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Identification and quantification of even and odd chained 5-n alkylresorcinols, branched chain-alkylresorcinols and methylalkylresorcinols in Quinoa (*Chenopodium quinoa*)

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ABSTRACT

Quinoa is a pseudocereal grown in the Andean region of South America that is of increasing interest worldwide as an alternative staple food. We have detected a complex mixture of both odd- and even-alkyl chain alkylresorcinols (AR), branched-chain alkylresorcinols (bcAR) and methylalkylresorcinols (mAR) in ethyl acetate extracts of quinoa. We quantified the content of AR in 17 commercial samples of quinoa, and found that the mean ± SD content of AR was 58 ± 16 μg/g, bcAR was 182 ± 52 μg/g, and mAR was 136 ± 40 μg/g. AR from quinoa could also be detected in plasma after eating quinoa, indicating that some of these unique AR could be used as biomarkers of quinoa intake in humans. Further work is required to understand the role of these ARs in the quinoa plant and whether any of the novel ARs may be of particular interest in human nutrition.

1. Introduction

Quinoa is a pseudocereal crop cultivated in the Andean region of South America. While quinoa is not botanically a cereal, it is used in a similar manner to the cereal grains, and is considered as a whole grain, according to the major definitions of whole grains (van der Kamp, Poutanen, Seal, & Richardson, 2014). While it has been an important food crop in the Andes region for centuries, it is of increasing interest outside of South America, both for its nutrient content and as a gluten-free grain (Graf et al., 2015). Quinoa is of nutritional interest, due to quinoa protein not being limiting for lysine, unlike cereal proteins, notably wheat, and it does not contain the protein gluten, which must be avoided in the management of coeliac disease (Zevallos et al., 2014). Less well recognised is that quinoa appears to have a particularly high amount of certain micronutrients compared to other cereal and pseudocereal grain, including folate and betaine (Ross, Zangger, & Guiraud, 2014; Schoenlechner, Wendner, Siebenhandl-Ehn, & Berghofer, 2010). This may be of importance for people following gluten free diets, as the major gluten-free cereals rice and corn are low in these compounds (Ross et al., 2014; Schoenlechner et al., 2010; Yazynina, Johansson, Jagerstad, & Jastrebova, 2008).

To date, there are only limited studies on the composition of quinoa, mainly focusing on macro- and micronutrients, as well as the content of saponins, which are mainly present in the outer seed coat (Graf et al., 2015; Schoenlechner et al., 2010). Following up on a chance finding, we present the first evidence that alkylresorcinols, long-chain phenolic lipids hitherto thought only to be present in important amounts in wholegrain wheat, rye and barley (Ross, 2012; Ross et al., 2003), are also present in quinoa. Several branched chain- and methylalkylresorcinol homologues are also identified for the first time in the published literature.

2. Materials and methods

2.1. Samples and chemicals

Quinoa was purchased from stores around Jönköping and Gothenburg, Sweden, Lausanne, Switzerland and San Diego, United States, leading to a total of 17 quinoa samples, including white, red and black coloured varieties. Where possible, country of cultivation is reported, though this was not always indicated on the packaging.

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Alkylresorcinol standards (compounds 1, 3–10; Supplemental Table 1) were purchased from Reseachem AG (Burgdorf, Switzerland). *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane (MSTFA + 1% TMCS) was from Thermo Scientific (Waltham, MA). All solvents used were LC-MS grade from Sigma (St Louis, MO).

2.2. Nomenclature

To simplify the description of the different alkylresorcinol homologues in this paper, we refer to the 5-n-alkylresorcinols with a saturated alkyl-chain as 'AR', 5-n-alkylresorcinols with a branched alkyl chain as 'bcAR', and 5-n-alkylresorcinols with a methyl group at the 2 position of the resorcinol ring as 'mAR' (Supplemental Fig. 1). When referring to all alkylresorcinols as a group, we use 'alkylresorcinol(s)'. Chain length numbering follows that used previously in the literature, and in the case of bcAR refers to the entire alkyl chain including the branched methyl group, and for mAR, refers to the chain length of the alkyl chain at the 5-position of the resorcinol ring.

2.3. Sample extraction

The sample extraction procedure followed a previously established method for rye grains (Ross, Kamal-Eldin, Jung, Shepherd, & Åman, 2001). Different extraction times were tested (1, 6 and 24 h) and it was found that both quinoa grains and flour were fully extracted after 6 h. Repeated extractions did not yield additional alkylresorcinols. The final protocol used is as follows: approximately 500 mg of cereal grains or flour were weighed into a 20-mL glass test tube with a screw cap. This weight of quinoa equals approximately 50–60 individual seeds. Internal standard was added (25 μL of 140 μg alkylresorcinol C19:0 $^2H_4$/mL in methanol) and 15 mL of ethyl acetate added as extraction solvent. Samples were extracted in a vertical rotator at 1 Hz for 6 h. The test tubes were centrifuged at 1000*g* for 10 min to remove any small particles from the extract, and 300 μL of the extract were pipetted into a glass GC vial with insert. The extract was evaporated in a centrifuge evaporator (MiVac, Genevac Ltd, Ipswich, UK), and an additional 300 μL of extract added and evaporated in the same way to further concentrate the sample. The extracts were derivatised in the vials with 50 μL of MSTFA + 1% TMCS at 60 °C for 1 h before being injected into the GC–MS/MS instrument. All quinoa samples were prepared in duplicate, and each batch included triplicate extracts of the same brown wheat flour control to ensure inter-batch repeatability. Each extract was analysed once and average values for the duplicate extractions of samples are reported.

2.4. GC–MS analysis

GC–MS analyses were carried out using a Shimadzu TQ8030 triple quadrupole mass spectrometer (Shimadzu Europa GmbH, Duisburg, Germany). For exploratory analyses, samples were analysed in full scan and single ion monitoring (SIM) mode, with SIM mode selected for *m*/*z* 268 and 282, indicative of the 1,3-dihydroxybenzene group of alkylresorcinols (Ross et al., 2001) and methyl alkylresorcinols (Adamski et al., 2013) respectively. Methyl alkylresorcinols with the same molecular ion as alkylresorcinols could be distinguished by the ratio of ions 282 and 268 (more intense signal at *m*/*z* 282 compared to *m*/*z* 268). All alkylresorcinols were quantified using SIM mode for their respective molecular ions (Fig. 1). Different alkylresorcinols with the same molecular ion were further identified based on their retention time. Up to four alkylresorcinols were identified for each molecular ion.

Quantification was carried out using a labelled internal standard (C19:0 $^2H_4$) compared to authentic alkylresorcinols standards C17:0, C19:0, C20:0, C21:0, C22:0, C23:0, C24:0, C25:0 and C26:0.

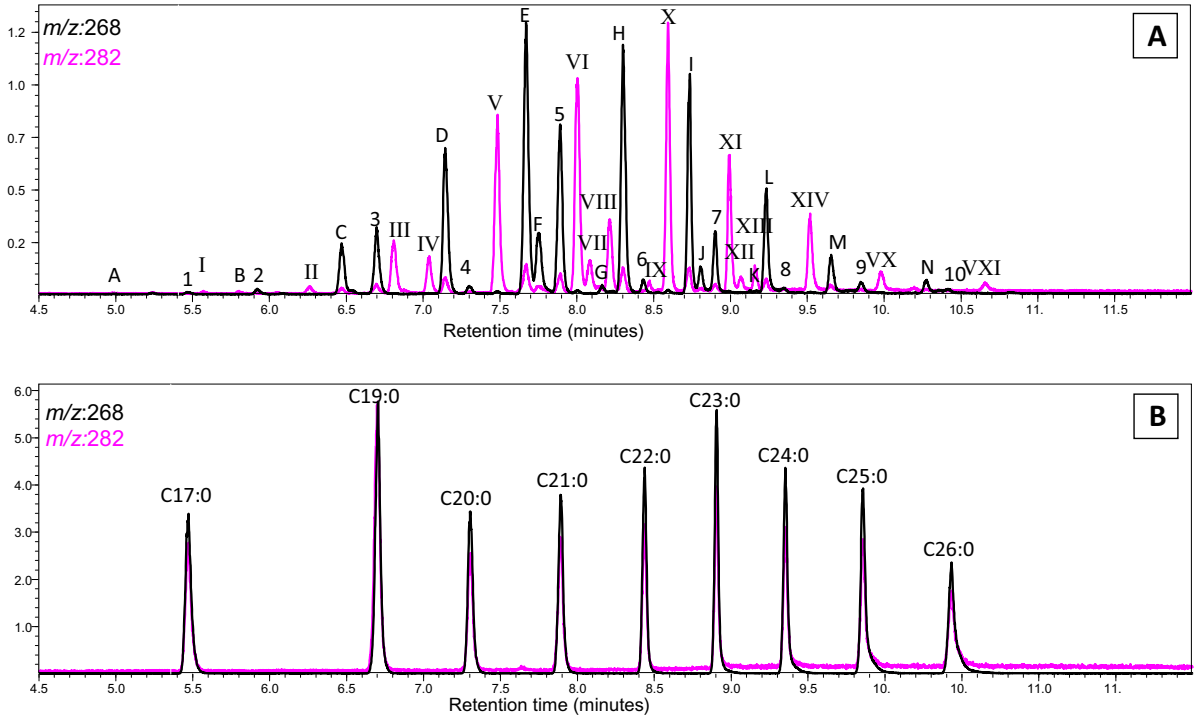

Fig. 1. (A) Single ion monitoring GC–MS chromatogram of white quinoa extract, with ions *m*/*z* 268 (black) and 282 (pink) extracted. Alkylresorcinols have a typical base mass fragment of *m*/*z* 268. The series of peaks with base mass fragmentation at *m*/*z* 282 are tentatively identified as methyl-alkylresorcinols. (B) Alkylresorcinol standards. Numbers and letters correspond to those in Supplementary Fig. 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)