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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.

titration with an automated titration system, Titrando 808 from Metrohm (AOAC 991.20, 2000).

Protein content was calculated using conversion Jones factors of 6.25 for pseudocereals and 5.95 for rice, according to (FAO, 1973). The results are expressed in g per 100 g of edible portion on fresh weight basis. The obtained protein values were compared with the sum of AA according to FAO recommendations (FAO Food nutrition, 2002).

2.3.2. Amino acids

Sample digestions were undertaken using a closed-vessel microwave digestion system, Milestone ETHOS 1 Series. At least three test portions (30 mg) were weighted into proper quartz digestion vials. One millilitre of cloridric acid (6 N) containing 0.5% phenol and 200 μL of internal standard (25 mM of D-Norvaline) were carefully added to each vial. The vials were closed and introduced into the microwave oven in anaerobic conditions using nitrogen gas purges and a vacuum pump. The microwave program was optimised and established (15 min to increase temperature to 160 °C, 10 min at 160 °C and 90 min to cool).

After complete hydrolysis the extracts were neutralised with 1 mL sodium hydroxide (6 N), and fillup to a total volume of 10 mL with deionised water. Hydrolysates were filtered through a common filter paper before derivatisation.

The derivatisation process was performed by adding 80 μL of buffer, 10 μL of sample and 20 μL of reconstituted derivatisation reagent in a chromatographic vial. The reaction was vortex mix and immediately heated to a constant temperature of 55 °C during 10 min.

Chromatographic determination was performed in an Acquity UPLC system from Waters in accordance to the equipment Manufacturer's application system guide note (Waters, 2006). The system is equipped with photodiode array (PDA) detector. A BEH C18 column (100 mm × 2.1 mm i.d., 1.7_m; Waters) at a flow rate of 0.7 ml/min was used, and the column temperature was kept at 55 °C. The injection volume was 1 μl and the detection wavelength was set at 260 nm. The mobile phase consisting in two eluents: AccQTag ultra eluent A diluted in 95% of deionised water and AccQ-Tag ultra eluent B. The following gradient conditions used were: 0–0.54 min, 99.9% A–0.1% B; 5.74 min, 90.9% A–9.1% B; 7.74 min, 78.8% A–21.2% B; 8.04 min, 40.4% A–59.6% B; 8.70–10 min, 99.9% A–0.1% B. Within 10 min of gradient chromatographic run time.

The quantification processing method was performed with a calibration curve prepared from a stock solution containing 2.5 mM of each amino acid, diluted to appropriate concentrations. D-Norvaline was added as internal standard to a final concentration of 45.5 pmol. Calculations were performed in the empower software from Waters®. Derivatised amino acids were identified and quantified by comparison with the retention times and areas ratios of standard amino acids mixture with the internal standard. The results are expressed in mg per 100 g of edible portion on fresh weight basis.

2.4. Quality assurance and quality control

Quality assurance tests that contribute to analytical quality of data, such as regular verification of instruments, performance, linearity, Limit of Quantification (LoQ) and Limit of Detection (LoD), were in place. Also laboratory competence is demonstrated through satisfactory participation in proficiency testing schemes launched by PT providers holds ISO 17043 certificate.

The EuroFIR data quality evaluation system QE scirep (Quality Evaluation of analytical data from SCIentific literature and laboratory REPorts) is part of EuroFIR Quality Evaluation System and aims to create tools to assist compilers to score value documentation. QE scirep is described elsewhere in detail (Oseredczuk & Westenbrink, 2013; Salvini, Oseredczuk, Roe, Møller, & Holden, 2009). Briefly is composed by seven categories (Food description; component identification; sampling plan; number of analytical samples; sample handling; analytical method; analytical quality control) in total 40 questions. The scoring of analytical data generated in this project was presented in previous work (Nascimento et al., 2014) and evaluated for all seven categories. The component identification, analytical method and analytical control, used in amino acids analysis and described here has the same quality index > 30 (rating 5 points per each of seven categories), as well as mineral and proximates data, published by Nascimento et al. (2014).

2.5. Statistic analysis

All data was analysed by one-way analysis of variance (ANOVA) using the nonparametric Tukey–Kramer multiple comparison test to identify significances between the origins and species. Statistical analysis was conducted by the Statistica 7 software (Statsoft Ibérica, Lisboa, Portugal). Statistical significance was established at a p-value < 0.05.

3. Results and discussion

3.1. Analytical data quality assurance

Quality control results for protein and amino acid analysis are indicated in Table 2. The analytical values are within the certified ranges for all amino acids and macronutrients. The sum of individual amino acids agree with protein content determined by Kjeldahl. For each amino acid analysis the variations between replicates agree well and are within certified values range, conferring an appropriate accuracy to the analytical method. Other parameters included in performance criteria were LoD and LoQ, determined according with Eurachem guidelines and Codex Alimentarius as the ratio to signal/noise of 3 and 10. The minimum specific level (ML) as defined by Codex Alimentarius can be assessed by LoD and LoQ. The matrix match LoD ranged between 0.7 mg/100 g (valine) and 3.9 mg/100 mg (tyrosine). The developed UPLC method fits the criteria of ML to measure content of each amino acid, usually found in concentrations higher than LoQ. Calibration curves for the simultaneous determination of 17 amino acids were constructed using the integrated analyte absorbance versus analyte concentration of multielementar solutions. A correlation coefficient ranging from 0.9996 (proline) to 0.9973 (glutamine) was obtained. The method resolution can be derivate from the retention time of two adjacent amino acid peaks. As one can observe from the retention time presented in Table 2 and Fig. 1 a clear peak separation was obtained for almost all amino acids. The smallest difference was obtained between cysteine and lysine, although peak overlapping was not observed.

3.2. EuroFIR quality criteria of amino acids

EuroFIR criteria regarding food analytical data for compilation and publication in the Food Composition Databanks was recently published. The present research work was designed in compliance with EuroFIR Data Quality Evaluation System (DQES) (Oseredczuk, Salvini, Roe, & Møller, 2009; Oseredczuk & Westenbrink, 2013) encompassing requirements of other systems such as DQES USDA (Bhagwat, Patterson, & Holden, 2009) and FAO (Charrondiere, Burlingame, Berman, & Elmadfa, 2011). The work fulfils almost all criteria, nevertheless sampling plan was not robust enough due to economical constrains and time line of the project and more laboratory data is necessary, namely for the quinoa available in Portuguese market. Although these constrains can be suppressed

because analytical values can be aggregated with high score if each raw data complies with quality criteria for food description, component identification, sample handling, analytical methodology, analytical quality control (Castanheira et al., 2011).

3.3. Contributions of pseudocereals to essential amino acids nutrient intake

Contributions of amino acid intake expressed in Protein Digestibility Corrected Amino Acid Score (PDCAAS) calculated according FAO/WHO/UNU 2007 reports, considering adults as target population, present in Fig. 2 (WHO, 2007). PDCAAS was calculated dividing the amino acid amount in 1 g of protein by the amino acid requirement pattern. The result (amino acid score) is then multipled by the protein digestibility: 92% for quinoa, 90% for amaranth, 80% for buckwheat and 88% for polished rice (Boye, Wijesinha-Bettoni, & Burlingame, 2012; Ferreira & Arêas, 2004; WHO, 2007).

3.4. Protein analysis

Pseudocereals presented a protein content (g/100 g of edible portion on fresh weight basis) of 13.1 (±0.1) g/100 g for buckwheat, 13.5 (±0.2) g/100 g for amaranth, 12.2 (±0.1) g/100 g for quinoa_J and 16.3 (±0.02) g/100 g for quinoa_S (Table 3). The quinoa results are shown separately evidencing significant differences between origins. Regarding all pseudocereals, only quinoa_S statistically differs from the other ($p < 0.05$). When compared with pseudocereals, the rice protein content was significantly lower (6.7 ± 1.1 g/100 g). All the values are evaluated under appropriate quality control procedures as a guarantee of reliability and further comparability. These values are in agreement with those reported by USDA (2011).

3.5. Amino acid method analysis

The method presented in Section 2.3.2 was used for qualifying amino acids in one of three categories: essential, conditionally essential and non-essential.

The choice of the chromatographic method was based on the ability to provide high selectivity, sensitivity, reproducibility and reduced analysis time. UPLC offers large advantage over HPLC, since only 10 min is needed to obtain the separation of seventeen amino acids with a clear resolution (Fig. 1) against HPLC which takes approximately 60 min to complete (Boogers, Plugge, Stokkermans, & Duchateau, 2008). This technique utilises 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate to transform primary and secondary amines into highly stable fluorescent derivatives yielded appropriate sensitivities within a low pmol range (Fiechter & Mayer, 2011). The microwave method used for acid hydrolysis creates an inert and anaerobic media (vacuum atmosphere in a nitrogen media) that prevents amino acid oxidation and degradation especially for the case of cysteine and methionine.

The amino acids under study in this research work are: His, Ile, Leu, Lys, Met, Phe, Thr and Val as essential amino acids, Cys, Tyr, Gly, Arg, and Pro as conditionally essential and Asp, Glu, Ala and Ser as non-essential.

Tryptophan (Try) needs an alkaline hydrolysis in anaerobic condition. Since the analytical facilities were not available and to avoid missing data borrowed values were taken from the USDA nutrient database (USDA, 2011), as suggested by Greenfield (Greenfield & Southgate, 1992). Amino acids profile is presented in Tables 3 and 4. Quinoa, amaranth and buckwheat revealed significantly higher ($p < 0.05$) content in almost all amino acids when compared to white rice. The only exceptions are for the case of Ala where rice does not differ from amaranth and quinoa_J and for the case of Met where no differences were identified between rice and quinoa_S.

Significant differences were found among quinoa_S and quinoa_J for the amino acids studied, except for Cys and Ser where similar values were observed. The variations for amino acids content can be explained by genetic or environmental growth conditions (Gonzalez, Konishi, Bruno, Valoy, & Prado, 2012; Miranda et al., 2012). Regarding amaranth and rice, no differences were identified between the different origins. The nutrient content of foods can be affected by cultivar type, although cultivar specific differences have received little attention to date.

Similarities were found between amaranth and quinoa_J and between quinoa_S and buckwheat, where results are equivalent for the case of 10 out of 17 amino acids analysed. Non-essential amino acids were mainly composed by Glu and Asp. The results for Glu ranged from 2751 mg/100 g to 1888 mg/100 g and for Asp ranged from 1421 mg/100 g to 923 mg/100 g, respectively for quinoa_S and for quinoa_J. These values are in accordance with

Table 2
Quality assurance results and method parameters.

AA	Retention time (RSD) (min)	Correlation coefficient (r^2)	Slope (B)	Sy/x	LoD (mg/100 g)	LoQ (mg/100 g)	Certified value ± U[a]	Analysed values[c]	Test material 2573 (Z-score)[d]
Ala	5.22 (0.05)	0.9990	8.64E−01	8.75E−01	1.13	2.59	2.12 ± 0.96	2.20 ± 0.05	–
Arg	3.64 (0.07)	0.9983	9.41E−01	1.47E+00	2.49	7.54	2.26 ± 0.52	2.41 ± 0.22	2.50
Asp	4.12 (0.04)	0.9979	1.56E+05	1.48E+00	2.68	8.12	5.29 ± 0.28	5.39 ± 0.12	–
Cys	6.65 (0.02)	0.9982	1.91E+05	2.18E+00	3.18	9.65	0.48 ± 0.14	0.39 ± 0.04	–
Glu	4.57 (0.03)	0.9973	1.54E+05	1.38E+00	2.82	8.56	14.3 ± 2.1	15.4 ± 0.90	–
Gly	3.77 (0.05)	0.9988	2.18E+05	1.42E+00	1.01	3.05	1.23 ± 0.13	1.30 ± 0.05	–
His	2.52 (0.10)	0.9980	2.32E+05	2.03E+00	2.78	8.43	1.73 ± 0.17	1.67 ± 0.10	1.60
Ile	7.81 (0.01)	0.9997	2.05E+05	6.49E−01	0.85	2.58	3.00 ± 0.61	3.14 ± 0.04	0.40
Leu	7.89 (0.01)	0.9984	2.24E+05	1.22E+00	1.23	3.72	6.16 ± 0.88	6.37 ± 0.05	0.30
Lys	6.71 (0.01)	0.9980	1.43E+05	1.43E+05	3.79	13.65	4.78 ± 0.77	4.96 ± 0.22	0.00
Met	6.97 (0.05)	0.9989	2.25E+05	1.44E+00	2.02	6.12	1.71 ± 0.28	1.81 ± 0.11	2.50
Phe	8.02 (0.01)	0.9974	2.46E+05	2.74E+00	3.08	9.34	3.48 ± 0.50	3.35 ± 0.33	1.50
Pro	5.82 (0.02)	0.9996	1.85E+05	6.23E−01	0.82	2.47	6.64 ± 0.73	6.73 ± 0.03	–
Ser	3.45 (0.08)	0.9994	2.06E+05	9.00E−01	1.00	3.03	3.80 ± 0.35	3.60 ± 0.09	–
Thr	4.91 (0.02)	0.9994	2.06E+05	8.85E−01	1.11	3.38	2.76 ± 0.54	2.77 ± 0.12	0.30
Tyr	6.87 (0.02)	0.9977	2.46E + 05	2.75E+00	3.94	11.94	3.16 ± 0.71	3.11 ± 0.26	2.30
Val	7.10 (0.02)	0.9996	3.02E+05	7.18E−01	0.73	2.21	3.67 ± 0.98	3.97 ± 0.02	−0.50
Moisture							1.98 ± 0.27[b]	1.84 ± 0.07	
Protein							66.1 ± 1.3[b]	66.0 ± 0.37	

[a] NIST 3244 – Ephedra – containing protein powder. National Institutes of Standards and Technology. Gaithersberg. MD. USA.
[b] NIST SRM 1846 – infant formula. National Institutes of Standards and Technology. Gaithersberg. MD. USA.
[c] AA units are expressed in % AA dry mass and protein units are expressed in g/100 g.
[d] Proficiency test – FAPAS – Food Analysis Performance Assessment Scheme.

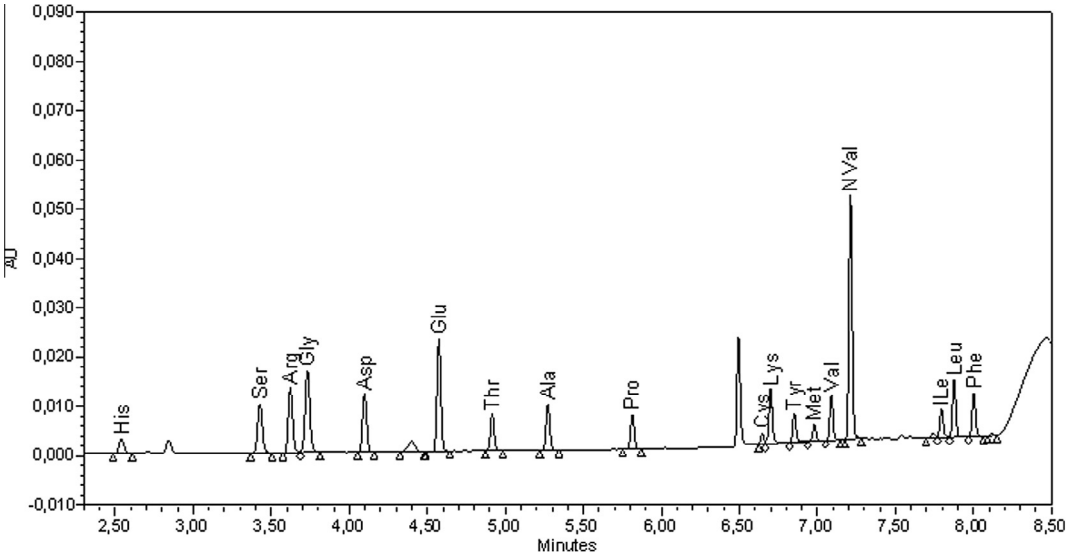
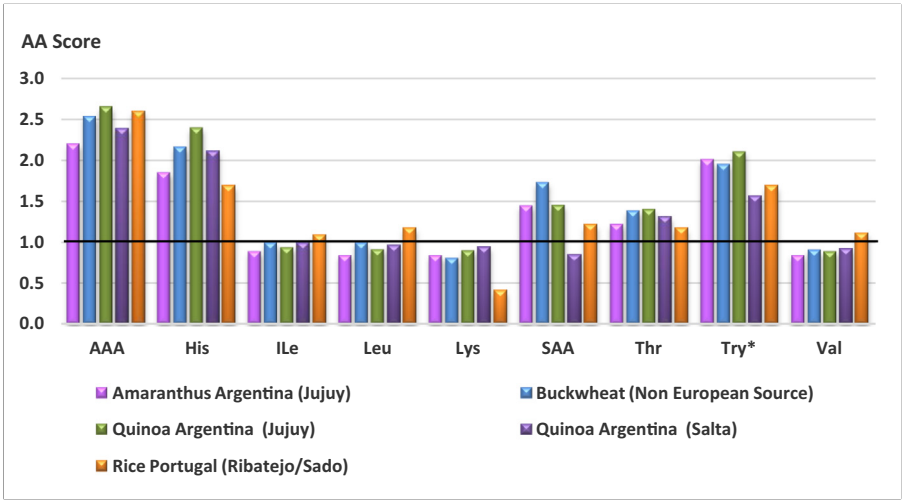


Fig. 1. Amino acid chromatogram of buckwheat.



AAA - Aromatic amino ácids (Phe+Tyr)
SAA- Sulfur Amino acids (Met+Cys)
*USDA National Nutrient Database for Standard Reference (2011)

Fig. 2. Protein Digestibility Corrected Amino Acid Score (PDCAAS).

the USDA National Nutrient Database, however a difference of 33% is observed in Ser for the case of amaranth.

Similar values were obtained for conditionally essential amino acids and essential amino acids excepting for Cys where significant differences between the three pseudocereals were found.

A relevant content was observed for Arg in all pseudocereals highest values in quinoa_S and in buckwheat, with values of 1501 mg/100 g and 1483 mg/100 g, respectively. Since Arg is required for the synthesis of other amino acids (Lupton, 2005) these values highlight the quinoa and buckwheat nutrient profile and are in agreement with other works reported in literature.

Since Arg and Gly are involved in the synthesis of other nitrogenous compounds that are important to physiological viability, these non-essential amino acids are important as part of the diet when optimum growth conditions are targeted as those detected in the North of Argentina (Young & Borgonha, 2000). Quinoa_J presents lower amino acid content than the other pseudocereals nevertheless much higher than rice. In spite of Cys and Tyr are designated as conditionally essential amino acids they were taken into account with Met and Phe, respectively for PDCAAS calculation (WHO, 2007).

Pseudocereals content of Leu and Phe amino acids revealed a similar pattern, evidencing the highest values, with 1013 mg/100 g (Leu) for quinoa_S and 862 mg/100 g (Phe) for buckwheat, and with the lowest statistical values for the case of amaranth, with 673 mg/100 g for Leu and 652 mg/100 g for Phe (Table 4). Leu plays an important role in the protein synthesis (Norton & Layman, 2006). In the study Leu content in pseudocereals is one of the highest, however for the case of amaranth Leu presents a 0.8 value for PDCAAS, qualifying as a limiting amino acid but near the end of the interval to be considered as such (Fig. 2). Although not in accordance with the results presented by (Wei, Hu, Zhang, & Ouyang, 2003), buckwheat Leu presents a score of 1.

For Lys, the lowest value is presented in rice (139 mg/100 g) as expected in accordance with Schaeffer and Sharpe (1997). Fig. 2 shows for Lys PDCAAS value is 1 for quinoa_S and 0.9 for buckwheat and quinoa_J. For amaranth the score was 0.8, whereas for rice the value did not exceed 0.4. After determining the scores

Table 3
Toatl protein, non-essential[(1)] and conditionally essential[(2)] amino acids content of cereals under study.

Samples	Ala[(1)]	Arg[(2)]	Asp[(1)]	Cys[(2)]	Glu[(1)]
	(mg per 100 g of edible portion on fresh weight basis)				
Amaranth (Jujuy)	429.0 ± 12.4	1254.6 ± 5.0	930.2 ± 29.9	154.8 ± 7.9	2195.9 ± 69.7
Amaranth (biological agric.)	415.8 ± 24.2	1113.0 ± 19.8	985.0 ± 73.5	176.8 ± 10.5	2031.9 ± 9.3
Mean ± sd	423.7 ± 16.6a	1197.9 ± 78.2a	952.2 ± 52.0a	163.6 ± 14.3a	2130.3 ± 102.6a
Quinoa (Jujuy)	465.8 ± 34.7ab	1101.2 ± 94.7a	923.7 ± 49.2a	165.1 ± 52.3	1888.0 ± 79.2a
Quinoa (Salta)	690.4 ± 38.1c	1501.4 ± 130.5b	1421.8 ± 95.0b	129.3 ± 24.1	2751.7 ± 187.8b
Mean ± sd	555.7 ± 120.8	1261.3 ± 231.0	1122.9 ± 265.5	147.2 ± 42.3b	2319.9 ± 480.6
Buckwheat (biological agric.)	540.4 ± 2.2b	1483.0 ± 125.9b	1309.1 ± 27.1b	242.8 ± 21.4c	2535.3 ± 31.1ab
Rice (Sado)	411.7 ± 59.4	658.8 ± 81.7	700.0 ± 119.3	44.6 ± 8.8	1568.6 ± 254.5
Rice (Ribatejo)	332.9 ± 50.2	550.1 ± 76.9	550.2 ± 94.2	41.2 ± 6.9	1202.0 ± 211.0
Mean ± sd	360.1 ± 64.9a	587.6 ± 93.3c	601.9 ± 124.6c	43.0 ± 7.9d	1328.4 ± 284.4c
Samples	Gly[(2)]	Pro[(2)]	Ser[(1)]	Tyr[(2)]	Protein
	(mg per 100 g of edible portion on fresh weight basis)				g/100 g
Amaranth (Jujuy)	912.0 ± 3.4	478.7 ± 9.5	703.6 ± 1.9	613.9 ± 18.6	13.4 ± 0.3
Amaranth (biological agric.)	978.7 ± 19.4	519.6 ± 10.2	855.2 ± 16.4	583.2 ± 116.8	13.6 ± 0.2
Mean ± sd	938.7 ± 37.9a	495.1 ± 23.9a	764.2 ± 83.4a	601.6 ± 62.2a	13.5 ± 0.2a
Quinoa (Jujuy)	648.1 ± 48.3b	432.6 ± 33.8a	593.8 ± 72.3	717.9 ± 57.5a	12.2 ± 0.1a
Quinoa (Salta)	939.7 ± 60.6a	613.5 ± 32.7b	698.7 ± 34.7	825.3 ± 47.8b	16.3 ± 0.0b
Mean ± sd	777.7 ± 161.8	504.9 ± 98.6	653.7 ± 79.0a	760.8 ± 75.3	14.2 ± 2.4
Buckwheat (biological agric.)	831.3 ± 64.6a	541.0 ± 22.5ab	771.5 ± 41.9a	718.9 ± 73.4a	13.1 ± 0.1a
Rice (Sado)	343.5 ± 32.7	3281.1 ± 43.9	394.9 ± 59.0	358.6 ± 45.8	7.4 ± 0.9
Rice (Ribatejo)	297.0 ± 37.3	277.0 ± 41.4	318.4 ± 46.8	330.8 ± 69.5	6.2 ± 1.0
Mean ± sd	313.0 ± 41.8c	294.7 ± 48.3c	344.8 ± 62.4b	340.4 ± 62.9c	6.7 ± 1.1c

Means within a column with the same superscript letter are not significantly different ($a = 0.05$).

Table 4
Essential amino acids and moisture content of cereals under study.

Samples	His	Ile	Leu	Lys	Met
	(mg per 100 g of edible portion on fresh weight basis)				
Amaranth (Jujuy)	392.6 ± 8.2	420.8 ± 1.1	682.2 ± 8.2	551.9 ± 32.8	291.5 ± 1.1
Amaranth (biological agric.)	453.2 ± 6.3	368.8 ± 4.3	659.3 ± 9.5	496.8 ± 48.7	348.0 ± 8.4
Mean ± sd	416.8 ± 33.8a	400.0 ± 28.6a	673.0 ± 14.6a	529.9 ± 45.2a	314.1 ± 31.2a
Quinoa (Jujuy)	475.0 ± 47.6a	371.8 ± 31.0a	713.3 ± 39.0a	481.1 ± 45.7a	257.3 ± 14.0a
Quinoa (Salta)	564.7 ± 49.0b	526.9 ± 34.7b	1013.0 ± 58.8b	755.4 ± 69.9b	199.1 ± 17.6b
Mean ± sd	514.9 ± 64.8	433.8 ± 85.7	833.2 ± 161.1	590.8 ± 155.3	231.4 ± 33.9
Buckwheat	532.1 ± 46.7ab	431.5 ± 8.0ab	846.0 ± 14.0ab	595.6 ± 57.2a	380.9 ± 33.7a
Rice (Sado)	206.6 ± 29.0	275.8 ± 41.0	585.2 ± 96.3	179.4 ± 55.8	168.9 ± 13.6
Rice (Ribatejo)	186.6 ± 45.8	232.1 ± 37.8	496.9 ± 79.0	118.4 ± 38.7	155.6 ± 43.6
Mean ± sd	193.5 ± 41.4c	247.2 ± 43.7c	527.3 ± 93.9c	139.4 ± 53.3c	160.2 ± 36.3b
Samples	Phe	Thr	Try[a]	Val	Moisture
	(mg per 100 g of edible portion on fresh weight basis)				g/100 g
Amaranth (Jujuy)	629.7 ± 15.5	422.8 ± 1.2	–	443.8 ± 5.7	9.7 ± 0.1
Amaranth (biological agric.)	685.2 ± 11.8	418.3 ± 18.9	–	440.8 ± 10.3	9.9 ± 0.0
Mean ± sd	651.9 ± 32.8a	421.0 ± 9.8a	181	442.6 ± 6.8a	9.8 ± 0.1
Quinoa (Jujuy)	614.9 ± 73.8a	426.3 ± 33.9a	–	458.2 ± 46.1a	10.5 ± 0.1
Quinoa (Salta)	791.2 ± 76.8b	534.1 ± 50.6b	–	634.7 ± 40.2b	10.9 ± 0.0
Mean ± sd	685.4 ± 115.2	469.4 ± 67.7	167	528.8 ± 100.1	10.7 ± 0.3
Buckwheat	862.1 ± 79.1b	521.7 ± 18.8b	192	580.7 ± 14.5ab	13.4 ± 0.0
Rice (Sado)	448.5 ± 49.5	226.6 ± 30.0	–	375.2 ± 60.4	12.9 ± 0.0
Rice (Ribatejo)	393.3 ± 55.9	194.0 ± 32.4	–	306.2 ± 53.2	13.0 ± 0.1
Mean ± sd	412.3 ± 59.2c	205.3 ± 34.8c	77	330.0 ± 64.1c	13.0 ± 0.1

Means within a column with the same superscript letter are not significantly different ($a = 0.05$).
[a] The values of tryptophan for amaranth, quinoa, buckwheat and rice (white, medium-grain, raw and unenriched) were obtained in literature (USDA, 2011).

using the USDA data, all pseudocereals reach a score equal or higher than the unit, for Leu and Lys. Regarding rice, as well as the case of all other cereals, Lys represents a limiting amino acid (Young & Pellett, 1994), with a low value for PDCAAS.

Met and Cys are the major sources of sulphur. For these two sulphur amino acids the scores are upper to 1, except for quinoa_S which attains a score of 0.9, near to the limit, showing that all are important sources of sulphur. Buckwheat presents the highest value for both Met and Cys amino acids, differing significantly with quinoa_S for the case of Met, and with both quinoa (S and J) and amaranth for the case of Cys (Table 4).

The amino acids Phe, Tyr and Thr presents a score higher than 1 regarding all the pseudocereals under study (Fig. 2).

Val is an aliphatic amino acid, from Leu and Ile family, both in structure and in function. For Val the score is equal or upper to 1 for buckwheat, quinoa_S and rice, whereas for amaranth and qui-

noa_J the scores are below the unit, respectively with 0.8 and 0.9. Comparing with the USDA table values for Val presents scores upper to 1 to all pseudocereals.

His it has been classified as a non-essential amino acid, but recently the scientific community has reviewed this classification, particularly due to its role in healthy development of children, since its deficiency affects growth (Young, 1998). When compared with rice (194 mg/100 g), the pseudocereals present the statistically significant highest values (532–417 mg/100 g), however His qualifies as no limiting amino acid for none of the samples.

Due to the fact that analytical resources were not available to determine Try, borrowed values were taken from USDA database and following the approach suggested by Southgate and Greenfield. Using these values for PDCAAS calculation as described by (WHO, 2007) a score higher than 1 was found for each of the pseudocereals which indicates Try is not limiting (USDA, 2011). Wei et al. (2003) evaluated amino acid profile and found similar values for Try in buckwheat, although reporting lower levels the score remains higher than 1.

4. Conclusions

A methodology to characterise amino acids in pseudocereals is developed in compliance with available Data Quality Evaluation Systems. Through the use of these systems reliable results can be guaranteed to assist users and compilers of analytical data for pseudocereals allowing compare and evaluate analytical data from several sources. According with the obtained results pseudocereals constitute a richest source of amino acids higher than rice and could be an alternative to rice in gluten-free diets. These considerations reinforce the importance of quinoa and buckwheat due to highest content of Lys found in these pseudocereals. Studies to understand the amino acids bioavailability and bioaccessibility in pseudocereals food products are ongoing. These results will be used to estimate amino acids dietary intake of Portuguese celiac patients and to understand their role in health status.

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