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Isolation of quinoa protein by milling fractionation and solvent extraction

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ABSTRACT

Quinoa is an attractive non-animal protein source, because of its absence of gluten and the favorable amino-acid profile. The objective of this study was the extraction of quinoa protein by two consecutive approaches. Initially, the protein-rich bran was isolated by optimizing the conditioning parameters of a milling fractionation procedure. In contrast to the protein content of the whole grain, 11.75% in dry base (db), the bran fraction contained 27.78% (db) protein when conditioned with 15% moisture at 20 °C for 20 h. Subsequently, an aqueous extraction was developed, resulting in a protein solubility of 60% at pH 10 and 20 °C for 1 h. For purification at the isoelectric point, the pH-value and the separation method were varied. After drying, an extraction of proteins from bran yielded 68% (db) as compared to 52% (db) protein from quinoa whole grain flour.

1. Introduction

The uptake of proteins is one of the major factors for maintaining human health. Because of the higher costs for animal protein production and the huge amount of resources which are required, plant-based protein sources are of growing interest. In addition, the incidence of allergies and intolerances concerning proteins from egg or milk has been rising. Currently, cereals and legumes represent the main alternatives, for both human and animal nutrition. However, the alcohol soluble gliadins and glutenins in wheat are attributed to the celiac disease (Thompson, 2001). Thus, the identification of novel and sustainable protein sources is of great relevance. The pseudocereal quinoa might be a suitable candidate, since it features high protein and mineral content with a well balanced amino acid profile and lacks gluten (Aufhammer et al., 1995).

Protein from these sources can be isolated by solvent extraction to serve as an ingredient in dietary supplements. Additionally, for gluten-free products the application of protein isolates can be beneficial for processing and quality improvement of bread, pasta, dietary supplements, baby foods and beverages. According to Segura-Nieto et al. (1999) and Gorinstein et al. (2002) proteins of amaranth, soybean, buckwheat, and quinoa are highly soluble and applicable in functional foods.

The type of milling product, processing parameters and suitable solvents have to be taken into consideration to achieve a maximum yield. Previously, a lot of studies have focused on the extraction of proteins from whole grain flours for e.g. amaranth (Salcedo-Chávez et al., 2002), while only few reported extraction from byproducts such as rice bran (Jiamyangyuen et al., 2005). The separation of different grain tissues enables the selection of a fraction which is naturally

Abbreviations: AACC, American Association of Cereal Chemists; db, dry base; ICC, International Association for Cereal Science and Technology; CIE, Commission Internationale d'Eclairage.

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rich in target substances. The germ of quinoa, for example, contains most of the protein (Valencia-Chamorro, 2003), while starch is present in negligible levels. Additionally, protein can be solubilized more easily when less starch is available, because especially small starch granula are difficult to separate. Since amaranth and quinoa starch granula are particularly small (Aufhammer et al., 1995) typical separation methods based on gravimetric or size differences are difficult to realize.

Nevertheless, in the case of quinoa and amaranth, milling presents a technological challenge because of their small kernel sizes: for quinoa 1.0–2.5 mm (Taylor and Parker, 2002) and for amaranth 1.0–1.5 mm (Bressani, 2003), respectively, depending on the plant variety. Thus, only few studies have focused on the production and application of milling fractions (Chauhan et al., 1992; Ando et al., 2002). Chauhan et al. (1992) compared two methods for reducing the saponine content by a pretreatment before milling the grains into whole grain flour, bran and white flour. However, there are no available data, focusing on the duration and the water content during a conditioning step. Similarly US patent 20100184963 from 2010 suggests the use of protein-rich fractions for the production of quinoa protein concentrate (Scanlin et al., 2010). Scientific data and validation of the proposed methods are not available. In the case of wheat and other typical cereals, conditioning is said to be one of the most important steps prior to grinding, which renders the outer cell tissues more elastic, resulting in a better separation from the endosperm. Depending on the morphology of wheat kernels, the target moisture content during conditioning varies between 15% and 16% with a resting time between 6 and 36 h (Cauvain, 2003; Fang and Campbell, 2003).

After choosing a suitable milling fraction for protein concentration, an extraction procedure has to be developed. Salcedo-Chávez et al. (2002) varied processing parameters for alkaline extraction from amaranth whole grain flour, determining solubilization at pH 8 to 11 and acidic precipitation at pH 4.5 to 5.0 as optimum conditions. Depending on the extraction conditions, physicochemical changes of the proteins may affect their intended functionality and bioavailability. Scilingo et al. (2002) analyzed the effect of extraction and precipitation conditions on the electrophoretic and calorimetric behavior of amaranth proteins. Results from this study indicated that pH-values >9 and <5 reduced thermal stability and increased protein denaturation. Similarities regarding protein solubility of amaranth and quinoa can be expected, because of the same family (Amaranthaceae) and a related seed structure.

The aim of this study was to optimize a fractionation process in order to separate quinoa flour from the bran fraction. Second, proteins of the bran fraction were further concentrated by optimizing an extraction process, aiming to reach a protein content between 50% and 90%. Different pH conditions for extraction were analyzed and the resulting solution was further purified and dried to obtain a stable product which can be used as dietary supplements and functional food ingredients.

2. Materials and methods

2.1. Origin and fractionation of quinoa

Organic Royal Quinoa grains (*Chenopodium quinoa*) from Bolivia were purchased from Ziegler & Co. GmbH (Wunsiedel, Switzerland). Due to the mechanical pretreatment (involving washing and friction) by the manufacturer, the quinoa bran fraction in this work was lacking the pericarp. Quinoa seeds were ground to whole grain flour in an ultra-centrifugal mill Retsch ZM 200 (Haan, Germany) with a mesh screen of 500 μm. Prior to fractionation, the moisture content of whole grain flour was determined thermo-gravimetrically according to AACC standard method 44-01 (American Association of Cereal Chemists (AACC), 2002). Conditioning was carried out in an airtight box and the initial moisture content of 12.3% was elevated to 14%, 15% and 16% by spraying water onto the seeds, followed by manual stirring. Resting time lasted 20 h and was shortened to 16 h for 15% seed moisture samples at room temperature (20 °C). Milling trials were performed in a Brabender Quadrumat Junior mill (Duisburg, Germany), which is a laboratory roller mill, separating the quinoa seeds into bran and white flour by sieving in a rotating sifter (mesh of 200 μm). The milling yield as percentage was determined by dividing the mass of the respective fraction by the total mass of milling products.

2.2. Quantitation of protein, ash and moisture

Milling fractions were analyzed for their moisture and ash content by employing standard methods of analysis, AACC 44-01 and 08-12, respectively (American Association of Cereal Chemists (AACC), 2002). The crude protein content was determined by the Kjeldahl procedure according to standard method AACC 46-10 using a conversion factor of $N \times 5.54$ (American Association of Cereal Chemists (AACC), 2002; Fujihara et al., 2008). Each test was performed in quadruplicate and expressed on dry weight basis (db).

Color characterization of milling fractions was measured using a Spectrophotometer fitted with an optical sensor from BYK Gardner GmbH (Geretsried, Germany) on the basis of the CIE L^*, a^*, b^* color system as describe by Edney et al. (2002) The instrument was calibrated using a white standard color calibration plate. The brightness, indicated by L^* (0 = black, 100 = white) was recorded. Three measurements from three different samples were performed.

2.3. Particle size distribution of milling fractions

The particle size distribution of flour was measured according to ICC standard method No 207 (International Association for Cereal Science and Technology (ICC), 1998). Milling products (each 100 g) were sifted in a sieving chamber from Bühler GmbH (Braunschweig, Germany) with a set of graded standard sieves (45 μm, 90 μm, 125 μm, 180 μm, 250 μm, 355 μm, 500 μm, 1000 μm, 1250 μm) for 10 min in the case of flours and for 5 min when sieving bran. The retained product on each sieve was weighed and expressed as percentage retention. Three measurements from three different samples were conducted.

2.4. Extraction of proteins from quinoa bran

After enriching the proteins in the bran fraction through conditioning, milling and sieving, further concentration was conducted by solvent extraction followed by an optional purification procedure as described in Fig. 1. Proteins were solubilized (see Fig. 1a) by preparing a suspension of quinoa bran and water in a ratio of 1:10 (w:v). The impact of the pH-value on protein solubility was determined by adding 1N NaOH to obtain a pH-range from 7 to 12 according to the