Industrial Crops & Products 121 (2018) 54–65

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Integrated process for sequential extraction of saponins, xylan and cellulose from quinoa stalks (*Chenopodium quinoa* Willd.)

Alicia Gil-Ramirez[a,1], Daniel Martin Salas-Veizaga[b,c,1], Carl Grey[b], Eva Nordberg Karlsson[b], Irene Rodriguez-Meizoso[a], Javier A. Linares-Pastén[b,*]

[a] Centre for Analysis and Synthesis, Department of Chemistry, Lund University, P.O. Box 124, 22100 Lund, Sweden
[b] Division of Biotechnology, Department of Chemistry, Lund University, P.O. Box 124, 22100 Lund, Sweden
[c] Instituto de Investigaciones Fármaco Bioquímicas, Universidad Mayor de San Andrés, La Paz, Bolivia

ARTICLE INFO

Keywords:
Quinoa
Saponins
Pressurized hot water extraction
Xylan and cellulose extractions
Integrated process
Biorefinery

ABSTRACT

World quinoa production is increasing due its high nutritional value. As a consequence, large quantities of stalks accumulate as unused byproducts. Here, we verify the presence of saponins in the stalks and present a biorefinery approach with quinoa stalks as feedstock, using an integrated processing scheme to separate saponins, xylan and cellulose. Saponins were extracted using pressurized hot water extraction (PHWE), optimized by a central composite experimental design (rotatable 2^2) with temperature and extraction time as factors. Xylan was extracted from the residual solid material after PHWE by an alkaline method using 0.5 M NaOH at 80 °C. Cellulose was purified from the remaining residuals using acetic and nitric acid at 120 °C, which resulted in recovery of white cotton-like cellulose, showing no need of further bleaching. The saponin yield was significantly increased at temperatures exceeding 110 °C, with highest amounts obtained at 195 °C (15.4 mg/g raw material). The yield in the following xylan extraction (maximum 120 mg/g raw material) was however significantly reduced when preceded by PHWE above 110 °C, indicating degradation of the polymer. Cellulose recovery (maximum 296 mg/g raw material) was less affected by variations in temperature and time in the preceding PHWE. The results obtained shows that tuning between saponin and xylan extraction is critical. This approach is foreseen to be applicable to the valorisation of residual fiber-rich biomass from various types of crops, besides quinoa.

1. Introduction

Quinoa has gained great global interest in recent years due its high nutritional value. Quinoa is not a cereal but belongs to the Amarantacea family (subfamily Chenopodiace). The seeds are rich in proteins, present in levels comparable or higher than in cereals (~15%). The seeds also contain essential amino acids, carbohydrates, lipids, vitamins and minerals, thus quinoa is considered as a complete nutritional food (James, 2009). In addition it is free of gluten and can substitute wheat in the diet of patients with celiac disease (Zevallos et al., 2014). In 2013, the Food and Agricultural Administration of the United Nations declared the "International Year of the Quinoa" highlighting the "quinoa capacity to adapt to different agro-ecological soils and its potential contribution in the fight against hunger and malnutrition" (www.fao.org/quinoa-2013).

Quinoa originates from the Andes, and was domesticated for the first time more than 5000 years ago (Bazile et al., 2013). Nowadays there are a broad variety of ecotypes distributed at different altitudes along the Andean system, from the sea level up to 4000 m' height. Most of the production takes place in the highlands of Bolivia, Peru and Ecuador (www.fao.org/faostat). Recently, the cultivations have expanded to include other geographical areas, such as Colorado and California in the USA, and to other continents, including Europe and Asia (*e.g.* China, India and some experimental cultivars in other countries) (Jacobsen and Bendevis, 2013). The world total production has almost been tripled from 52.6 thousand tons in 2000 to 148.7 thousand tons in 2016 (www.fao.org/faostat). This agricultural expansion and intensification generates of course huge amounts of byproducts, mainly seed coats and stalks.

These byproducts have potential to be feedstock for manufacturing of high value products. For instance, seed coats are rich in saponins (85–90% w/w) (Muir et al., 2002; Ruiz et al., 2017; San Martín et al., 2008) and a number of saponin extraction processes have been reported (Verza et al., 2012; Woldemichael and Wink, 2001; Zhu et al., 2002).

* Corresponding author.
E-mail address: javier.linares_pasten@biotek.lu.se (J.A. Linares-Pastén).
[1] Both authors have contributed equally.

https://doi.org/10.1016/j.indcrop.2018.04.074
Received 17 January 2018; Received in revised form 17 April 2018; Accepted 23 April 2018

The stalks, however, remain much less explored. Stalks are a natural source of lignocellulosic biomass, which include lignin and a variety of abundant polysaccharides such as cellulose and xylan. In addition, a recent study involving extraction of quinoa xylan for production of xylooligosaccharides (XOs) in our laboratory (Salas-Veizaga et al., 2017) revealed foaming in initial washing steps (unpublished data), suggesting the presence of saponins. Therefore, quinoa stalks are a source of xylan, cellulose and potentially saponins.

All saponins found in quinoa are triterpene glycosides and have potential value in brewing, cosmetics and detergent production industries due to their foaming properties at low concentrations (0.1% w/w) (Rojas et al., 2011). Nowadays, saponins are used as pesticide and fungicide products, due to their antibiotic and antifungal properties. Moreover, these compounds have several other reported biological activities, including anti-inflammatory, anticancer, antidiabetic and hypocholesterolemic properties (Rojas et al., 2011).

Xylan is a group of heteropolymers with a backbone made from β-1,4-linked xylose residues substituted to different extent by *e.g.* arabinose and glucuronic acids (Linares-Pastén et al., 2018). Xylans are attractive renewable polymers for the development of new materials, including nanocomposites, hydrogels, bioplastics and others, and thus have a broad potential for applications in the industry (da Silva et al., 2012). Xylan can also be transformed into XOs, which have shown prebiotic properties; promoting the growth of health beneficial bacteria, and therefore useful for the development of functional food and feed (Faryar et al., 2015).

Cellulose is a polysaccharide consisting of a linear chain of several hundreds to many thousands of β-1,4-linked D-glucose units. Many properties of cellulose depend on its degree of polymerization, which varies with origin (Klemm et al., 2005). The main application of cellulose is in production of paper and paperboard. Smaller quantities are also converted into a wide variety of derivative products *e.g.* cellophane, rayon or viscose as well as different types of composites and nano-structures (Klemm et al., 2005; Pandey et al., 2013).

Individual extraction protocols focusing on obtaining xylan and cellulose have been reported from numerous agricultural resources. However, cellulose has previously not been extracted from quinoa stalks and the first xylan or xylooligosaccharides extractions from quinoa stalks have only recently been reported involving either alkaline extraction combined with enzymatic hydrolysis (Salas-Veizaga et al., 2017) or SO_2-catalyzed steam pretreatment (Carrasco et al., 2015). Saponins have to our knowledge not previously been reported from quinoa stalks. In addition, no attempts have previously been made to develop an integrated process scheme for this product combination. Hence, the aim of the current study was for the first time to establish an integrated processing scheme, involving established extraction methodologies (PHWE, alkaline extraction of xylan and acidic solubilisation of cellulose) to create an overall biorefinery solution for valorisation of quinoa stalks.

2. Material and methods

2.1. Raw material

Stalks of quinoa, variety Real Blanca, were collected in the Andean plateau in Bolivia (Salas-Veizaga et al., 2017). The stalks were size reduced with a knife-milling machine, sieved to 1–1.7 mm pieces and stored at −20 °C under darkness in order to avoid material contamination and/or degradation.

2.2. Standards and reagents

Diosgenin 93% purity, vanillin 99% purity and sulfuric acid (a 96% solution in water) were purchased from Sigma-Aldrich (Steinheim, Germany). Chloroform stabilized with 0.6% ethanol and butan-2-ol were supplied by VWR Chemicals (Rheinland, Germany) and Merck (Darmstadt, Germany) respectively. Ethanol 99% purity was purchased from Solveco Group (Rosenberg, Sweden). Ultrapure water (18,2 MΩ/cm) was provided through a Milli-Q instrument (Millipore, Billerica, MA, USA). Xylan from birchwood (Xylose residues ≥90 %), xylan from oat spelts (Xylose ≥70 %) and α-cellulose were purchased from Sigma-Aldrich (Steinheim, Germany).

Table 1
Details of the central composite design (2^2 + star) for PHWE extraction of *C. quinoa*. *Star points, **central points (replicates).

Sample	Temperature (°C)	Time (min)
S1	170	60
S2*	110	1
S3*	195	35
S4*	110	70
S5	50	60
S6**	110	35
S7	50	11
S8*	25	35
S9**	110	35
S10	170	11

2.3. Experimental design for the pressurized hot water extraction (PHWE) process

A central composite and rotatable design 2^2 with star points (α = 1.414) and two central points was selected for the PHWE of saponins, which is the first step of the sequential extraction (Table 1). Two factors, temperature and time of extraction, were selected for this study. The range of values introduced in the model were 50–170 °C and 11–60 min respectively. The experimental conditions generated, including star points, covered the values normally reported in the literature (Engelberth et al., 2010; Güçlü-Üstündağ et al., 2007; Wan et al., 2006) and were within the equipment limitations. Saponins, xylan and cellulose yields (mg/g raw material) were studied as variable responses. Estimated response surfaces were plotted for each response and optimal extraction conditions were set by multiple linear regressions using Statgraphics Centurion XVI software (Statpoint Technologies, Warrenton, Virginia, USA). The model was validated through regression plots of observed vs. predicted yields of saponins, xylan and cellulose after PHWE.

2.4. Extraction processes

2.4.1. Extraction of saponins

Conventional extractions. Ten grams of quinoa stalks were suspended in water-ethanol (1:1) solvent. Two different temperatures and extraction times were tested. In a first experiment, the extraction suspension was heated at 30 °C for 72 h. In a second experiment, the extraction solution was heated at 60 °C during 1 h by reflux.

Pressurized hot water extraction (PHWE). The PHWEs were obtained using an Accelerated Solvent Extraction (ASE 350) equipment (Dionex Corporation, USA), that operate at 200 °C as maximum temperature. Quinoa stalks (1 g) were placed in stainless steel cells (10 mL) previously loaded with cellulose filters. No inert material (i.e. glass beads) was used since neither channelling effects nor material collapses were expected because of the large enough particle size (preliminary extractions were done to confirm this hypothesis, data not shown). The cells were filled with ultrapure water, heated up to a set temperature and submitted to pressure in static mode for a selected time. After that, the cell was rinsed with fresh solvent (flush volume of 60%), the solvent was purged with N_2 for 100 s and the extract was automatically collected. Finally, aqueous extracts were freeze-dried and light protected at −20 °C until use. Once PHWE extraction was done, the remaining material was recovered and kept at −20 °C until their use in the forthcoming steps of the sequential extraction. Extractions were carried