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Distribution of phenolic antioxidants in whole and milled fractions of quinoa and their inhibitory effects on α-amylase and α-glucosidase activities

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ABSTRACT

Whole grain quinoa and its milled fractions were evaluated for their phenolic composition in relation to their antioxidant properties and inhibitory effects on α-amylase and α-glucosidase activities. Compositional analysis by HPLC–DAD showed that the distribution of phenolic compounds in quinoa is not entirely localised in the outer layers of the kernel. Milling of whole grain quinoa resulted in about 30% loss of total phenolic content in milled grain. Ferulic and vanillic acids were the principal phenolic acids and rutin and quercetin were predominant flavonoids detected in whole grain and milled fractions. Quinoa milled fractions exhibited numerous antioxidant activities. Despite having relatively lower phenolic contents, dehulled and milled grain fractions showed significantly ($p \leqslant 0.05$) higher metal chelating activity than other fractions. Furthermore, extracts of bran and hull fractions displayed strong inhibition towards α-amylase [IC_{50}, 108.68 μg/ml (bran) and 148.23 μg/ml (hulls)] and α-glucosidase [IC_{50}, 62.1 μg/ml (bran) and 68.14 μg/ml (hulls)] activities. Thus, whole grain quinoa and its milled fractions may serve as functional food ingredients in gluten-free foods for promoting health.

1. Introduction

Quinoa (*Chenopodium quinoa*) is one of the most important pseudocereals from the Andean origin, cultivated extensively at mountain altitudes in Peru and Bolivia. It is a gluten-free grain and considered easy to digest (Gallagher, Gormleya, & Arendt, 2004). Because of its excellent nutritional characteristics, interest in quinoa is growing in other parts of the world. Although most quinoa is still cultivated in the Andean region of South America, it is also cultivated in the USA, China, Europe, Canada, and India (Jacobsen, 2003). In addition to nutritional importance, quinoa grains contain significant amounts of phytochemicals including phenolic compounds (Hirose, Fujita, Ishii, & Ueno, 2010). Phenolic compounds are diverse and widely occurring groups of phytochemicals in plant foods having beneficial effects on health. They are of considerable interest due to their antioxidant properties and their ability to scavenge free radicals and reactive oxygen species (Chandrasekara & Shahidi, 2011). These can prevent degenerative diseases such as cardiovascular diseases, cancers, obesity, diabetes and Alzheimer's disease through antioxidative action and/or the modulation of different enzyme activities (Scalbert, Manach, Morand, Remesy, & Jiménez, 2005; Shahidi & Chandrasekara, 2013).

Diabetes mellitus is characterised by the excessive accumulation of free glucose in blood, which is linked to the onset of vascular diabetic complications and triggers the generation of free radicals and oxidation-related damage to various organs (King & Loeken, 2004). Alpha-amylase and α-glucosidase are key enzymes involved in the digestion of carbohydrates in the small intestine (McDougall & Stewart, 2005). Inhibitors of these enzymes delay the breakdown of starch and lower the postprandial blood glucose levels in diabetic patients. Synthetic inhibitors are being used clinically for effective control of hyperglycemia in type II diabetic patients. However, these synthetic antidiabetic drugs have adverse side effects in humans compelling to find natural and safer alternatives (Bischoff, Puls, Krause, Schutt, & Thomas, 1985). Phenolic compounds from several plant sources including food grains and legume seeds have been shown to inhibit α-amylase and α-glucosidase activities and allow for better control of blood glucose levels (Kim, Hyun, & Kim, 2011; Qin, Wu, Yao, & Ren, 2013; Ranilla, Apostolidis, Genovese, Lajolo, & Shetty, 2009; Shobana, Sreerama, & Malleshi, 2009; Sreerama, Sashikala, Pratape, & Singh, 2012). Therefore, these phytochemicals offer an attractive strategy for the control of enzyme activities involved in the starch breakdown and intestinal glucose absorption.

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Quinoa grains contain various phenolics such as quercetin, kaempferol and rutin and their derivatives, vanillic acid, ferulic acid and ferulic acid-4-glucoside (Gomez-Caravaca, Lafelice, Verardo, Marconi, & Caboni, 2014; Gomez-Caravaca, Segura-Carretero, Fernandez-Gutierrez, & Caboni, 2011; Hirose et al., 2010; Tang et al., 2015). In addition, whole grain quinoa in diet was shown to reduce most of the adverse effects exerted by high fructose on lipid profile and glucose levels in rats (Paśko, Zagrodzi, Bartoń, Chlopicka, & Gorinstein, 2010). Furthermore, consumption of quinoa instead of typical products with or without gluten significantly reduced blood sugar, insulin and triglyceride levels in normal as well as celiac patients (Berti, Riso, Monti, & Porrini, 2004). These studies suggest the potential of quinoa in the prevention of oxidative stress and in controlling the activities of enzymes involved in the regulation of glucose homeostasis.

The grains of quinoa are being used in several forms such as flour, toasted and added to soups, or made into bread (Caperuto, Amaya-Farfan, & Camargo, 2000). With increasing interest in grain diversification, it is usually processed by milling to improve its nutritional properties. This results in different types of milled fractions such as bran (pericarp) and milled grain. Although milling of quinoa improves nutritional properties by removing the antinutrients particularly saponins, it might also decrease the levels of phenolic compounds and their bioactivities in milled products, as shown for other grains (Liyana-Pathirana & Shahidi, 2007; Sreerama, Sashikala, & Pratape, 2010). Recently, Gomez-Caravaca et al. (2014) reported a decrease of free and bound phenolic compounds after pearling of whole quinoa. Given the emerging role of quinoa as a healthy alternative to commonly used ingredients in gluten-free products, milled grain and its by-products have the potential to be used as ingredients in the preparation of specialty products for human consumption. However, there have been no systematic studies on the distributions of phenolic compounds in different milled fractions of quinoa as related to their contributions to the antioxidant activity and their influence on enzyme activities associated with hyperglycemia. Therefore, the aim of this work is to evaluate the distribution of phenolic compounds in different milled fractions and whole grain quinoa in relation to their antioxidant properties and inhibitory activities against α-amylase and α-glucosidase. These studies may provide useful information for the effective utilisation of quinoa-milled fractions as functional food ingredients for promoting health.

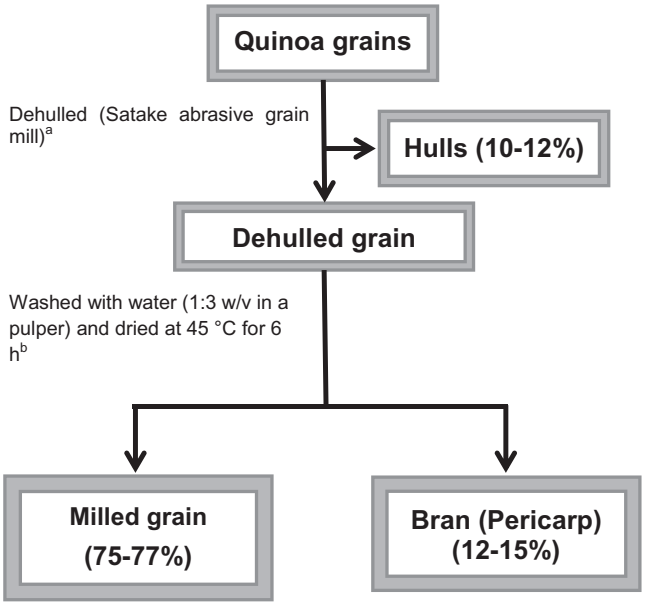

Fig. 1. Flow chart for the milling and separation of milled fractions of quinoa. [a] Cleaned quinoa grains were successively passed twice into the grain mill. [b] Bran fraction was collected with the washed water from pulper and dried.

2. Materials and methods

2.1. Materials

White quinoa grains grown locally were procured from Ananthapur, Andhra Pradesh, India and stored at 4 °C until use. Folin-Ciocalteau reagent, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), gallic acid, 3,4-dihydroxybenzoic acid, vanillic acid, chlorogenic acid, *p*-coumaric acid, sinapic acid, ferulic acid, quercetin, rutin, myricetin, daidzein, luteolin, apigenin, naringenin, kaempferol, rat intestinal acetone powder, porcine pancreatic α-amylase and 4-nitrophenyl α-D-glucopyranoside (PNPG) were obtained from Sigma–Aldrich Chemicals Pvt. Ltd., Bangalaru, India. Sodium carbonate, sodium nitrite, ferric chloride, HPLC grade methanol and acetonitrile were supplied by Merck (Darmstadt, Germany). All other reagents were of analytical grade.

2.2. Methods

2.2.1. Milling and separation of milled fractions of quinoa

Quinoa grains were cleaned, and size graded to obtain uniform size grains. A portion of the graded grains were dehulled on a Satake abrasive grain mill (Model TM05, Satake Corporation, Japan) successively in two passes to obtain hulls and dehulled grain fractions. Thereafter, a portion of dehulled grain fraction was washed with excess water (1:3 w/v) in a pulper (Sen Berry & Co., India), and bran was collected with the washings. Both dehulled grain and bran fractions were dried at 45 °C for 6 h. Pearling degree of 30% was used to obtain different milled fractions. Fig. 1 shows the flow chart for the milling and separation of milled fractions of quinoa. Fractions were weighed and expressed as a proportion of the total initial sample weight. The hulls, dehulled grain, bran and milled grain fractions, in addition to whole grain, were ground into fine powder to pass through a 250 μm sieve using a coffee grinder. The flours were defatted (hexane 1:5 w/v, 2 h, three times at room temperature and air-dried for 12 h) and stored at −20 °C in polythene pouches until further analysis.

2.2.2. Extraction and quantification of total phenolic, total flavonoid and condensed tannin contents

Total phenolics were extracted with 80% methanol containing 1%HCl (1:50 w/v) in a shaking water bath at 50 °C for 3 h (3× times) as described by Sreerama et al. (2012). Resulting slurry after each extraction was centrifuged at 4000*g* for 10 min and the supernatants were collected. Combined supernatants were desolventized in vacuo at 40 °C. The resulting concentrated solutions were freeze dried and stored at −20 °C until their use for further analysis. Extraction yields for whole grain quinoa and each milled fraction were calculated as follows:

$$\text{Extraction yield} = (\text{Freeze dried weight of extract} / \text{weight of defatted flour used for extraction}) \times 100$$

The total phenolic content (TPC) of methanol extracts of quinoa whole grain and its milled fractions was determined using the Folin-Ciocalteu colorimetric method of Singleton, Orthofer, and Lamuela-Raventos (1999) with modifications as described by Sreerama et al. (2012). Results were expressed in mg of ferulic acid equivalents (FAE) per gram of sample. The total flavonoid content (TFC) was measured by the aluminium chloride colorimetric assay