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Quinoa seed coats as an expanding and sustainable source of bioactive compounds: An investigation of genotypic diversity in saponin profiles

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ABSTRACT

Saponins (SAPs) are a diverse family of plant secondary metabolites and due to their biological activities, SAPs can be utilised as biopesticides and as therapeutic compounds. Given their widespread industrial use, a search for alternative sources of SAPs is a priority. Quinoa (*Chenopodium quinoa* Willd) is a valuable food source that is gaining importance worldwide for its nutritional and nutraceutical properties. SAPs from quinoa seed coats could represent a new sustainable source to obtain these compounds in high quantities due to the increasing production and worldwide expansion of the crop. This research aims to characterise saponins of seed coat waste products from six different quinoa varieties for their potential use as a saponin source. Gas chromatography (GC)- and Liquid chromatography (LC)- with mass spectrometry (MS) were applied for qualitative and relative quantitative analysis of saponins. GC–MS led to the identification of three main aglycones, oleanolic acid (Ole), hederagenin (Hed), and a phytolaccagenic acid (Phy), while LC–MS enabled characterization of 24 SAPs with varying sugar moieties. Hed was the most abundant aglycone, followed by Phy and Oledepending on the genotype. Saponin distribution and relative abundances are discussed in the light of genotype provenance and agronomic features. Improved knowledge on the phytochemicals present in quinoa varieties might help in finding valuable and sustainable uses for quinoa SAPs in agroindustry as biopesticides as well as in the production of food and pharmaceuticals.

1. Introduction

Currently, the major industrial source of SAPs is the bark of quillay (*Quillaja saponaria* Molina) accounting for 80% of the world market (San Martín, 2000). Unfortunately, the increasing commercial use of quillay SAPs is causing damage to Chilean forests (San Martín et al., 2000; Schlotterbeck et al., 2015). In the pharmaceutical industry, SAPs are used in the synthesis of hormones, contraceptives, anti-inflammatories, expectorants, and diuretics (Vincken et al., 2007; Augustin et al., 2011). In addition, SAPs have found wide application in food and cosmetic production (Price et al., 1987; Sparg et al., 2004). In agriculture, SAPs have potential as biopesticides and growth promoters (Andresen and Cedergreen, 2010; Andresen et al., 2015). For example, molluscicidal and vermicidal activity has been reported in complex mixtures of SAP derivatives obtained from alkali-treated quinoa seed coats (San Martín et al., 2008) and from SAP-rich by-products of tea oil production (Potter et al., 2010). Increased consumer demand for natural products with beneficial physico-chemical (e.g., surfactant) and biological (e.g., biocidal, antimicrobial) properties makes steroidal and triterpenoid SAPs promising compounds for industrial applications.

Triterpenoid SAPs are a diverse group of compounds characterized by the presence of a triterpenoid aglycone backbone ($C_{30}H_{48}$) with one or more sugar moieties attached to them via glycosidic and/or ester bonds (Khakimov et al., 2016b). Triterpenoid SAPs are ubiquitous in the plant kingdom (Sparg et al., 2004; Vincken et al., 2007). Triterpenoid aglycones found in plants include dammaranes, tirucallanes, oleananes, lupanes, hopanes, ursanes, taraxasteranes, cycloartanes, lanostanes, cucurbitanes, and steroids (Vincken et al., 2007). Biosynthesis and heterogeneity of SAPs depend on plant species and tissue type (Augustin et al., 2011). Moreover, environmental, agronomic factors as well as post-harvest treatments, such as storage and processing seem to influence SAP composition of plants (Fenwick et al., 1991; Yoshiki

Abbreviations: SAPs, saponins; Ole, oleanolic acid; Phy, phytolaccagenic acid; Hed, hederagenin; GC, Gas Chromatography; LC, Liquid Chromatography; MS, Mass Spectrometry

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et al., 1998; Szakiel et al., 2011). Even though some research has been devoted to reveal the relationship between biological properties and chemical structure (Dini et al., 2001a,b; Francis et al., 2001; Güclü-Üstündağ and Mazza, 2007; Khakimov et al., 2012), this aspect deserves further investigation.

In foods, SAPs may have a bitter and astringent taste depending on chemical structure, and consequently SAP levels in food products are reduced to decrease bitterness (Price et al., 1987; Gómez-Caravaca et al., 2014). This is done through dehulling and washing of seeds, or by using so-called sweet varieties with low SAP levels as in the case of quinoa (Ward, 2000; Dini et al., 2002). Quinoa (*Chenopodium quinoa* Willd) has become an extremely popular food in the last 20 years, especially in Europe and North America, due to the increased interest in vegetarian diets, its high nutritional quality, and the increasing number of people with gluten intolerance and celiac disorder (Rubio-Tapia et al., 2009; Abugoch, 2009; Vega-Gálvez et al., 2010).

Quinoa cultivation continues to expand worldwide, and the potential of quinoa in the food and healthcare sector is huge (Bazile et al., 2016; Jacobsen, 2003). Therefore, it is of high relevance to gain further knowledge on the SAP profiles of various quinoa varieties. It has been shown that SAP content of quinoa is mainly genotype-dependent (Ward, 2000; Miranda et al., 2014). The major part of SAPs are present in the outer layers of the seed, and protect it from pests and herbivores (birds and insects) and microorganisms (Ridout et al., 1991; Abugoch, 2008). When properly handled to remove the bitter SAPs, which are the major anti-nutritional factors, quinoa seeds have a mild flavour (Maradini et al., 2015). Compared to wheat, quinoa seed has higher protein content, but especially a more favourable amino acid profile (Repo-Carrasco et al., 2003; Stikic et al., 2012). Quinoa has higher levels of energy, calcium, phosphorus, iron, dietary fiber and B-vitamins than cereals (Abugoch, 2009). In addition to its nutritional profile, new targets in research are focused on quinoa by-products in order to add value to its secondary metabolites, such as the SAPs that are removed prior to consumption and are, therefore, a waste product.

Bitter varieties of quinoa contain more SAPs (4.7–11.3 g kg^{-1} dry matter) than sweet varieties (0.2–0.4 g kg^{-1} dry matter) (Mastebroek et al., 2000), thus they are more widely cultivated (San Martín et al., 2008; Stuardo and San Martín, 2008). The coat represents about 8–12% w/w of the quinoa seed and is the main storage tissue of SAPs (up to 86% of the total amount in seeds) (Ando et al., 2002). In total more than 30 SAPs have been identified from quinoa plants that possess mainly five different triterpenoid aglycones including Ole, Hed, Phy, and Serjanic acid (SA) (Mastebroek et al., 2000; Dini et al., 2001a,b, 2002; Zhu et al., 2002; Kuljanabhagavad et al., 2008; Kuljanabhagavad and Wink, 2009). The major sugar moieties of SAPs are glucose, arabinose, galactose, glucuronic acid, xylose, and rhamnose (Woldemichael and Wink, 2001; Zhu et al., 2002). Recently published genome sequencing of quinoa allowed the identification of the transcription factor that is high likely to control the triterpenoid saponins synthesis in seeds (Jarvis et al., 2017). The finding is expected to ease future research on selection for sweet varieties of quinoa. In general, the high genetic variability in quinoa represents a precious resource, which can be exploited for selecting and breeding cultivars adapted to the most diverse soil and climatic conditions (Zurita-Silva et al., 2014).

The present study reports, for the first time, the results of a comparative analysis of SAP profiles in powdered seed coats, rather than whole seeds of six different varieties of quinoa originating from Bolivia or selected and bred in Denmark. The main objective of the study was to screen a complexity and relative amounts of SAPs as well as their corresponding triterpenoid aglycones backbones from the quinoa seed coat waste products using comprehensive GC–MS and LC–MS/MS analysis.

Table 1
An overview of quinoa seed samples, harvested in 2014, investigated in this study.

Variety	Acronym	Origin	Place of Cultivation (Lat;Long)	Quality	Seed colour
Sample 1	P1	Bolivia	Uyuni (BO)	bitter	white
Sample 2	P2	(BO)	20°28′12″S;	bitter	white
Real	QR		66°48′50″W	bitter	white
Titicaca	Ti	Denmark (DK)	Taastrup (DK)	bitter	light brown
Puno	Pu		55°40'13.2"N;	bitter	light yellow
Vikinga	Q124		12°18'13.4"E	sweet	light brown

2. Materials and methods

2.1. Seed material

Seeds were collected in 2014 from six different varieties of quinoa (Table 1). QR is a *Real*-type variety originating from Bolivia; P1 and P2 are mixed samples of Bolivian material. Titicaca (Ti) and Puno (Pu) are registered varieties that were selected and bred in Denmark from varieties originated in southern Chile and Peru (Jacobsen, 1998; Adolf et al., 2012). Q124 is a Danish variety bred for low SAP content, now registered under the name *Vikinga*. The seed coats powder of *Real* varieties was obtained from seeds harvested in Bolivia, while Danish varieties were obtained from seeds harvested in Denmark.

2.2. Sample preparation

Twenty-five mg DW of the outer layer of quinoa seeds (obtained after mechanical dehulling using a mill) were powdered and extracted following the procedure described by Khakimov et al. (2016a,b) with some modifications, using 80% methanol in the ratio of 25:1 (w/v). The mixture was vortexed for 30 s, incubated at 70 °C for 3 h with agitation at 1400 rpm using a ThermoMixer F2.0 (Eppendorf, Hørsholm, Denmark), and cooled to room temperature. Extracts were then vortexed for 30 s, and centrifuged at 16k g for 10 min. In order to identify the triterpenoid aglycones and characterise sugar moieties of SAPs, the supernatant was separated into aliquots as follows: 0.1 mL for LC–MS/MS analysis; 0.37 mL for HCl (acidic) hydrolysis and 0.37 mL for basic (NaOH) hydrolysis based GC–MS analysis. Basic hydrolysis was applied prior to GC–MS in order to cleave sugar moieties linked via ether bonds, while the acidic hydrolysis removed sugar moieties linked via both ether and glycosidic bonds. The general procedure for SAP extraction is summarized in Fig. 1.

Aliquots consisting of 0.37 mL of supernatants were completely dried in a ScanVac (Labogene, Lynge, Denmark) operating at 45 °C and 200 g for 2–3 h. For the acidic hydrolysis, 0.5 mL of 2 M HCl was added, the mixture was vortexed for 30 s and then incubated at 100 °C for 1.5 h in the ThermoMixer (1400 rpm). The mixture was cooled to room temperature. For basic hydrolysis, 0.5 mL of 2 M NaOH was added to the dried aliquots of 0.37 mL supernatant; the mixture was vortexed for 30 s and incubated at 25 °C for 1.5 h with mixing at 1400 rpm as described above. Thereafter, 0.1 mL of 12 M HCl was added to adjust the pH ≤ 2. Both acidic and basic extracts were then transferred to fresh 15-mL Falcon tubes. Free aglycones, released by hydrolysis, were extracted by addition of diethyl ether at an extract:ether ratio of 1:4 (v/v). This procedure was repeated twice and the combined ether fractions were washed with MilliQ water (ether:water, 2:1, v/v) in order to remove the residual acid or base. The washed ether fraction was transferred into a new 15-mL tube, dried under fume hood and re-suspended in 0.25 mL of 80% methanol. After centrifugation (16k g for 3 min) of the final methanol extracts, 0.1 mL aliquot was used for LC–MS/MS and 0.05 mL was used for GC–MS. Prior to GC–MS, samples