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Amino acid contents and toxically relevant arsenic of rice varieties consumed in Portugal

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ABSTRACT

This study evaluated amino acids, antioxidant capacity, and arsenic species present in Indica and Japonica rice cultivars (*Oryza* sativa L.) grown in similar regions, as well as brown rice. Essential amino acids in brown rice exhibited a greater abundance when compared to white rice. Lysine was the only essential amino acid with higher concentration in white rice than in brown rice. Results showed antioxidant activity of 0.46 IC_{50} (mg mL^{-1}) and TPC 0.11 mg eq. GA/g of sample. Total arsenic content in rice samples was about 200 μg/kg. HPLC-ICP-MS determinations revealed iAs as the predominant species in bran samples. Through Spearman's correlation, negative correlations between six amino acids and total arsenic content in japonica white rice were observed. Sulphur amino acid cysteine presented the highest negative correlation ($\rho = -0.758$, p-value = 0.011). Based on our results, a regular benefit-risk assessment for rice products to enhance the consumption choice is recommended.

1. Introduction

There are many sub-varieties of rice (*Oryza* sativa L.) grown, as it provides a basic source of energy, protein and other nutrients to half the world's population. The sub-varieties of Indica and Japonica present different nutrient profiles mainly in amino acid content, which is due to climate and soil conditions [1].

Amino acids content variation in rice is observed either on Essential Amino Acids (EAA) or non-Essential (n-EAA) and this is not reflected in Food Composition Databanks (EuroFIR). Therefore, representative values of rice AA are extremely necessary to obtain a reliable estimation of their intake, giving focus on EAA which are not synthesised by the human body. EAA play an important role in human nutrition due to their contribution to protein biosynthesis and sensory traits of products. A wrong estimation of EAA intake could constitute a serious nutrition problem for target population, like vegetarians, infants or celiac patients who consume high amounts of rice in their diets [1,2].

Epidemiological-nutrition studies have indicated that bioactive compounds found in rice can provide protective effects and reduce the risk of developing non-transmissible chronic diseases related to oxidative stress, such as cancer [3], cardiovascular or neural diseases. Nevertheless, these studies also recommend more research on the profile of phenolic compounds, because they can be related to cultivars, soils and climatic conditions and may have a protective effect against environmental contaminants [4,5].

Among environmental contaminants, arsenic is highly assimilated by rice roots and it accumulates in grains. Therefore, health risks associated with arsenic exposure due to rice consumption need to be evaluated, as rice is considered the major arsenic dietary source. Many epidemiological studies reported that a high risk of bladder, lung, skin, and prostate cancer is associated with arsenic exposure. The International Agency for Research on Cancer (IARC) places inorganic As (iAs) in Group 1, classifying it as a genotoxic substance. On the other hand, dietary exposure to organic arsenic is unlikely to constitute a risk to health [6,7]. Since the total concentration of arsenic does not elucidate on toxicity, reliable speciation analysis is needed. HPLC-ICP-MS is a very usefully technique to identify arsenic species in rice, which belong to the designated toxic relevant arsenic (TRA) – arsenious acid (As(III)), arsenic acid (As(V)), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and other non-toxic species such as Arsenobetaine (AsB) [8,9].

Recently, Dewivedi [2] demonstrated that TRA modifies AA content in rice grain with consequences on a real intake of amino acids. Other studies indicated that anti oxidant system could be involved [5,10]. However, the mechanism by which arsenic exerts

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its effects on amino acid reduction is not fully understood, and more studies are needed to verify if it changes from variety to variety and/or from location to location. However, environmental variation seems more prominent [7]. As a consequence, this has implications on human diet and health. Furthermore, data from analytical studies of rice arsenic species is sometimes not concordant. It happens in Portugal the largest consumer of rice in Europe [11,12].

The present work aimed to study the effects of arsenic in the amino acid profile of cultivars usually produced in Portugal. The antioxidant activity of the rice fraction under study is also discussed.

2. Materials and methods

2.1. Sample collection and sample preparation

Thirty-nine rice samples representative of the brands and varieties most consumed in Portugal, both from national production and imported, were collected for analysis. Twenty-two white rice samples from Indica (n = 12) and Japonica (n = 10) varieties produced in Portugal were collected from a supermarket and rice-milling factories. Seventeen brown rice samples from organic (n = 8) and non-organic (conventional) (n = 9) farming were purchased only at the supermarket. Each sample unit (500 g) was homogenised and milled separately, using a high-speed grinder (knife mill GRINDOMIX GM 200, equipped with titanium knives to prevent contamination), and stored in vacuum bags at room temperature until analysis. All these samples were analysed for amino acid and arsenic content. Samples collected from the milling factories (grain, bran and husk) were also analysed for anti-oxidant activity.

2.2. Determination of amino acid content

Amino acids were characterised according to their nutritional role by acidic hydrolysis and chromatographic analysis, applying a procedure previously set up by our group [1]. Acidic hydrolysis was carried out using a microwave digestion system (Milestone ETHOS 1 Series) under a vacuum atmosphere in a nitrogen media, followed by the amino acid derivatization with 6-aminoquinoly-N-hydroxysuccinimidyl carbamate. Then chromatographic analysis was performed using an Acquity UPLC system equipped with a photodiode array detector (PDA) from Waters.

An aqueous hydrochloric acid (HCl) 0.1N (Merck) was used to prepare a stock solution of 2.5 mM and 25 mM D-Norvaline (Sigma-Aldrich), constituting the internal standard of the method.

Also, a solution of HCl (6N) containing phenol (0.5%) from Merck was used in microwave hydrolysis. Waters® AccQ reagent kit, containing 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate was used as a derivatizing compound, sample dilution buffer and eluent A and B as mobile phase, all obtained from Waters Corporation Company.

The amino acids contents were quantified against working standard solutions 2.5 mM for each amino acid, prepared from an Amino Acid Standard Hydrolysate provided by Waters® 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), aspartic acid (Asp), glutamic acid (Glu), alanine (Ala) and serine (Ser).

Results were expressed as mean and standard deviation of three replicates in mg/100 g of the edible portion on fresh weight basis.

2.3. Determination of antioxidant activity

The antioxidant activity of the samples was determined by measuring: (i) the scavenging effect on DPPH radical, and (ii) the total phenolic content of an extract of the bioactive compounds contained in the sample.

2.3.1. Extraction of the bioactive compounds

The extraction of bioactive compounds was carried by applying the method of Butsat et al. [13] with some modifications. Rice samples were extracted twice with 80% methanol (Merck) for 24 h in an incubator with agitation, and then filtered using a GHP 0.2 μm 45 mm filter. The filtered obtained were concentrated by using a rotary evaporator (Büchi) and then lyophilized (Heto HSC 500) to dryness. Crude extracts were dissolved in 10 mL of methanol and stored at 4 °C, until further analysis.

2.3.2. Scavenging effect on DPPH radical

Different concentrations of extracts in methanol were prepared and a reaction was started with the addition of DPPH· (Aldrich) at 0.1 mM. The reaction mixture stood in the dark for 100 min and then the absorbance was measured at 517 nm (Evolution 300 UV–Visible spectrophotometer, Thermo electron Corporation), using methanol as blank.

The results of three replicates were expressed as mean and standard deviation, in milligram of extract per millilitre of reaction mixture (mg mL^{-1}).

2.3.3. Determination of total phenolic content (TPC)

The total phenolic content was determined through Folin-Ciocalteu reagent method. 250 μL of Folin-Ciocalteu reagent (Merck) was added to 3.70 mL of water and 50 μL of rice extracts, and let react for 5 min. Then, the solution was neutralised with 1 mL of 15% (m/v) Na_2CO_3 (Merck) and incubated for 30 min at 40 °C in a water bath. The absorbance was measured at 760 nm against a blank in the same conditions using a UV–Vis spectrophotometer (Evolution 300, Thermo electron Corporation). Gallic acid (Aldrich) was used as a standard. TPC was expressed as the mean and standard deviation of three replicates in milligram of gallic acid equivalents per gramme of sample. The calibration curve was prepared for a working range: 10–200 μg mL^{-1} and a linear equation with a correlation coefficient greater than 0.9997 was obtained.

2.4. Determination of total arsenic and arsenic species

2.4.1. Total arsenic

Sample preparation to determine total As was performed using the following chemicals: nitric acid (70% Instra Analysed, Baker), hydrogen peroxide 30%. A 100 mg L^{-1} stock solution of total As (CCS-4, Inorganic Ventures) was used to prepare the calibration curve.

For total As analysis, approximately 300 mg of samples and reference material NMIJ-7503a were weighed into a 50 mL polypropylene tube. 2 mL of HNO_3 were added, left overnight and on the day after 1 mL of H_2O_2 was added. Samples were digested using a graphite heating block system (DigiPrep), applying the following thermal program: (a) 20 min ramp at 45 °C, (b) 40 min hold at 45 °C, (c) 20 min ramp at 85 °C, (d) 160 min at 85 °C. The tubes were completed to 50 mL with ultrapure water (18.2 mΩ cm, Millipore system). The samples were analysed for total As by ICP-MS (7500cx, Agilent Technologies) using H_2 as collision gas. The standard calibration curve was performed using the standard addition method: a mono-elemental standard at different concentrations was added to the rice samples and used to correct the signals, in order to account for matrix effects as well as to quantify arsenic content. Quality control for total digestion and

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