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Fatty acid profile, phytochemicals and antioxidant activity from barnyardgrass *(Echinochloa crus-galli)*

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Abstract

The objective of this study was to evaluate the chemical composition, fatty acid profile, phytochemical compounds and antioxidant activity of barnyardgrass *(Echinochloa crus-galli)* in order to evaluate its possible use for food. Barnyardgrass samples were obtained from rice crops and grains with and without husk were evaluated. The morphology, chemical composition, fatty acid profile, phytochemical compounds and the antioxidant activity by the methods of 2,2-difenil-1-picrilhidrazil (DPPH*) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS*) of barnyardgrass grains were determined. The grains with husk presented higher content of lipids 6.7%, minerals 7.8%, dietary fibers 40.8%, total phenolic compounds 399 mg equiv. catechin/100g and antioxidant activity as compared to grains without husk. The results suggest that barnyardgrass grains with and without husk are promising as a source of nutrients for use in both human and animal nutrition.

Introduction

The barnyardgrass *(Echinochloa crus-galli)* is an invasive plant from Poaceae family, responsible for significant losses in productivity from diverse cultures and is considered one of the main weeds of importance in world agriculture. According to Rao *et al.* (2007), the occurrence of E. crus-galli has been reported in 22 countries in dry rice crop and in 15 countries in irrigated rice crop. The barnyardgrass has difficult control due to its high competitiveness mainly on rice cultivation, and other vegetables such as cotton, corn, sorghum, peanuts, sugarcane and cassava.

The use of herbicides is a common practice to control barnyardgrass, however, is not complete efficiency and has limited application due to constant concern with continued use, limited availability of new and effective herbicides, as well as concerns about environmental pollution (Chauhan and Abugho, 2013). The barnyardgrass is harvested together with the rice and then separate the industrial processing of rice. The barnyardgrass grains are discarded or used as animal feed, reaching up to 2% of the total harvested rice production. Although the main worldwide appeal is to minimize this development in order to reduce losses in agriculture, studies report that several species of Echinochloa are used for forage, production of alcohol, paint and even for human consumption, however the application is in a few countries.

In our research group, a recent study was conducted on use of *E. crus-galli* starch (Bartz *et al*., 2015) to get better use of this grain; however there are no studies on lipid composition and bioactive compounds of this species. In order to better use of barnyardgrass grains *(Echinochloa crus-galli)* in food and feed is important to elucidate its physical and chemical composition and bioactive properties. Thus, the aim of this study was to evaluate the morphology, chemical composition, fatty acid profile, phytochemical compounds and antioxidant activity of barnyardgrass *(Echinochloa crus-galli)*.

Material and Methods

Material

The barnyardgrass grains *(E. crus-galli* Beauv.) were obtained by manually harvesting the grains from irrigated rice fields, located in the State of Rio Grande do Sul, Brazil. The barnyardgrass samples were dehusked, sun-dried and removed of impurities. The grains were dehusked using rice mills (Kepler Weber S. A., Brazil) and the husk were separate through air

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machines by suction. The yield of dehusked grains was calculated from the ratio of the mass of grain with husk and final mass of dehusked grains. The grains were ground in a Perten 3110 grinder (Perten knife grinder, model Laboratory Mill 3100, Sweden) for further analysis.

Characterization and chemical composition of barnyardgrass grains

The morphology of the grains with and without husk was evaluated by scanning electron microscope (JEOL JSM-6610LV, USA) under an acceleration voltage 10 kV and magnification of 40x. The grains were fixed on stubs with double sided tape and coated with gold using a sputter coating (sputtering, Deston Vacuum Deskv, USA). The dimensions (length, width and thickness) of grains were determined using 60 grains.

The moisture and ash contents were determined by gravimetric method, the lipid content determined by soxhlet and protein by Kjeldahl, according to methods described by AOAC (2005). Soluble and insoluble dietary fibers were determined by gravimetric enzymatic method (AOAC, 2005), and other carbohydrates by difference.

Fatty acids content

The lipids of barnyardgrass grains were extracted by the Bligh and Dyer method, with some modifications (Bligh and Dyer, 1959). A 20 g sample was used and the moisture content adjusted to 80% with distilled water. The fatty acids were derivatized according to methodology proposed by Hartman and Lago (1973). A 100 mg sample of the oil was weighed in a test tube, added 0.5 mL of 0.1 $mol.L^{-1}$ KOH in methanol, and the samples remained in the water bath (Quimes, Dubnoff, England) at 60°C for 90 min. After reaching room temperature, was added 1.5 mL of 1 $mol.L^{-1}$ H_2SO_4 and again brought to a water bath for 90 min. After cooling, was added 2 mL of hexane and the samples were stirred using vortex for 30 s. The phase of hexane was collected and dried with nitrogen flow to obtain the methyl esters. For analysis, a solution was prepared with methyl nonadecanoate acid (C:19) HPLC standard in hexane at a concentration of 2 $mg.mL^{-1}$ (internal standard). Then esters, 30 mg, were diluted in 1 mL of the internal standard solution. The determination of fatty acid profile was performed using gas chromatography GC/FID 2010 (Shimadzu, Japan), equipped with split/splitless injector, ionization detector (FID), autoinjector AOC-20i and column SPTM -2560 (100 m x 0.25 mm x 0.20 μm) (SUPELCO, USA). Hydrogen was used as carrier gas at a flow rate of 1.2 $mL.min^{-1}$; Split: 1: 100. The temperature employed was 140°C for 5 min, and subsequently at 4°C.min^{-1} until reaching 240°C remained at this temperature for 10 min. The temperatures of the injector and detector were 260°C. Qualitative analysis was performed by comparing the retention time of the pattern 37 FAME mix (Sigma-Aldrich) and quantitative analysis by area normalization corrected using as internal standard the methyl nonadecanoate (Sigma-Aldrich-C19:0).

Tocopherol

The lipids of barnyardgrass grains were extracted by the Bligh and Dyer method, with some modifications (Bligh and Dyer, 1959). Then a sample of 150 mg of oil was weighed into a 5 mL volumetric flask and the volume measured with isopropanol. The solution was homogenized and centrifuged for 6 min at 9000 x g with subsequent HPLC analysis. The column used was octadecyl shim pack CLC-ODS column (5 μm, 4.6 mm x 150 mm) at 25°C. The mobile phase consisted of methanol, isopropanol and acetonitrile isocratic elution system, covering 10 min in the proportion 40:50:10, and later changing to 65: 30: 5. After 2 min the ratio of the mobile phase returned to baseline by the end of the race 15 min, operating at 290 nm excitation and 330 nm emission. The identification of compounds was performed by comparing with chromatographic patterns of alpha (α), gamma (γ), and delta (δ) tocopherols (Chen and Bergman, 2005).

Oryzanol

The lipids of barnyardgrass grains were extracted by the Bligh and Dyer method with modifications (Bligh and Dyer, 1959). A sample of 0.75 g of oil was weighed in a 5 mL volumetric flask and the volume measured with isopropanol: acetonitrile (7:3). An aliquot of 1 mL was transferred to an eppendorf tube and underwent centrifugation at 9000 x g for 6 min. Subsequently the samples were evaluated in a HPLC coupled with a fluorescence detector (HPLC-FL) (HPLC Shimadzu-SLC-10AVP, England) with automatic injection, quaternary pump, degasser oven and the mobile phase (Model DGU). The mobile phase used was methanol: acetonitrile: isopropanol (40:50:10) at a flow rate of 1 $mL.min^{-1}$, the minimum temperature of the column was 25°C and maximum 30°C, with a total run time of 30 min. The control equipment and data processing were performed by ClassVp software.

Secondary metabolites

Extract preparation

Secondary metabolites were extracted from 2 g sample with 10 mL of acetone:water solution 7:3 (v/v). The samples were subjected to constant agitation for 20 min, followed by centrifugation at 7500 x g for 15 min. This procedure was repeated twice to ensure complete extraction. Finally, the supernatants were united and used for the following evaluations (Nasar-Abbas *et al*., 2008)

Total phenols

The content of phenolic compounds present in the extracts was determined by the Folin-Ciocalteu method, according to the methodology proposed by Zieliński and Kozłowska (2000). In falcon tubes was added 100 µL extract, 400 µL of distilled water and 250 µL of Folin-Ciocalteu reagent 1 N and after 8 min was added 1.25 mL of sodium carbonate 7%. The reaction was conducted under the light for a 2 h interval, after the absorbance was measured in a spectrophotometer at 725 nm. The results were interpolated from a standard curve of gallic acid and expressed as mg equivalents per 100 g of sample.

Free tannins and non-tannic phenolic compounds

A sample of 100 mg polyvinyl polypirrolidona was weighed into falcon tubes, 1 mL of extract and 1 mL of distilled water, followed by vortexing. Subsequently the mixture was cooled to 5°C for 15 min and centrifuged at 7500 x g for 15 min (Nasar-Abbas *et al*., 2008). For determination of non-tannic phenolic compounds was performed by colorimetric reaction proposed Zieliński and Kozłowska (2000). The tannins were obtained by the difference between the content of non-tannic phenolic compounds and content of phenolic compounds present in the sample. The results were expressed in mg of gallic acid equivalents per 100 g of sample.

Condensed tannins

The condensed tannins were determined according to the method described by Díaz et al. (2010). An aliquot of 0.5 mL of extract was transferred to test tubes with 3 mL acidified butanol (butanol: HCl; 19: 1 mL) and 100 µL of ferric reagent. The mixture was brought to boiling water bath (Fisatom, Model 550, São Paulo), approximately 100°C for 30 min. The absorbance was recorded at 550 nm in a spectrophotometer and the results expressed in mg of leucocyanidins per 100 g of sample.

Flavonoids

Flavonoids were determined according to the method described by Zhishen *et al.* (1999), with some modifications. In falcon tubes were added 0.5 mL of extract, 2 mL of distilled water and 0.15 mL of $NaNO_2$ (5%), allowing to react for 5 min. Then was added 0.15 mL of $AlCl_3$ (10%) and the reaction continued for 6 min. After they were added 1 mL of NaOH 1 $mol.L^{-1}$ and 1.2 mL of distilled water and the absorbance was measured at 510 nm. The results were expressed in mg of equivalent per 100 g of sample using catechin standard curve

Anthocyanins

The anthocyanin content was determined according to the method described by Francis (1982), with some modifications. In falcon tubes was weighed and 1 g of sample added 30 mL of ethanolic solution acidified with HCl (85 mL ethanol to 15 mL of HCl 1.5 $mol.L^{-1}$). The mixture was subjected to constant mechanical stirring for 1 h. Subsequently, the absorbance of the samples was measured at 525 nm and the content of anthocyanin expressed as cyanidin-3-glucoside mg per 100 g of sample.

Antioxidant activity by DPPH* method

The antioxidant activity of the extracts was determined by DPPH* method according to Brand-Williams *et al*. (1995). Briefly, 100 µL of appropriately diluted extract was added to 3.9 mL of freshly made 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical solution (60 µM) that was previously diluted in methanol until an absorbance of 1.100 ± 0.02 was reached at 517 nm. After 2 h of incubation at room temperature, the absorbance at 517 nm was measured. Results were expressed mg of trolox equivalent per 100 g of sample.

Antioxidant activity by ABTS* method

The antioxidant activity of the extracts was also evaluated against ABTS* radical, according to the method described by Re *et al*. (1999). ABTS* was dissolved in water to a concentration of 7 mM. ABTS* radical cation ($ABTS^+$) was produced by reacting ABTS* stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark room for 16 h before use. The $ABTS^{*+}$ solution was diluted with 45% ethanol to an absorbance of 0.700 ± 0.02 at 734 nm. The $ABTS^{*+}$ solution (3.9 mL; absorbance of 0.700 ± 0.02) was added to 0.1 mL of the diluted extract and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6 min, and the absorbance was immediately recorded at 734

nm using a UV spectrophotometer. The antioxidant activity was expressed as mg equivalent trolox per 100 g sample.

Statistical analysis

Analytical determinations for the samples were performed in triplicate and standard deviations were reported. Means were compared by T Student test.

Results and Discussion

Morphology and size of the grains

The morphology of barnyardgrass grains is showed in Figure 1. The Figure 1 shows images of grains with and without husk. Figures 1a and 1c show barnyardgrass grains with husk, the palea and lemma form a protective layer of caryopsis, while Figures 1b and 1d show only the caryopsis after separation of husk. Figures 1a and 1b show the images of the side of the germ and Figures 1c and 1d show the images of the opposite side to the germ.

The grains with husk had a mean length of 2.92 ± 0.40 mm, width of 1.47 ± 0.14 mm and thickness of 0.95 ± 0.10 mm, and the grains without husk had a mean length of 1.57 ± 0.06 mm, width of 1.37 ± 0.04 mm and 0.78 ± 0.08 mm thickness. Echinochloa grains with husk have pronounced apexes, being responsible for the difference in length among species, and are related to genetic variability. The width of the caryopsis difference can be attributed to the level of surface removal during stripping operation as well as related to the degree of maturation of barnyardgrass grains. The stripping operation of the grain promoted average yield of 43.6 ± 4.7%, lower than the result reported for others cultivated Poaceae as rice and oat. However, factors such as lack of specific equipment for separation of husk of barnyardgrass as well as the small size of grains and strongly adhered husk, may have contributed to this difference.

Chemical composition

The chemical composition of barnyardgrass grains with and without husk is shown in Table 1. The husk of barnyardgrass grains consists mainly of fibers, ashes and lipids and other components are concentrated in the fraction of the caryopsis. The moisture content is an important factor for conservation of grain quality. The barnyardgrass grains were dried in the sun and had low moisture contents, which are indicated to prevent and biochemical deterioration by microorganisms during storage. The grain moisture without husk was larger than the grains with husk, however did not exceed 13%, maximum moisture of storage for most cereals.

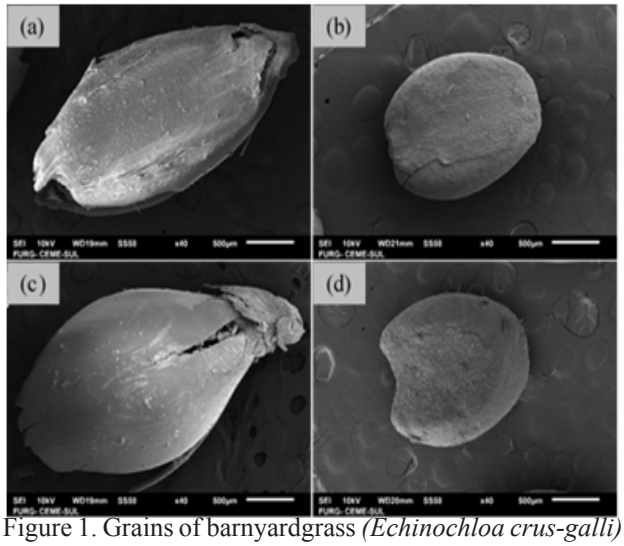

Figure 1. Grains of barnyardgrass *(Echinochloa crus-galli)* with husk, side of germ (a) and without husk, side of germ (b); with husk, opposite side of germ (c) and without husk, opposite side of germ (d).

The dehusked barnyardgrass grains have a higher protein content as compared to the grains with husk. The barnyardgrass can be an alternative source of protein to the diet, as compared to the protein intake of rice, which is a food consumed daily by the population, this stands out due to its higher content (11.7%) in grains without husk, in addition the grains with husk also have high protein content (8.7%).

The lipid content of the barnyardgrass ranged from 6.7% to 4.9% in grains with and without husk, respectively. According to Shahidi and Chandrasekara (2013), millets generally have higher fat content when compared to other cereals on average have values between 3.5 and 5.2% for whole grain dehusked. The presence of antioxidants in the lipid fraction of cereals has been widely studied and has been associated with a decrease in total cholesterol, increasing HDL cholesterol and in the prevention of cardiovascular diseases, which makes it interesting to consider barnyardgrass as a source of bioactive compounds (Rong, Ausman and Nicolosi, 1997; Iqbal, Bhanger and Anwar, 2005).

Minerals are essential nutrients for the effective functioning of bodily activities. The ash content of the barnyardgrass was 7.8% in the grain with husk and 1.5% in grain without husk (Table 1). This variation is due to the high content of silicon present in the silica of husk, while dehusked grain is commonly found phosphorus, potassium and magnesium, but in lower quantities (Juliano and Bechtel, 1985). Other Echinochloa species showed values higher than those found in this study with values ranging from 2.7 to 4.2% of minerals (Veena *et al*., 2005).

While the husk of barnyardgrass grains is mainly composed of dietary fiber, the caryopsis consists

Table 1. Chemical composition of grains of barnyardgrass with and without husk.

Composition (%)	Grains of barnyardgrass	
	With husk	Without husk
Moisture	10.7 ± 0.0 [b]	12.2 ± 0.1 [a]
Protein	8.7 ± 0.0 [b]	11.7 ± 0.2 [a]
Lipids	6.7 ± 0.5 [a]	4.9 ± 0.2 [b]
Ash	7.8 ± 0.1 [a]	1.5 ± 0.0 [b]
Dietary fiber	40.8 ± 2.6 [a]	3.1 ± 0.2 [b]
Soluble fiber	3.3 ± 1.2 [a]	0.8 ± 0.2 [b]
Insoluble fiber	37.5 ± 1.4 [a]	2.3 ± 0.1 [b]
Other carbohydrates	25.3 ± 0.3 [b]	66.6 ± 0.1 [a]

Results are the means of three determinations ± standard deviation. Values accompanied by different letters in the same row statistically differ ($p<0.05$).
Other carbohydrates: determined by difference.

Table 2. Percentage of fatty acids in the form of methyl esters in oil of barnyardgrass grains without husk.

Fatty acid	Fatty acid content (%)
Caprylic (C8:0)	Nd
Capric (C10:0)	Nd
Lauric (C12:0)	Nd
Myristic (C14:0)	0.06
Palmitic (C16:0)	13.01
Palmitoleic (C16:1)	0.17
Stearic (C18:0)	1.00
Oleic (C18:1)	22.88
Linoleic (C18:2)	61.03
Linolenic (C18:3)	1.47
Arachidic (C20:0)	0.22
Behenic (C22:0)	Nd
Erucic (C22:1)	Nd
Lignoceric (C24:0)	0.18

Nd: not detected

mostly of other carbohydrates (25.3%), in which the starch is the major component, accounting for 66.6% carbohydrate present in grain without husk, and present 3.1% of fibers. The content of soluble and insoluble fractions of dietary fiber of barnyardgrass grains with and without husk is shown in Table 1. The higher insoluble fiber content of barnyardgrass grains with husk is due to presence of cellulose, lignin and hemicellulose insoluble portion mainly concentrated in the husk. While the soluble fraction is probably composed of pectins, gums, mucilages and some of the soluble hemicellulose. The fibers have important functions, since its use has been associated with reduction of food retention time in the intestine, increase in the retention time in the stomach causing a feeling of satiety, reducing the risk of gastric ulcers, among others (Dhingra *et al*., 2012; Foschia *et al.*, 2013).

The barnyardgrass, red and black rice grains were considered invasive plants in traditional rice cultivation. Currently these crops has been grown and used for human consumption, with high market value. The composition of the barnyardgrass suggests a possible use for human consumption and better use, given that currently most of the production of this grain is discarded or intended to feed. However, due to the grain irregularities and color, the development of a suitable processing to improve the quality of barnyardgrass grains to entire consumption it is necessary, since these characteristics may limit its acceptability. According to Shahidi and Chandrasekara (2013), the use of millets in food has a tendency to expand in the market in the production of organic products, due to their resistance to pests and diseases, and for the preparation of gluten-free products.

Fatty acids, tocopherols and gamma oryzanol

The fatty acid profile in dehusked barnyardgrass oil is shown in Table 2. The barnyardgrass grains contain a significant fraction of unsaturated fatty acids, corresponding to 85.6% of the fatty acids present. These grains also contain monounsaturated fatty acids (23.0%) and saturated smaller amount (14.5%). Linoleic acid is an essential fatty acid since it is not synthesized by the human body and must be ingested in the diet; in this study the polyunsaturated fatty acids was predominant in the sample, reaching approximately 62.5%. This value is higher than that found for other grains oils such as canola (20.1%), rice (36.3%) and soybean (56.0%) and similar to corn oil (60.4%) (Zambiazi *et al.*, 2007).

According to the Department of Health England the ratio of polyunsaturated and saturated fatty acids should be more than 0.45 in order to prevent damage from cardiac diseases. The ratio observed for the fatty acids present in the barnyardgrass grains was 4.3, a value considered high when compared to Yoshida *et al.* (2011) that evaluated the fatty acid profile of two rice cultivars and this ratio was approximately 1.7%. The results suggest the high quality of fatty acids present in barnyardgrass grains and possible indications for human consumption.

Tocopherols, better known as vitamin E are phenolic antioxidants, consisting of a chromanol ring and a side chain with 16 carbons (Wanyo *et al*., 2014).

Table 3. Tocopherols and oryzanol contents in oil of barnyardgrass grains without husk.

Compounds	Content (mg/100g)
α-tocopherols	115.6
γ-tocopherols	1.3
δ-tocopherols	3.6
Σ (α,γ,δ) tocopherols	120.5
Gamma oryzanol	6.0

Σ – Total tocopherols.

In this study 3 forms of tocopherols were quantified in dehusked barnyardgrass grains. The predominant form was α-tocopherol, corresponding to 96% of the forms found (Table 3).

Yoshida *et al.* (2011) evaluated tocopherols in rice oil and observed similarities between 5 distinct cultivars, and 96% was also found α-tocopherol, similar to the obtained in this study for barnyardgrass. However, the total content obtained for barnyardgrass exceeded 50% the content present in rice bran oil, standing out as a promising source of α-tocopherol. Studies show that the main source of vitamin E to the diet is by intake of oils; however it is interesting to note that there is a constant search for healthy foods and moderate consumption of oils and fats. Whereas that the barnyardgrass is consumed daily by the population, the intake of 100 g per day, will contribute approximately 7,7 times the recommended daily amount (15 mg α-tocopherol/day). These results are related to the raw sample, therefore, more studies are need to recommend the amount of sample after cooking and thus to evaluate the influence of cooking processes.

In the analysis of gamma oryzanol, was used Bligh and Dyer method for extracting lipids by being a cold-extraction method, and thus keeping the quality of the compound of interest. The average content of gamma oryzanol in dehusked barnyardgrass was 0.06 mg/g of sample. This compound is a complex mixture of ferulic acid, esterified sterols and triterpene alcohols (Rogers *et al.*, 1993). The presence of gamma oryzanol in food has been widely studied due to its pharmacological properties, such as cholesterol regulator, anti-inflammatory activity, and is considered a potent antioxidant both for food, pharmaceutical products (Rong, Ausman and Nicolosi, 1997; Iqbal, Bhanger and Anwar, 2005).

Currently studies are focused on rice bran as a source of gamma oryzanol. Lilitchan *et al.* (2008) evaluated several varieties of rice and they reported that the highest content was found in the bran fraction due to its high fat content, varying between 18.2 and 21.2% on a dry basis and oryzanol range from 1.9 to 3.1 mg/g of bran.

Table 4. Secondary metabolites and antioxidant activity present in grains of barnyardgrass with and without husk.

Secondary metabolites	Grains of barnyardgrass	
	With husk	Without husk
Phenols total (mg equiv. gallic acid/100g)	399.0 ± 1.9 ª	96.7 ± 5.7 ᵇ
Total flavonoid (mg equiv. catechin/100g)	102.7 ± 4.9 ª	27.3 ± 0.6 ᵇ
Total tannin (mg equiv. gallic acid/100g)	206.2 ± 4.3 ª	39.8 ± 3.8 ᵇ
Condensed tannins (mg equiv. leucocyanidin/100g)	6.9 ± 1.2 ª	0.3 ± 0.1 ᵇ
Anthocyanins (mg cyanidin/100g)	5.6 ± 0.3	Nd
Vanillic acid (mg equiv. vanillic acid/100g)	4.9 ± 0.0	Nd
ABTS*	491.5 ± 0.6 ª	165.1 ± 6.4 ᵇ
DPPH*	285.3 ± 6.1 ª	72.17 ± 3.3 ᵇ

Results are the means of three determinations ± standard deviation. Values accompanied by different letters in the same row statistically differ ($p<0.05$).
Nd: not detected.

Secondary metabolites

The composition of secondary metabolites in barnyardgrass grains with and without husk is presented in Table 4. Currently has been recommended to consume foods rich in bioactive compounds, phenolic compounds are among these, due to their pharmacological properties (Verschoyle et al., 2007; Arab, Alemzadeh and Maghsoudi, 2011).

The contribution of total phenolic compounds was greater in barnyardgrass samples with husk reaching about 4 times the amount found in caryopsis (Table 4). Chethan and Malleshi (2007) found 6.2% of total phenolic compounds in the husk of finger millet and 0.8% in the endosperm, higher than those found in this study. This difference can be attributed to genetic variability, degree of maturity, cultivation conditions, among others. According to McDonough and Rooney (2000) the ferulic acid and p-coumaric acid are the main occurrence of phenols in millets, being considered of great importance in the diet due to its antioxidant potential.

The flavonoid, subclass of phenolic compounds (Bakar *et al*., 2009), also showed low content when compared to studies about samples of finger millet which showed 210 mg/100 g of defatted flour. Watanabe (1999) reported that the major flavonoids in *E. crus-galli* grains are tricin and luteolin. Luteolin and their glycosides have beneficial health properties, being highlighted its antioxidant activity, anti-inflammatory and prevention of some types of

cancer and tricin, for its anti-tumor and anti-leukemic properties (Han *et al*., 2007).

The condensed tannins are phenolic compounds considered to be potent inhibitors of enzymes due to its complexation with enzymatic proteins causing low digestibility (Silva and Silva, 1999). The content of tannins was about 20 times higher in grain with husk when compared to grains without husk. According to Dykes and Rooney (2006), the condensate tannin content of finger millet from brown species contain higher content (0.12 to 3.47%) when compared the white species (0.04 to 0.06%). The condensed tannin content in barnyardgrass grains without husk was 0.34 mg equiv. of leucocyanidin/100 g of sample (Table 4), approximately 8.5 times higher as compared to the results cited by the authors for finger millet species white.

The anthocyanins content and vanillic acid of barnyardgrass was detected only in the fraction of the grains with husk (Table 4). The higher amount of bioactive compound in the husk is possibly due to action of the grain protection against various external factors and therefore the need to produce these bioactive. Although the content of secondary metabolites in barnyardgrass grains was low when comparing with other species of millet, the results suggest that barnyardgrass can contribute to the daily intake of bioactive. Furthermore, studies are needed to verify the stability this compounds on conventional cooking processes of the grains, and their availability during digestion.

Antioxidant activity

The antioxidant activity of barnyardgrass grains with and without husk was evaluated by reducing the radical DPPH* and ABTS* (Table 4). Although there are several methods to evaluate the antioxidant activity, they have advantages and limitations. Thus, it is necessary to use more than one method of analysis to that the approach can be representative of the antioxidant activity of the product (Carocho and Ferreira, 2013).

The extracts of barnyardgrass grains showed antioxidant activity against both radical evaluated, due to the radical phenol (hydroxyl substituent of the aromatic ring) stabilize the free radical by donating hydrogen atoms. The highest antioxidant activity was observed for the extract obtained from barnyardgrass grains with husk (Table 4), which can be explained by the higher contribution of bioactive compounds.

The efficiency of the phenolic substances as antioxidants depend largely on their chemical structures, relative orientation and the number of hydroxyl groups linked to the aromatic ring (Sánchez-Moreno *et al*., 1998), which would need to identify the compounds present in the barnyardgrass grains for this relationship to be further clarified. The use of antioxidants is important because the human organism is constantly exposed to a variety of oxidizing agents that can cause damage DNA and proteins, and lipid oxidation of the membranes and increased risk of degenerative diseases. Thus, sufficient amounts of consumption of antioxidants is necessary to inhibit or delay the oxidative stress induced by these free radicals (Adom and Liu, 2002; Sun *et al*., 2002).

Conclusion

The study of the nutritional characteristics of barnyardgrass grains has shown that this plant has the potential for use both as food and feed. The barnyardgrass grains with husk had higher content of lipids, minerals, fiber, bioactive compounds and antioxidant activity when compared to the grains without husk. The dehusked barnyardgrass grains are also good sources of protein, unsaturated fatty acids, tocopherols and gamma oryzanol. However, by the evaluation of the characteristics of the grains, probably products obtained from the grains with husk may have a higher nutritional potential, since that they have about 13 times more dietary fiber and more secondary metabolites and antioxidant activity when compared to the dehusked grains.

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