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Analytical Methods

Protein content and amino acids profile of pseudocereals

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ABSTRACT

Quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus caudatus*) and buckwheat (*Fagopyrum esculentum*) represent the main protein source in several diets, although these pseudocereals are not currently present in the FCDB nutrient profile information. The aim of this work is to characterise the AA profile of these pseudocereals and compare them with rice. Total protein content revealed to vary from 16.3 g/100 g (quinoa Salta) to 13.1 g/100 g (buckwheat) and lower values were found in rice samples (6.7 g/100 g). For pseudocereals the most abundant essential AA was leucine. Quinoa-Salta evidences the highest leucine content (1013 mg/100 g) and the minor methionine content (199 mg/100 g). Buckwheat was the cereal with the highest phenylalanine content (862 mg/100 g). Rice (*Oryza sativa*) presents the lowest content for all AA. Results showed pseudocereals as the best source of AA. EuroFIR guidelines where strictly followed and proved to be a crucial tool to guarantee data interchangeability and comparability.

1. Introduction

Amaranth (*Amaranthus caudatus*) and quinoa (*Chenopodium quinoa*) known as pseudocereals, were considered major crops used by the Pre-Colombian cultures in Latin-America for centuries. As a consequence of the invasion and the conquest by the Spanish, cultivation and consumption of these crops were suppressed and thereafter only continued on a minor scale. Attending to their good nutritional properties, the interest on these grains has risen again. Buckwheat was originated from Central Asia and was transferred by nomadic people to Central and Eastern Europe. Today, buckwheat (*Fagopyrum esculentum*) is celebrating something of a comeback due to the demand for gluten-free diets, and the total area of soil dedicated to its crop amounts to 2.5 million hectares, representing a production of 2 million tonnes of grain per year (Fabio, Schoenlechner, Siebenhandl, & Berghofer, 2008).

Amaranth, quinoa and buckwheat are recommended for celiac disease patient diets by the World Gastroenterology Organization, since they are gluten free cereals. In addition, all these gluten free grains are also recommended as base ingredients for baby food recipes as an alternative to rice (*Oryza sativa*) due to their low allergenicity (WGO, 2012).

Some studies reported the fact that some cultivars of quinoa could activate the adaptative immune response in some patients with celiac disease (Bergamo, Maurano, Mazzarella, Gianfrani, & Rossi, 2011; Zevallos, Ellis Julia, Ciclitira, Tanja Suligoj Herencia, & Irene, 2012). However, a recent *in vivo* study, which included a panel of adult celiac patients, indicates that celiac patients safely tolerate a daily ingestion of 50 g of quinoa during a period of 6 weeks (Zevallos, Herencia, & Ciclitira, 2014).

The nutritional value of pseudocereals is mainly connected to their proteins that are an important group of bio macromolecules involved in physiological functions (Gorinstein et al., 2002). The protein content is 13.4–16.5% for amaranth 12.0–18.9% for buckwheat and 12.1–14.5% for quinoa (Alvarez-Jubete, Arendt, & Gallagher, 2010; Christa & Soral-Śmietana, 2008; Nascimento et al., 2014). Compared with common cereal grains, the protein content is significantly higher than maize (*Zea mays*) (10.2%), and comparable to whole-grain wheat (*Triticum* spp.) (13.2%). These pseudocereals contain minor protein content when compared with legume seeds such as bean (*Phaseolus vulgaris*) with 23.6% or soya (*Glycine max*) with 36.1% (USDA, 2011).

The most important aspect of a protein, from a nutritional point of view, is its essential amino acids (EAA), because they have carbon skeletons that cannot be synthesised by humans, therefore they must be provided through the diet. For this reason essential amino acids are more important for growth and maintenance of

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metabolic needs, than the remaining non-essential amino acids. Besides these two categories, a third category is also considered as "conditionally essentials" amino acids, meaning that they are not normally required in the diet, but under specific physiological or pathological conditions the human body cannot synthesise them in adequate amounts, and in this context their intake becomes therefore indispensable (WHO, 2007). Digestibility is also a relevant factor for the nutritional value of proteins. EAA content can be used to estimate the Protein Digestibility Corrected Amino Acid Score (PDCAAS) that measures the protein quality in human nutrition according to different stages of life (WHO, 2007).

The composition data values for pseudocereals are usually obtained from Food Composition Databanks (FCDB). A research in several databases showed that only United States Department of Agriculture (USDA) FCDB has analytical data for amino acid profile in these pseudocereals.

Requirements for data interchange have been recently published in Europe by the EuroFIR AISBL platform. These requirements were also applied in Nascimento et al. (2014) work.

The main goal of this work was to determine the amino acid profile for quinoa, amaranth and buckwheat, as well as to compare amino acid profile of rice, the largest consumed gluten free cereal in Portugal (FAOSTAT, 2014).

2. Materials and methods

2.1. Samples and sample preparation

The sampling used in this research is part of a study that started in 2010 and from which the first scientific results were reported in (Nascimento et al., 2014). Complete seed samples of amaranth from Jujuy and quinoa from Salta (quinoa_S) and Jujuy (quinoa_J) were obtained from the Cooperative of Producers CAUQUEVA-Tilcara – Argentina. Buckwheat and amaranth seeds originated from biological agriculture were obtained in the Portuguese market from a non European source. Samples of white polished rice were obtained from local factories in Portugal, having their origin from their main crop geographies – Ribatejo and Sado. According to a selective sampling plan five primary samples of each species and geographical region were taken. Quinoa and rice samples were collected in three consecutive years, amaranth samples were collected in two consecutive years and samples of buckwheat were collected in one year. The samples were immediately prepared after receipt in the laboratory according to the procedure described by Table 1. Each primary sample was milled using a high speed grinder (knife mill GRINDOMIX GM), homogenised and analysed separately. The laboratory samples were stored in vacuum bags at room temperature until further processing. Two test portions were analysed for moisture and protein content and at least three test portions were taken for amino acid composition analysis.

2.2. Reagents and chemical standards AA analysis

All reagents were of high analytical grade. Ultrapure water obtained from a Milli-Q purifier (Millipore, Eschborn, Germany), was used for the preparation of all solutions. Aqueous hydrochloric acid (HCl) 0.1 N was used to prepare a stock solution of D-Norvaline at a concentration of 2.5 mM to add to standard solution and a concentration of 25 mM to add to samples. Also, a solution of HCl 6 N containing 0.5% phenol was used to dilute the samples before microwave hydrolysis. Waters® AccQ Fluor reagent kit, containing 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate as derivatising compound, sample dilution buffer and eluent A and B as mobile phase, all obtained from Waters Corporation Company.

Working standard solutions were prepared from an Amino Acid Standard Hydrolysate provided by Waters®, containing 2.5 mM of each amino acid including histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), valine (Val), cysteine (Cys), tyrosine (Tyr), glycine (Gly), arginine (Arg), proline (Pro), acids aspartic acid (Asp), glutamic acid (Glu), alanine (Ala) and serine (Ser).

2.3. Analysis

Moisture was determined by the method of AOAC (AOAC 952.08, 2000). Two test portions (5.0 g) were weighed into a predried weighed crucibles and placed in a dry air oven from Heraeus Instruments, Hanau, Germany, at 102_C ± 2_C for 2 h. The crucibles were removed and cooled in a desiccator and then weighed. This process was repeated until constant weight was obtained.

2.3.1. Total protein

Test portions (1.0 g) of each sample were analysed in duplicate for total nitrogen content, according to the Kjeldahl method. This method contemplates three different steps: digestion, distillation and titration. In this process, most organic nitrogen containing samples are digested with sulphuric acid in combination with a copper catalyst to ammonium sulphate using a block digestion system Foss Tecator 2006 Digestor (Höganäs, Sweden). The ammonium is then liberated by raising the pH with a Foss 2800 KjeltecAutoDistillation unit (Foss Tecator), and measured by

Table 1
Sampling.

Sample	Species	Crop			Sample Preparation/Sample Handling
		Geographical region	Number of primary samples[a]	Years	
Quinoa	*Chenopodium quinoa* Willd.	-Salta -Jujuy	5 5	2011–2013	Each sample was washed with tap water with the aim to eliminate bitter taste and toxic saponins. Washed grains were dried at 45 °C for 12 h and stored in vacuum bags at room temperature until processing
Amaranth	*Amaranthus* spp.	-Jujuy -Biological agriculture south American	5 5	2011 2012	Each sample was homogenised and milled. The analytical samples were stored in vacuum bags at room temperature until processing.
Buckwheat	*Fagopyrum esculentum*	Biological agriculture Non European Source (China and India)	5	2013	
Rice	*Oryza sativa*	-Ribatejo -Sado	5 5	2010–2012	

[a] Sample unit of 500 g analysed separately.