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Nutritional and bioactive compounds of adzuki bean cultivars using chemometric approach

Compostos nutricionais e bioativos de duas cultivares de feijão adzuki utilizando abordagem quimiométrica

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ABSTRACT

Azuki beans are small red grains rich in several essential nutrients used in traditional dishes in Asia that, nowadays present many applications around the world. This work aimed at evaluating angularis and niponensis cultivars from south region of Brazil. Both cultivars presented proximal composition similar to literature and the majority fatty acids were 18:2n-6, 16:0 and 18:3n-3. All samples showed polyunsatured fatty acids prevalence and nutritional indices and ratios considered adequate for biological system maintenance of a healthy organism. The grains presented significant contents of tocopherols and vitamin E activity, resulting in a high contribution to the dietary reference intake. Significant contents of iron, manganese and zinc were also found in the azuki beans, and they are very important mainly due to their function as cofactors in metabolic reactions. Phenolic compounds and flavonoids corroborated with other studies and contributed to the antioxidant activity. The principal components multivariate analysis allowed distinguishing the cultivars, and the two principal components could explain 92.28% of data variance.

Index terms: *Vigna angularis*; alpha-linolenic; principal component analysis; vitamin E; antioxidant activity.

RESUMO

O feijão adzuki consiste de pequenos grãos de cor vermelha, ricos em diversos nutrientes essenciais e que são utilizados em pratos tradicionais na Ásia e, atualmente têm várias aplicações ao redor do mundo. Neste trabalho, objetivou-se avaliar os cultivares angularis e niponensis produzidas na região sul do Brasil. Ambas os cultivares apresentaram valores de composição proximal similares aos encontrados na literatura e os ácidos graxos majoritários foram 18:2n-6, 16:0 e 18:3n-3. Todas as amostras apresentaram prevalência de ácidos graxos poli-insaturados, bem como razões e índices nutricionais adequados. Os grãos apresentaram teores significativos de tocoferóis e atividade de vitamina E, resultando em alta contribuição para a ingestão de referência diária. Concentrações de ferro, manganês e zinco também foram importantes, em razão das suas funções como cofatores em reações metabólicas. Compostos fenólicos e flavonoides corroboraram com outros estudos e contribuíram para atividade antioxidante. A análise multivariada de componentes principais permitiu distinguir as cultivares, e os dois componentes principais selecionados puderam explicar 92,28% da variância total dos dados.

Termos para indexação: *Vigna angularis*; alfa-linolênico; análise de componentes principais; vitamina E; atividade antioxidante.

INTRODUCTION

Adzuki, azuki and small red beans are the common names for several cultivars of *Vigna angularis* (Willd.) Ohwi & Ohashi spice. There is a large number of varieties of adzuki beans around the world, mainly in Asiatic Countries, and their characteristics may depend on grain size and color, genetic factors, cultivar type, cultivation and harvest time, climate and region where it was cultivated. This bean has been used in traditional Japanese confections, such as *wagashi*, *youkan*, *manju* and *amanatto*. In Chinese Medicine, adzuki beans have commonly been used to treat diuretic functions, and other disease such as dropsy and beriberi. Its medical application has been reported also in Korea (Yousif et al., 2003; Yoshida et al., 2009). Furthermore, adzuki beans, as well as many other types of beans, are considered source of carbohydrates, protein, fiber, vitamins and minerals (Palombini et al., 2013; Gohara et al., 2014a; Lam-Sanchez, 1990).

The lipid fraction of adzuki beans is composed mostly of unsaturated fatty acids (Yoshida et al., 2008) and presents low contents of saturated fatty acids. This is considered a positive point, according to the Institute of Medicine (2002/2005), who established that the consumption of saturated fatty acids should be avoided in a balanced diet. Important unsaturated fatty acids were found by Yoshida et al. (2010), their results ranged from 31.9 to 32.7% of linoleic fatty acid (18:2n-6) and from 25.4 to 26.8% of alfa-linolenic (18:3n-3). These fatty acids are considered essentials because they cannot be metabolized by the human body and must be obtained from the diet (Ratnayake; Galli, 2009).

The bioactive compounds in the adzuki bean seed coat have received significant interest because of their health-promoting antioxidant properties (Lin; Lai, 2006). The presence of antioxidant compounds such as flavonoids and tocopherols in foods is very important; the latter may reduce the risk of heart diseases, type 2 diabetes and cancer (Carocho; Ferreira, 2013). Maruyama et al. (2008) suggested that the consumption of the adzuki bean is linked to a reduced risk of lifestyle related diseases in humans. The isomers of tocopherols show different activities of vitamin E, and the isomer α-tocopherol is the most biologically active. These compounds are found only in plant foods (Yada; Lapsley; Huang, 2011).

Principal component analysis (PCA) is a very useful chemometric tool. This technique allows organization of data in a simpler and easier way to understand structure, and it's possible to extract additional information when compared to univariate analysis. The principal components (PC) are mutually orthogonal, and the explained variance decreases with the increase in the number of PC (Correia; Ferreira, 2007). This method has been applied in many studies to characterize foodstuffs and natural products (Souza et al., 2015; Pagamunici et al., 2014; Silva et al., 2015; Fuchs et al., 2013; Nishiyama et al., 2014; Souza et al., 2014).

This study investigated the chemical characterization of the two principal cultivars of adzuki bean most consumed in Brazil, both produced in Maringa city, in Parana State. The grains were analyzed concerning their proximal and fatty acids composition, isomers of tocopherols, minerals, bioactive compounds and nutritional aspects. The PCA was applied to extract more information from the data.

MATERIAL AND METHODS

Sampling

Adzuki beans (*Vigna angularis*) cultivars: angularis and niponensis, were acquired directly from the producer. The grains were cultivated in the region of Maringa city, Parana State, Brazil (23.4000º S, 51.9167º W); on September/October 2013. The mean altitude of the region is 596 m and September is the beginning of Spring Season. The harvest of azuki beans was performed on December 2013, when the levels of rainfall ranged from 50 to 100 mm (Instituto Nacional de Meteorologia - INMET, 2012). Sampling consisted of 3 batches of 5 kg, harvested at intervals of 10 days. All samples of adzuki beans were ground separately in a hammer mill to obtain a flour which was sieved to obtain a particle-size distribution between 14 and 16 mesh. Each sample of the two varieties: angularis and niponensis, were vacuum packed, protected from light and stored in a freezer at -18 ºC until analysis.

Proximal composition and crude energy

The moisture, ash and crude protein contents were determined according to Cunniff (1998) using factor 6.25 to convert the percentage of nitrogen into crude protein content (Gohara et al., 2014b). The total lipids were extracted and determined according to Bligh and Dyer (1959). The total carbohydrates were calculated by difference.

The caloric value was determined using the indirect method, which is based on the application of conversion factors for the main nutrients of the product (carbohydrates, crude protein and lipids), according to Holands et al. (1994). The results were obtained in cal g^{-1} of food and converted to kJ kg^{-1} product.

Fatty acid composition

The determination of fatty acid composition was performed using the lipid fraction extracted from the grain; these lipids were derivatized and converted into fatty acid methyl esters (FAME) according to Hartman and Lago (1973). The FAME were separated in gas chromatograph CP-3380 (Varian, USA) fitted with a flame ionization detector and a CP 7420-select Fame fused-silica capillary column (100 m x 0.25 mm x 0.25 µm cyanopropyl). The gas flows were: carrier gas hydrogen 1.4 mL min^{-1}, make-up gas nitrogen 30 mL min^{-1}, synthetic air 300 mL min^{-1} and flame gas hydrogen 30 mL min^{-1}; the sample was injected in split ratio of 1:100. The injector and detector temperature was 235 °C. The column temperature was maintained at 165 °C for 4 min, increased to 185 °C at 4 °C min^{-1} and maintained for 5 min, then increased from 185 °C to 225 ºC at 10 °C min^{-1} and maintained for 10 min. The same chromatographic conditions were used previously to analyze other plant foods (Souza et al., 2013).

The retention times were compared to those of standard methyl esters (Sigma, USA) for identification of fatty acids. The quantification of fatty acids was performed using tricosanoic acid methyl ester (Sigma, USA) as an internal standard, following Joseph and Ackman (1992). The peak areas were determined with software Star 5.0 (Varian, USA) and the concentrations were expressed in mg FA per g of total lipid. The limits of detection (LOD) and quantification (LOQ) were estimated by triplicate analysis of diluted methyl arachidate standard solution (1.0 mg mL^{-1}), considering the signal-noise rate relative to the background signal as 3 and 10, respectively (Analytical Methods Committee, 1987). The sums of some fatty acids (FA) classes were also determined: total fatty acids from n-6 and n-3 series (called as n-6 and n-3, respectively); total saturated fatty acids (SFA); total monounsaturated fatty acids (MUFA); and total polyunsaturated fatty acids (PUFA).

Indices of the nutritional quality of lipids

A better approach to the nutritional evaluation of fat is the utilization of some indices based on the functional effects of fatty acid composition. The fatty acids requested to calculate the indices were: 12:0 (lauric acid), 14:0 (myristic acid), 16:0 (palmitic acid), 18:0 (stearic acid), 18:1n-9 (elaidic acid), 18:2n-6 (linoleic acid), 20:4n-6 (arachidonic acid), 18:3n-3 (alpha-linolenic acid), 20:5n-3 (eicosapentaenoic acid), 22:5n-3 (docosapentaenoic acid), 22:6n-3 (docosahexaenoic acid), and the sums n-6 (total omega-6 fatty acids), n-3 (total omega-3 fatty acids) and MUFA (total monounsaturated fatty acids). The indices determined in this study were: Index of Atherogenicity (IA) = [(12:0 + (4 x 14:0) + 16:0)] / (MUFA + n-6 + n-3), and Index of Thrombogenicity (IT) = (14:0 + 16:0 + 18:0) / [(0.5 x MUFA) + (0.5 x n-6) + (3 x n-3) + (n-3:n-6)], according to Ulbricht and Southgate (1991); and ratio between Hypocholesterolemic and Hypercholesterolemic fatty acids ratio (HH) = (18:1n-9 + 18:2n-6 + 20:4n-6 + 18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3) / (14:0 +16:0), according to Santos-Silva, Bessa and Santos-Silva (2002).

Tocopherols isomers analysis

All samples were saponified and the isomers of vitamin E were extracted according to the methodology described by Souza et al. (2014). Under stirring and protected from light, 50.0 mL of ethanol, 5.0 mL of aqueous solution of ascorbic acid 10% (w/v), 10 mL of aqueous solution of potassium hydroxide 60% (w/v) and 25 mL of water were added to 2.00 g of the ground sample. The unsaponifiable material extraction was performed with hexane and water. The organic phase containing the tocopherol fraction was collected and the solvent was evaporated under vacuum at 50 °C. The residue was dissolved in methanol to obtain an extract solution.

The tocopherol isomers (δ-tocopherol, (β+γ)-tocopherol and α-tocopherol) were determined using high efficiency liquid chromatography (Varian) with a C18 column (Microsorb, 250 mm × 4.6 mm, 5 μm particles) fitted with a scanning UV/Vis detector. The mobile phase used was methanol/dichloromethane in the ratio 85:15 (v/v); and the flow rate was 0.8 mL min^{-1} (Kornsteiner; Wagner; Elmadfa, 2006). The tocopherols were quantified using external standard method, according to Instituto Adolfo Lutz (1985). The sum of isomers β-tocopherol and γ-tocopherol was determined, since the separation of these is not possible by this methodology (Kornsteiner; Wagner; Elmadfa, 2006). The LOD and LOQ were estimated by triplicate analysis of standards calibration curve for each isomer of tocopherols, considering the signal-to-noise ratio relative to the background signal as 3 and 10, respectively (Analytical Methods Committee, 1987).

Vitamin E activity

The activity of vitamin E in the samples was performed according to Kornsteiner, Wagner and Elmadfa (2006): the value found for each isomer, in milligrams, was multiplied by the equivalent factor for α-tocopherol (α-TE). For α-tocopherol, α-TE = mg x 1.0; for (β + γ)-tocopherol, α-TE = mg x 0.25; and for δ-tocopherol, α-TE = mg x 0.01.

Mineral quantification

For the mineral composition analysis, all the azuki beans samples were digested by the dry method (Association Of Analytical Chemists-AOAC, 1995) and Ca, Cu, Fe, Mn, and Zn were quantified in an atomic absorption spectrophotometer AA240FS (Varian, USA) as mg of mineral per 100 g of sample using technical parameters of calibration according to Table 1. The LOD and LOQ were estimated by triplicate analysis of standards calibration curve for each mineral, considering the signal-to-noise ratio relative to the background signal as 3 and 10, respectively (Analytical Methods Committee, 1987).

Calculation of the dietary reference intake

The Dietary Reference Intake (DRI) is a percentage estimate of the daily nutrient requirements per age and gender, established by the Institute of Medicine (2000) for individuals aged over 12 months. The DRI of vitamin E was determined as the mean amount in 100 g portion of azuki beans.

Table 1: Technical parameters of calibration for atomic absorption spectrophotometer.

Element	Wavelength/nm	Slit width/nm	Gas - Flow (L min^{-1})	Burner height (mm)
Ca	239.9	0.2	Nitrous oxide – 11.0; Acetylene – 6.35	13.5
Cu	324.8	0.5	Air – 13.5; Acetylene – 2.0	13.5
Fe	248.3	0.2	Air – 13.5; Acetylene – 2.0	13.5
Mn	279.5	0.2	Air – 13.5; Acetylene – 2.0	13.5
Zn	213.9	1.0	Air – 13.5; Acetylene – 2.0	13.5

Extract preparation and evaluation of bioactive properties

The methanolic extracts were obtained from the varieties of adzuki beans. Each sample (10 g) was submitted to stirring process with 100 mL of methanol (m/v) at 25 °C and 150 rpm for 4 h and subsequently filtered through a filter paper (Whatman No. 4). Each extract was evaporated at 45 ºC (rotary evaporator Büchi R-210, Flawil, Switzerland) to remove the methanol. The dried extract was maintained in amber vials with nitrogen atmosphere (N_2). After, the extracts were redissolved in methanol solvent (final concentration 5 mg mL^{-1}) for antioxidant activity evaluation. The final methanolic solutions obtained were further diluted to different concentrations to be submitted to distinct bioactivity evaluation in vitro assays.

The results for 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assays were expressed in IC_{50} values (sample concentration providing 50% of antioxidant activity) for DPPH radical-scavenging. These assays were evaluated using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, VT, USA), and the results were calculated as a percentage of DPPH discoloration using the formula: $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution containing the sample at 515 nm, and A_{DPPH} is the absorbance of the DPPH solution.

Total phenolic compounds were determined in triplicate according to the method proposed by Shahidi and Naczk (1995). A 0.25 mL aliquot of extract solution (2.5 mg mL^{-1} in methanol) was mixed with 0.25 mL of Folin–Ciocalteu's reagent previously diluted with water 1:1 (v/v), 0.5 mL of a saturated sodium carbonate solution and 4 mL of water. The reaction tubes, in triplicates, were wrapped in aluminum foil and kept at 25 ºC for 25 min in the dark. The tubes were centrifuged for 10 min and the absorbance of the supernatant fraction was measured at 725 nm using a spectrophotometer (Cary Win UV 50, Varian). Gallic acid (GA) was used as a standard and the results were expressed as gallic acid equivalents (mg GAE 100 g^{-1} sample).

Flavonoids analyses were performed according to the method proposed by Buriol et al. (2009). The same solution used in total phenolic assay was utilized for total flavonoids assay, with addition of aluminum chlorate solution at 5% (m/v). The yellow complex formed was measured at 425 nm. The results were expressed in quercetin equivalents (mg QE 100 g^{-1} sample).

Statistical and multivariate analysis

Proximal composition, crude energy, fatty acid composition, indices of the nutritional quality of lipids, tocopherols isomers, vitamin E activity, mineral quantification and antioxidant proprieties were carried out in triplicate for the three different batches. The results were compared using the t Student's test with 5% ($p<0.05$) significance level for rejection of the null hypothesis.

In multivariate analysis, the individual values of each replicate of the three batches analyzed (n = 9) were divided into arrays of data. The samples were arranged in rows (n = 18) and the results of the analyses were selected for principal component analysis (PCA; n = 19) in columns. These data used for PCA were: PUFA:SFA (ratio between polyunsaturated fatty acids and saturated fatty acids), n-6:n-3 (ratio between total omega-6 fatty acids and omega-3 fatty acids), and HH (ratio between Hypocholesterolemic and Hypercholesterolemic fatty acids) ratios; nutritional indices of lipids (index of atherogenicity and thrombogenicity – IA and IT); mineral composition (Ca, Cu, Fe, Mn and Zn); isomers of tocopherols (α-tocopherol, (β + γ)-tocopherol and δ-tocopherol); vitamin E activity; and antioxidant proprieties (DPPH radical-scavenging activity, total phenolic and total flavonoids compounds). The data were pre-processed by auto-scaling. This process was subsequently applied to the principal components analysis using the algorithm NIPALS. It was decomposed into a two-dimensional graph of scores (samples) and loadings (variables). The statistical software Statistica, version 7.0 (Statsoft, 2007), was used with a 5% ($p<0.05$) significance

level for rejection of the null hypothesis. This same significance level was used to select principal components for the Principal Components Analysis.

RESULTS AND DISCUSSION

The results of the proximal composition and crude energy analyses (Table 2) were similar to those reported by Palombini et al. (2013) and Lam-Sanchez et al. (1990) for different cultivars of beans. Durak et al. (2013) found similar contents of protein, carbohydrates and total lipids (25.00, 55.00% and 0.45%, respectively) for adzuki beans. Angularis cultivar presented lower contents ($p<0.05$) of crude protein and moisture than niponensis cultivar. Yousif et al. (2003) studied the effect of storage of adzuki beans and their results suggested that detrimental changes occur in starch and protein of adzuki beans stored under unfavorable conditions; therefore, the proximal composition can influence the characteristics of beans and increase the shelf life of angularis cultivar. Nutritional composition of beans is also important to make some enriched food products. A recent study using adzuki flour in chocolate cakes showed a great contribution of these beans to increase contents of ash and crude protein in the final product (Gohara et al., 2014b).

Table 3 shows the conditions used to gas chromatography, high performance liquid chromatography and atomic absorption spectrophotometer. These analytical

Table 2: Proximal composition and crude energy in adzuki beans cultivars.

Parameters	Adzuki beans cultivars	
	Angularis	Niponensis
Moisture (g $100g^{-1}$)	13.07b±0.13	14.48a±0.08
Ash (g $100g^{-1}$)	3.85a±0.03	3.59b±0.04
Crude protein (g $100g^{-1}$)	20.36b±0.30	22.94a±0.22
Total lipids (g $100g^{-1}$)	0.45a±0.02	0.44a±0.03
Carbohydrates (g $100g^{-1}$)	62.26a±0.33	58.55b±0.24
Crude energy (kJ Kg^{-1})	1400.82a±0.04	1381.37b±0.06

Results expressed as mean ± standard deviation for analysis in triplicate of three batches. Means followed by the same letters in rows do not differ by t Student's test ($p < 0.05$). n = 9 replicates.

Table 3: Conditions of the selecting operation system for gas chromatography, high performance liquid chromatography and atomic absorption spectrophotometer.

Parameters	LOD	LOQ
	Fatty acid (mg g^{-1})	
	0.15	0.50
	Isomers of tocopherols (mg g^{-1})	
δ-Tocopherol	$4.55x10^{-5}$	$1.52x10^{-4}$
β+γ-Tocopherol	$3.46x10^{-5}$	$1.15x10^{-4}$
α-Tocopherol	$1.03x10^{-4}$	$3.44x10^{-4}$
	Minerals (mg g^{-1})	
Ca	$3.47x10^{-5}$	$1.15x10^{-4}$
Cu	$1.16x10^{-4}$	$7.31x10^{-2}$
Fe	$1.44x10^{-2}$	$4.79x10^{-4}$
Mn	$2.33x10^{-4}$	$7.76x10^{-4}$
Zn	$1.00x10^{-3}$	$3.33x10^{-3}$

LOD: limits of detection; LOQ: limits of quantification.

parameters allowed the detection and quantification of all the compounds showed in Tables 4, 5 and 6.

Table 4: Fatty acid absolute quantification, sums, ratios and indices of the nutritional quality of lipids in adzuki beans cultivars.

Fatty acid	Adzuki beans cultivars	
(mg g^{-1})	Angularis	Niponensis
14:0	2.21a±0.02	1.30b±0.03
14:1n-7	1.31a±0.02	0.88b±0.05
16:0	225.55a±2.00	226.84a±3.40
16:1n-7	2.84a±0.03	1.77b±0.06
17:0	2.88a±0.09	2.56b±0.04
18:0	56.67a±0.63	27.01b±0.37
18:1n-9	79.60a±0.98	40.88b±0.47
18:1n-7	18.29a±0.31	18.19a±0.21
18:2n-6	351.81a±3.36	390.91a±31.09
18:3n-3	160.58b±1.92	173.32a±3.09
20:0	7.97a±0.24	3.09b±0.02
18:3n-6	0.98a±0.04	0.77b±0.03
22:0	7.49b±0.29	11.55a±0.34
24:0	2.55b±0.04	4.99a±0.26
Sums and ratios of fatty acids		
SFA	305.33a±2.14	277.33b±3.44
MUFA	102.04a±1.03	61.72b±0.52
PUFA	513.37b±3.87	584.13a±31.14
n-6	352.78b±3.36	410.81a±31.09
n-3	160.58b±1.92	173.32a±1.75
n-6/n-3	1.46a±0.02	1.42b±0.08
PUFA/SFA	1.68b±0.01	2.11a±0.05
Index of the nutritional quality of lipid		
IA	0.38a±0.01	0.36b±0.01
IT	0.40a±0.01	0.34b±0.01
HH	2.60a±0.01	2.65a±0.01

Results expressed as mean ± standard deviation for analysis in triplicate of three batches. Means followed by the same letters in rows do not differ by t Student's test ($p < 0.05$). SFA: total saturated fatty acids, MUFA: total monounsaturated fatty acids, PUFA: total polyunsaturated fatty acids, n-6: total omega-6 fatty acids and n-3: total omega-3 fatty acids. IA: Index of atherogenicity. IT: Index of thrombogenicity. HH**:** ratio between Hypocholesterolemic and hypercholesterolemic fatty acids. n = 9 replicates.

The fatty acid composition of both cultivars of adzuki beans was similar, as shown in Table 4.

The majority fatty acids were 18:2n-6, 16:0 and 18:3n-3. Fatty acids classes have been largely studied by many researchers, as well as the effect of these compounds to the human body; the relationship between fatty acids quality and the body function may be verified using some nutritional indices and ratios (Ratnayake; Galli, 2009; Ulbricht; Southgate, 1991; Santos-Silva; Bessa; Santos-Silva, 2002; Durak et al., 2013). The nutritional indices calculated in this study with adzuki beans, IA and IT, presented better values than new formulations of some foodstuffs (hamburguer, food bar and cookies) containing whole flours of promising vegetables, such as chia and flaxseed (Yoshida et al., 2009; Souza et al., 2015; Pagamunici et al., 2014a; Pagamunici et al., 1024b). According to PCA analysis (Figure 1), IA and IT presented great contribution (0.9853 and 0.9929, respectively) to PC1 and influenced the separation of adzuki cultivars. Niponensis cultivar showed lower indices than angularis and its lipid fraction can be considered healthier (Ulbricht; Southgate, 1991).

The PUFA:SFA and n-6:n-3 ratios also presented high contribution (0.9923 and -0.9947) on PC1 to separate the two cultivars. Niponensis cultivar presented higher PUFA:SFA ratio and n-6:n-3 ratio closer to 1:1. According to Simopoulos (2011), the prevalence of polyunsaturated fatty acids is associated with a lower risk of cardiovascular disease, which makes the lipid quality of niponensis cultivar better than angularis.

According to Table 4 it was not verified statistical difference ($p<0.05$) between the values of HH ratios of the two samples. HH ratios presented a great value and significant contribution on PC2 (0.7322, Figure 1) for both cultivars of adzuki beans. Higher values for HH ratio are important to human health due to the hypocholesterolemic effects (Simopoulos, 2011).

As shown in Table 5, higher contents of tocopherol isomers and vitamin E activity were found in angularis cultivar. PCA presented positive contribution of δ-tocopherol (0.8932), (β+γ)-tocopherol (0.9859), α-tocopherol (0.7401) and vitamin E activity (0.7368) on PC1, causing the separation of angularis cultivar (Figure 1). Yoshida et al., (2009) found slightly lower results for δ-tocopherol in adzuki beans. Besides, other studies analyzing common beans (*Phaseolus vulgaris*) found lower contents of total tocopherols and lower activity of vitamin E than adzuki beans cultivars (Boschin; Arnold, 2011). The isomer α-tocopherol is the most biologically active form of vitamin E (Yada; Lapsley;

Huang, 2011), and it is related to the protection of unsaturated lipids present in biological systems, due to its lipophilic characteristic (Taipina et al., 2009). The DRI showed that adzuki beans present a high contribution of vitamin E for all populations analyzed. These levels ranged from 31% for women in lactation until upper 100% for children (1-3 years) (Institute of Medicine, 2000).

Table 6 showed greater contents of minerals Ca, Cu, Mn and Zn in angularis cultivar. This fact can be clearly observed in Figure 1, where the high contributions of the minerals Ca (0.9832), Cu (0.9721), Mn (0.9824) and Zn (0.8562) in PC1 could distinguish angularis cultivar. Niponensis cultivar presented high contribution of Fe (-0.9824, Figure 1 and Table 6). However, all samples presented great levels of iron, manganese and zinc, which are classified as trace minerals. These minerals are extremely important for the maintenance of biological systems because they act as cofactors in metabolic reactions (Hathcock, 2004).

The *in vitro* antioxidant properties of adzuki cultivars beans were evaluated and presented in Table 7. The antioxidant potential of extracts obtained from the two adzuki beans cultivars was similar to the results found for eight Brazilian bean cultivars (Palombini et al., 2013a). The total phenolic compounds of the adzuki beans cultivars were lower than the results found by Zhao et al. (2014) for some common legumes, but very similar to results obtained by Palomini et al. (2013b) for seven Brazilian rice cultivars; and for new cultivars of pseudocereals (Palombini et al., 2013c). Total phenolic compounds presented high contribution on PC1 (0.9823, Figure 1) to Angularis cultivar. On the other hand, Table 7 showed higher contents of total flavonoids in Niponensis than Angularis cultivar, and this antioxidant sort presented high contribution on PC1 (-0.8390) to separate Niponensis cultivar. The lowest concentration necessary for 50% inhibition of DPPH (Table 7) was obtained for Angularis cultivar, while Nioponensis cultivar presented the worst results.

Principal component analysis allowed the selection of PC1 and PC2, which explained 92.28% of the data variance of the parameters analyzed (Figure 1). PC1 showed the greatest data explanation (81.59%) and could make a clear separation of adzuki cultivars.

Table 5: Tocopherol composition of azuki beans cultivars analyzed.

Cultivars	δ-Tocopherol (mg 100 g^{-1})	β+γ-Tocopherol (mg 100 g^{-1})	α-Tocopherol (mg 100 g^{-1})	Total tocopherol content (mg 100 g^{-1})	α-TE
Angularis	4.28a±0.02	1.44a±0.06	0.84a±0.03	6.57a±0.07	1.22a±0.06
Niponensis	4.00b±0.15	1.05b±0.07	0.83a±0.01	5.88b±0.17	1.14b±0.08

Results expressed as mean ± standard deviation for analysis in triplicate of three batches. Means followed by the same letters in columns do not differ by t Student's test ($p < 0.05$). α-TE: α-tocopherol equivalents. n = 9 replicates.

Table 6: Mineral composition of adzuki beans cultivars.

	Mineral (mg $100g^{-1}$)				
Adzuki beans cultivars	Ca	Cu	Fe	Mn	Zn
Angularis	276.45a±20.77	1.11a±0.11	3.98b±0.37	2.39a±0.22	2.96a±0.44
Niponensis	95.60b±1.86	0.47b±0.01	6.05a±0.23	0.49b±0.02	2.05b±0.05

Results expressed as mean ± standard deviation for analysis in triplicate of three batches. Means followed by the same letters in columns do not differ by t Student's test ($p < 0.05$).

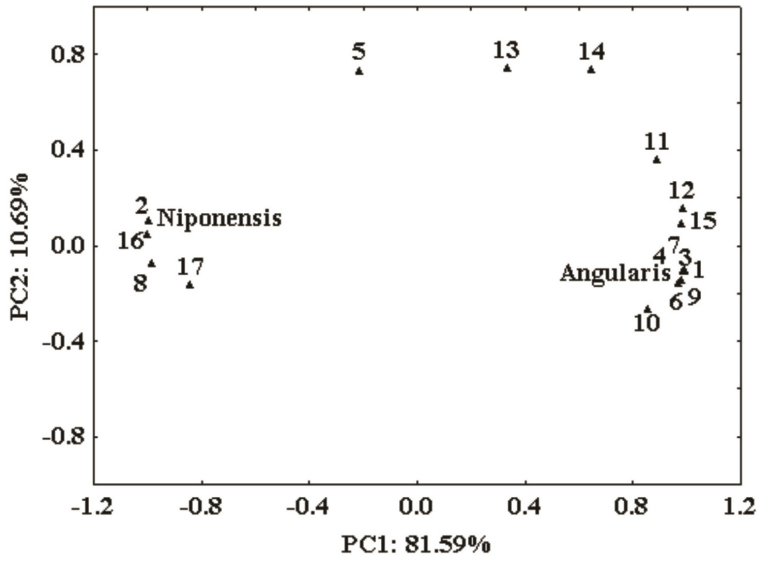

Figure 1: Principal components analysis of select data to characterize the adzuki beans cultivars. PC: Principal component. Scores (samples): var. Angularis and var. Niponensis. Loadings (analysis): 1 – PUFA:SFA (ratio between total polyunsaturated fatty acids and total saturated fatty acids); 2 – n-6:n-3 (ratio between total omega-6 fatty acids and total omega-3 fatty acids); 3 – IA (index of atherogenicity); 4 – IT (index of thrombogenicity); 5 – HH (ratio between hypocholesterolemic and hypercholesterolemic fatty acids); 6 – Calcium; 7 – Copper; 8 – Iron; 9 – Magnesium; 10 – Zinc; 11 – δ-tocopherol; 12 – (β + γ)-tocopherol; 13 – α-tocopherol; 14 – Vitamin E activity; 15 – Total phenolic compounds; 16 – DPPH radical-scavenging activity; 17 – Total flavonoid compounds.

Table 7: Antioxidant properties of the adzuki beans cultivars.

Adzuki beans	Folin-Ciocalteu assay (mg GAE 100 g^{-1} sample)	Flavonoids (mg QE 100g^{-1} sample)	EC_{50} values (mg mL^{-1}) DPPH radical-scavenging activity
Angularis	81.87a±4.39	7.21b±0.83	148.83b±4.83
Niponensis	59.94b±1.57	9.03a±0.70	243.41a±3.01

Results expressed as mean ± standard deviation for analysis in triplicate of three batches. Means followed by the same letters in columns do not differ by t Student's test ($p < 0.05$). GAE: Gallic acid equivalents; QE: quercetin equivalents. EC_{50}: Extract concentration corresponding to 50% of antioxidant activity. n = 9 replicates.

CONCLUSIONS

Cultivars of adzuki beans are excellent sources of many essential compounds. The nutritional indices of the lipid fraction showed that these grains present anti-atherogenic, anti-thrombogenic and hypocholesterolemic effects, and the ratios PUFA: SFA and n-6:n-3 were considered appropriate for biological system maintenance of a healthy organism. The content of tocopherols was higher than amounts found in common beans. The values of DRI enable the evaluation of the nutritional potential of adzuki beans. Both cultivars presented good levels of iron, manganese and zinc, which are classified as trace minerals and responsible for the maintenance of biological systems. The antioxidant properties were very similar to the other kinds of beans, rice and pseudo-cereals cultivars from the literature. The multivariate analysis allowed distinguishing batches of angularis and niponensis cultivars, as well as evaluating the importance of the parameters. The traditional use of adzuki beans in the eastern dietary is promising due to their intrinsic characteristics.

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