in a cooking pot. At the end of steaming, the bars reached a temperature of 80–85 °C which is also sufficient to inhibit undesired enzymes. Baking of BBBs was carried out on aluminum foil sheets brushed with little amount of sunflower oil in a pre-heated electric oven at 200 °C (fan assisted) and left for 5 min at constant temperature. After all, the unprocessed BBBs and cooked samples were immediately frozen overnight (−20 °C) until freeze-drying for at least 96 h (−20 °C, vacuum: 0.1 bar) using an Alpha 1-4 LSC system (Martin Christ GmbH, Osterode, Germany).

Table 1
Composition of vegetarian broccoli-based bars incorporating different protein sources.

Ingredients	Fresh vegetarian bars ingredients (%)		
	cp-BBB	sb-BBB	fb-BBB
Blanched broccoli	25	25	25
Peeled soaked chickpea	25	–	–
Hydrated soy bean flour (1:2)	–	25	–
Peeled soaked faba bean	–	–	25
Blanched sweet potato	12	12	12
Whole barley flour	10	10	10
Blanched carrot puree	8	8	8
Green leafy herbs mix[a]	7	7	7
Red pepper paste	5	5	5
Fresh onion	5	5	5
Salt	1.25	1.25	1.25
Fresh garlic	0.75	0.75	0.75
Traditional spices mix[b]	1	1	1

BBB: vegetarian broccoli-based bars formulated with soaked chickpea (cp), dehydrated soy bean flour (sb), soaked faba bean (fb).
[a] Green leafy vegetables herbs (coriander, dill, and parsley; 2:1:1).
[b] See Materials and methods.