BUCKWHEAT, QUINOA AND AMARANTH: GOOD ALTERNATIVES TO NUTRITIOUS FOOD

HELJDA, KINO A I AMARANTUS: DOBAR IZBOR ZA PRIPREMU NUTRITIVNO BOGATJE HRANE

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ABSTRACT

The purpose of this paper is to characterize the amino acid profiles of wholegrain buckwheat, quinoa and amaranth proteins. The content and protein quality of these pseudocereals were compared mutually and with those of wholegrain wheat as a widespread cereal in human nutrition. Amaranth seeds showed the highest total amino acid (TAA) content, followed by wholegrain buckwheat and quinoa, which showed the lowest TAA content compared to the other two pseudocereals (180.85, 162.46 and 125.37 g per 100 g protein, respectively). The protein digestibility, protein efficiency ratio, protein chemical score and essential amino acid index of the pseudocereals under consideration ranged from 79.25 – 84.58 %, 2.9 – 6.17 %, 16.24 – 47.59, and 45.68 – 68.36, respectively. The results obtained indicate that buckwheat, quinoa and amaranth are promising raw materials that can meet the daily requirements of most essential amino acids, and can be used for the development of a range of value-added bakery products.

Keywords: buckwheat, quinoa, amaranth, protein, amino acid.

REZIME

Porast svesti ljudi o uvođenju zdravijih navika u ishrani doprinela je da potrošači zamene kaloričnu hranu sa funkcionalnom i hranom koja poseduje veću nutritivnu vrednost. Poslednjih godina naročito velika pažnja posvećena je unapređenju nutritivnog profila bezglutenskih proizvoda. Među pseudožitima, heljda, kino a i amarantus privukli su najviše interesovanja imajući u vidu da ova pseudožita ne sadrže gluten, imaju visok sadržaj masnih kiselina, vitamina, minerala i prehrambenih vlakana. Osim navedenog, ona se smatraju nutritivno veoma bogatim sirovinama zbog visokog sadržaja proteina i dobro izbalansiranog profila aminokiselina. Istraživanja u okviru ovog rada bazirana su na karakterizaciji profila aminokiselina proteina heljde, kine a i amarantusa. Sadržaj i kvalitet proteina ovih pseudožita upoređen je i sa istim parametrima određenim u integralnom pšeničnom brašnu. Za analizu aminokiselina korišćena je tehnika tačne hromatografije visoke rezolucije (HPLC). Najviši sadržaj aminokiselina određen je u amarantusu (180,85 g/100 g proteina) i heljdi (162,46 g/100 g proteina). Sadržaj aminokiselina u kinoi (125,37 g/100 g proteina) bio je značajno niži u poređenju sa druga dva pseudožita, ali nanivou sadržaja određenog u pšenici (134,20 g/100 g proteina). Svarljivost proteina, efikasnost proteina (PER), hemijski proteinski skor (CS) i indeks esencijalnih aminokiselina (EAAI) analiziranih pseudožita, heljde, kine a i amarantusa, bio je u sledećim opsezima 79,25 – 84,58%, 2,9 – 6,17%, 16,24 – 47,59 i 45,68 – 68,36. Dobljeni rezultati ukazuju da su heljda, kino a i amarantus nutritivno vredne sirovine koje mogu da zadovolje dnevne potrebe za većinom esencijalnih aminokiselina, a kao takve mogu imati dobrobiti za zdravlje ljudi ukoliko se konzumiraju inkorporirane u različite prehrambene proizvode.

Ključne reči: heljda, kino a, amarantus, proteini, aminokiselina.

INTRODUCTION

The practice of healthier lifestyles has increased worldwide, thus consumers have made a shift from high-calorie foods towards functional foods and foods providing more nutritional impacts. Due to increasing protein requirements caused by the global population growth, the enrichment of food products with proteins has become a topical issue. Plant proteins have the same quality as the proteins based on meat and dairy substitutes, while having lower costs (Šimurina et al., 2015). There is a constant need in the food industry for nutritionally powerful alternative grains that can be incorporated into various food products, particularly gluten-free products. Most of the “new” grains are actually ancient crops, grown commercially in Central and South America (López et al., 2018). Due to great genetic variability, most of these grains can adapt and grow in the most detrimental environmental conditions (Aziz et al., 2018).

Pseudocereals have recently attracted increased attention. These seeds can be milled into flour and used as a cereal crop. Among pseudocereals, buckwheat (*Fagopyrum esculentum* and *Fagopyrum tartaricum*), quinoa (*Chenopodium quinoa*) and amaranth (*Amaranthus* sp.) have been extensively studied over

the last two decades (Abderrahim et al., 2015; Jambrec et al., 2015; Motta et al., 2019; Sakač et al., 2011; Škrobot et al., 2017). Although they are not as widespread as wheat, the interest in these grains has grown rapidly as all of them feature the total absence of gluten, possess high levels of fatty acids, vitamins, minerals, dietary fibres and polyphenols (Pellegrini et al., 2018; Sedej et al., 2011). Moreover, amaranth, quinoa and buckwheat are classified as highly nutritious food because of high protein contents and a well-balanced essential amino acid profiles (Mota et al. 2016).

The nutritional value of pseudocereals is mainly connected to their proteins (Berghofer and Schoenlechner, 2007). Proteins are an important group of biomacromolecules that are involved in physiological functions. Natural vegetable proteins are convenient materials due to their safety, high biocompatibility, nutritional value and low cost. Discovering new vegetable proteins rich in essential amino acids is very important for the food industry, as well as the information on their health effects on foods. Therefore, the objective of this study was to investigate the amino acid profiles of buckwheat, quinoa and amaranth, three pseudocereals very present in human nutrition nowadays, and to compare them with the content of amino acids

of wheat, a world widespread cereal and the staple food in Serbia.

MATERIAL AND METHOD

Samples

Common wholegrain wheat flour, wholegrain buckwheat flour, amaranth and white quinoa seeds were purchased from local markets in Novi Sad, Serbia. For each type of the grains examined, a representative sample consisted of a mixture of grains purchased from three randomly chosen suppliers. For the purpose of analysis, amaranth and white quinoa seeds were reduced to a fine dried powder (~20 mesh) and mixed to obtain a homogeneous sample. Flours were mixed directly.

Nutritional value

Nutritional value was determined according to the Association of Official Agricultural Chemists procedures (AOAC, 2000) using the methods for determining moisture (AOAC, 925.09), fat content (AOAC, 922.06) and protein content (AOAC, 920.87). Total starch content was determined according to ISO 10520 (1997), whereas the total carbohydrates were calculated as the sum of total starch and sugar content. Energy content was determined as follows: Energy (kcal) = $17 \times (\text{g protein} + \text{g carbohydrates}) + 37 \times (\text{g fat})$.

Amino acid analysis

The amino acid analysis was carried out using the high performance liquid chromatography (HPLC). Milled samples (50 mg) were subjected to the acidic hydrolysis (6M HCl with addition of 0.1% phenol and 3% thioglycolic acid) in closed vessels. Further sample preparation included the pre-column derivatization using *o*-phthalaldehyde (OPA) and 9-fluorenylmethylchloroformate (FMOC), according to the method proposed by Agilent Technology 5990-4547EN protocol (Henderson and Brooks, 2010). Chromatographic separation was achieved using the Eclipse Plus C18 column, 5.0 μm , 3.0 x 250 mm. Amino acids were detected with the Diode Array Detector (DAD) (Agilent Technologies, United State) on 338 and 262 nm, and Fluorescence Detector (FLD) (Agilent Technologies, United State) (parameters for excitation set on 340 nm, and emission set on 450 nm). Components spectre was recorded in the range from 190 to 400 nm, R500/100.

Prediction of protein quality

In vitro protein digestibility

In vitro protein digestibility was determined using the pepsin method described by Duodu et al. (2002). Briefly, the incubation mixture, after pepsin (from porcine stomach mucosa) digestion, was filtered using the Whatman No. 4 filter paper, which enabled a better separation of insoluble proteins. The residue on the filter paper was analysed for nitrogen using the Kjeldahl method. Protein digestibility was calculated by expressing the difference between the total nitrogen and residual nitrogen as a percentage of the total nitrogen.

Chemical score (CS)

The protein chemical score (CS, %) was calculated as the ratio of the restrictive amino acid to the concentration of this amino acid in the standard protein (Khatab et al., 2009) (Equation 1):

$$CS = (a_i/a_s) \times 100 \quad (1)$$

where: a_i – content of amino acid with the shortest supply (restrictive amino acid) in the analysed sample, and a_s – content of this amino acid in the standard protein as (FAO/WHO, 1973).

Essential amino acid index

The essential amino acid index (EAAI) was calculated according to Khatab et al. (2009) Equations (2, 3):

$$EAAI = 10^{\log EAA} \quad (2)$$

$$\log EAA = 0.1[\log (a_1/a_{1s} \times 100) + \log (a_2/a_{2s} \times 100) + \dots + \log (a_n/a_{ns} \times 100)] \quad (3)$$

a_1 – a_n are the content of amino acids including Lys, Met + Cys, Thr, Ile, Trp, Val, Leu, His, and Phe + Tyr in the test samples, while a_{1s} – a_{ns} are the essential amino acid requirements in the protein standard (FAO/WHO, 1973).

Protein Digestibility Corrected Amino Acid Score (PDCAAS)

The Protein Digestibility Corrected Amino Acid Score (PDCAAS) was estimated using the regression equation (4) proposed by Alsmeyer et al. (1974):

$$PDCAAS = 0.08084 \times \Sigma[AA_7] - 0.1094 \quad (4)$$

where: $\Sigma[AA_7] = [\text{Thr}] + [\text{Val}] + [\text{Met}] + [\text{Ile}] + [\text{Leu}] + [\text{Phe}] + [\text{Lys}]$.

Statistical analysis

The means of the results obtained were submitted to the one-way analysis of variance (ANOVA). Significant differences between the means of the treatments were observed by the Tukey's HSD (honestly significant difference) test ($p < 0.05$) using the STATISTICA (Data Analysis Software System), v.13.2 (2016), Stat-Soft, Inc, USA.

RESULTS AND DISCUSSION

Nutritional value

One of the more relevant characteristics of pseudocereals is their high protein content (Table 1). According to the results obtained, the pseudocereals examined showed significantly ($p < 0.05$) higher contents of total proteins compared to the wholegrain wheat, whereas buckwheat exhibited the highest total protein content (15.24 g per 100 g). Another important aspect of the nutrient value of pseudocereals is the fat content and composition, especially the fatty acid profile. In the present study, the fat content of the analysed pseudocereals was more than twice that of wheat (2.03 – 4.30 %), whereas amaranth showed the highest fat content (7.05 g per 100 g). The fat content of pseudocereals and its composition are important factors that have to be taken into account during grains storage and handling as fat deterioration can adversely affect food quality and shelf-life (Alvarez-Jubete et al., 2009). The carbohydrate content of amaranth and quinoa was significantly lower in comparison to the other samples, causing lower energy value of these samples. The results obtained within this study are comparable with those reported by Alvarez-Jubete et al. (2009), which showed significantly higher contents of proteins and fat for amaranth and quinoa in comparison to that established in wheat.

Table 1. Nutritional composition of wholegrain wheat, wholegrain buckwheat, amaranth and quinoa

Samples	Protein (%)	Carbohydrate (%)	Fat (%)	Energy (kJ)
Wholegrain wheat flour	13.60±0.30 ^a	73.55±0.10 ^c	1.64±0.25 ^a	1542.23
Wholegrain buckwheat flour	15.24±0.14 ^b	73.63±0.55 ^c	3.33±0.12 ^b	1633.49
Amaranth	14.31±0.31 ^b	65.39±0.70 ^b	7.05±0.10 ^d	1615.75
Quinoa	14.35±0.15 ^c	60.50±1.05 ^a	6.13±0.15 ^c	1499.26

Values are means ± SD of three measurements. Values in the same row followed by the same letter are not significantly different at $p \leq 0.05$.

Amino acid analysis

The amino acids composition of the samples examined is shown in Figures 1 and 2. The amino acids content was expressed as a percentage of total protein. The total sum of amino acids was 162.46, 180.85, and 125.37 g per 100 g protein in buckwheat, amaranth and quinoa, respectively, indicating that buckwheat and amaranth possessed higher concentrations of amino acids in comparison to the wholegrain wheat flour (134.20 g per 100 g protein). In contrast to wheat, the proteins of pseudocereals are composed mainly of cytoplasmatic proteins (globulins and albumins), of which amino acid are high in Lys and low in Glu and Pro. However, wheat storage proteins (Alvarez-Jubete et al., 2009) are high in Glu and Pro, but low in Lys, Arg, Thr and Try. In like fashion, the results obtained in the present study indicate that the predominant amino acids of wheat proteins were glutamic acid and proline, and that the pseudocereals under consideration showed higher contents of lysine. The sum of essential amino acids was 87.92, 105.0, and 61.68 g per 100 g protein in buckwheat, amaranth and quinoa, respectively, indicating that buckwheat and amaranth possessed higher concentrations of essential amino acids in comparison to the wholegrain wheat flour (62.78 g per 100 g protein). Comparing pseudocereals, the highest content of EAA was found in amaranth with Val as the predominant EAA, and CS of 935.4% (Table 2). Conversely, quinoa possessed the lowest EAA content, with Leu as the limiting amino acid with CS of 16.24%. According to Nowak et al. (2016), sulphur amino acids (Cys and Met) are limiting amino acids in amaranth, quinoa and buckwheat. Contrary to this report, Cys was the predominant EAA in quinoa in the present study, and the second dominant EAA in amaranth. The discrepancies established in those findings may be attributed to the fact that the protein content and amino acid composition depend on the genotype and growing conditions (Delgado et al., 1999). The limiting amino acid of buckwheat was Ile with CS of 22.57 %, followed by Cys (48.33 %). However, His and aromatic amino acids (Tyr and Phe) presented the highest values of CS. This last finding is in agreement with those presented by Motta et al. (2019) and Nowak et al. (2016). Buckwheat protein was rich in essential amino acids such as Lys, sulphur amino acids (Cys and Met), aromatic amino acids (Tyr and Phe), Try, Val and His, compared with the FAO/WHO (1973) requirements since their contents were higher than that in the reference protein. Moreover, their content was higher in comparison with their corresponding content in wholegrain wheat protein, as well. On the other hand, Ile and Leu were slightly deficient in buckwheat protein, compared to the standard pattern.

Fig 1. Essential amino acids composition of wholegrain wheat flour, wholegrain buckwheat flour, amaranth and quinoa

Fig 2. Non-essential amino acids composition of wholegrain wheat flour, wholegrain buckwheat flour, amaranth and quinoa

Protein quality estimation

An adequate supply of dietary protein is crucial since proteins are the major structural component of all body cells, and are essential to maintaining cellular integrity and function, health and reproduction (Food and Nutrition Board, I. O. M., 2005). Vegetable proteins are considered useful components due to their high biocompatibility, nutritional value and low cost, particularly in foods rich in essential amino acids (Gorinstein et al., 2002). The scoring patterns proposed by FAO/WHO expert Committee were designed for evaluating the adequacy of proteins for different age groups, and they should be based mainly on the estimated amino acid requirements for the specific age group. The proposed patterns are expressed as mg of amino acid per gram of protein. The amino acid values were selected so that proteins having the amino acid composition matching or exceeding the scoring pattern should meet the requirements for all the essential amino acids when consumed in the amount that will meet the total requirement for high quality protein (FAO/WHO, 1973).

Table 2. Protein digestibility and protein quality parameters – protein digestibility, chemical score (CS), essential amino acid index (EAAI) and protein efficiency ratio (PER)

Sample	Protein digestibility (%)	CS (%)	EAAI (%)	PDCAAS
Wholegrain wheat	85.62	20.99 (Ile)*	56.07	2.75
Wholegrain buckwheat	83.26	22.57 (Ile)*	68.36	3.35
Amaranth	84.58	47.59 (Ile)*	59.76	6.17
Quinoa	79.25	16.24 (Leu)*	45.68	2.90

* CS were presented for restrictive amino acids

The calculated protein quality parameters of the samples analysed are shown in Table 2. The values obtained were in the range from 2.75 to 6.17 for PDCAAS. According to the results obtained, it is evident that amaranth possessed the highest protein efficiency ratio. The protein digestibility of wheat, buckwheat and amaranth were similar, whereas quinoa showed slightly lower protein digestibility.

CONCLUSIONS

A comparative study of pseudocereals (buckwheat, amaranth and quinoa) and wheat flour (the global staple food) showed the superiority of pseudocereals relative to the protein content and its quality. Regarding lysin, the limiting amino acid of a majority of plant proteins, buckwheat, amaranth and quinoa possess almost twice as much of the recommended lysin value. Conversely, the fat content of pseudocereals under consideration was more than twice as high as that of wheat, which poses an issue to be tackled if these seeds are to be incorporated in foods, especially regarding storage conditions and the shelf-life of products. Accordingly, buckwheat, amaranth and quinoa are promising raw materials that can be used for the development of a range of value-added bakery products.

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