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Chemical and bromatological analyses of different corn (*Zea mays*, l.) Seeds varieties

Bruna Juber de Araújo, Dôuglas Nunes, Carla Lima, Enyara Rezende Morais, Cristina Fürstenau

Laboratório de Bioquímica Vascular, Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia, Campus Patos de Minas, Patos de Minas, Minas Gerais, Brazil.

Abstract
Corn (*Zea mays*, L.), a cereal of great importance in agriculture all over the world, is rich in several nutrients and presents phytochemicals with high antioxidant power. Concentration of total phenolic compounds and centesimal composition of five different corn varieties from native germplasm were determined. Phenolic extract was obtained from processed samples and its total content was determined by Folin- Ciocalteau reagent. Bromatological profile of all corn varieties was characterized by the index of dry matter (DM), crude protein (CP), ether extract (EE) and ash (A). Higher concentration of (poly) phenols was found in V1 red color variety (180.8 ± 10.63 mg EAG/100g) with potential vascular applications. Also, V1 (red) and V4 (white) varieties exhibited higher CP content when compared to other varieties and might represent an interesting increment in human and animal diets. Chemico-bromatological profile of these corn varieties was performed for the first time and may serve as background for further and deeper studies.

Keywords: corn, phenolic compounds, centesimal composition, antioxidant potential.

1. Introduction
Corn (*Zea mays*, L.) consists of a cereal widely grown throughout the world territory. Corn may be considered the second largest agriculture product, being soybean the first one. The huge production and consumption of this grain in Brazil is due to its different uses, ranging from daily consumption in Brazilians' meals as grits, popcorn, sweet, oil, flour, butter, glucose syrup and flakes cereals, until the crops used in animal feed silage industries, since corn is one of the major ingredients in poultry, cattle, fish and pork feed [9]. Data from the 7th survey of grains held by Companhia Nacional de Abastecimento (CONAB) point out that Brazil, especially the Midwest, the Southest and the South regions, produced about 91.468,400 tons of this grain during 2016/ 2017 [7]. Also, recent data from CONAB estimates that 30.313,3 tons of corn will be produced in the first 2017 season, and additional 63.522,3 tons in the second season of the same year [8]. Nutritional value of corn, which is attributed to chemical compounds present in corn grains, is another relevant factor that contributes to the high corn consumption [9].

The mean composition of a corn dry seed consists in 72% starch, 9.5% proteins, 9% fibers and 4% oil and its structure is composed by 82% endosperm, 11% germ (embryo), 5% pericarp (rind) and 2% tip [18]. Besides being an important source of carbohydrates, proteins and vitamins precursors, corn has also been reported as some grain rich in phytochemical compounds such as carotenoids, anthocyanins and other flavonoids. Altogether, these phytochemical substances are named as phenolic compounds which are obtained from vegetal and microbial sources and are been related to high antioxidant activity [15, 26].

Phenolic compounds are linked to different functional properties in human health, with emphasis in their ability to scavenge free radicals [10] that accounts for their antioxidant capacity. Also, some epidemiologic and experimental studies have demonstrated that phenolic compounds may develop an important role in preventing cardiovascular diseases as well as cancer, diabetes and neurodegenerative illnesses when obtained from diet [11, 24]. In this sense, Haida *et al*. (2015) reported the high concentration of phenolic compounds in guava (*Psidium guajava* L.), indicating the use of this fruit as an adjuvant in the treatment of degenerative and inflammatory pathologies, as well as a reducer of cardiovascular diseases due to its antioxidant properties. Simão *et al*. (2015) showed that antioxidant compounds may also help in obesity treatment throughout the usage of medicinal plants like gorse (*Baccharis trimera*, L.), "marmelinho" (*Tournefortia paniculata*) and others as sources of phenolic compounds.

The interaction between genotype versus enviroment has directly influenced in the amount of carotenoids present in corn seed endosperm and may also be related to yellow and orange color of corn grains [20]. González-Muñoz and colleagues (2013) showed that dark corn seeds presented high antioxidant activity while yellow corn seeds were efficient to inhibit α-amylase activity, a key enzyme in the development of hyperglycemia condition. Thus, it is clear that the quantification of corn phenolic compounds may help to reveal an interesting use this grain in the treatment of several pathologies as previously mentioned.

Bromatological analyses are essential to the knowledge of nutritional and chemical composition of a determined food. The amount of dry matter (DM), crude protein (CP), ether extract (EE) and ash (A) may be evaluated in order to determine the concentration of some nutrients, oil and minerals. To do so, Weende are the most common bromatological methods used in food analysis and give adequate information about food chemical composition [21].

Therefore, considering the economic, social, cultural and nutritional importance of corn grains, the goal of the present study is to determine the concentration of total phenolic compounds as well as the centesimal composition of different corn seeds varieties, whose colors vary from red, yellow and

white. The chemical and bromatological characterization of these seeds will help in their future utilization in a study that aims to elucidate the contribution of corn as an adjuvant in systemic arterial hypertension (SAH) treatment.

2. Materials and methods

2.1 Samples

Representative samples (200 g) of mature seeds of five different varieties of corn (*Zea mays*, L.) from local native germplasm (BA125 - BRA031194; BRS4104; MG089 - BRA052825; MG069 - BRA052612; MG020 - BRA052299) were made provided by the bank of germplasm of Empresa Brasileira de Pesquisa Agropecuária – Milho e Sorgo (EMBRAPA), located in Sete Lagoas – MG. The seeds were pre-selected based on their color that ranged from red, through yellow, and white colors, and by the precursor content of vitamin A (BRS4104). Distinct corn seeds may represent different amounts of antioxidant compounds. All varieties are suitable for human and animal consumption. Is important to mention that these seeds consist of varieties found in bank of germplasm and were evaluated for the first time about their composition and chemical profile, which may favor the cultivation of such varieties, and also strengthen your direct use in food and feed, as well as in industry. Also, analyses may contribute for storing relevant in the gene bank. Samples were stored at 10 C° until used.

To facilitate the understanding, varieties were numbered from 1 to 5 and categorized in a visual scale of color as shown in Figure A1. Variety 1 (V1) - BA125-BRA031194 red color, Variety 2 (V2) - BRS4104 yellow color and precursor of vitamin A, Variety 3 (V3) - MG020-BRA52299 yellow color, Variety 4 (V4) - MG089-BRA052825 white color and Variety 5 (V5) - MG069-BRA052612 white color.

2.2 Samples Processing

Samples of each variety (50 g) were ground through a domestic blender to form a fine powder.

2.3 Preparation of Phenolic Extract

In order to obtain the phenolic fraction from corn seeds, about 1 g of each dry and grounded sample was used, to which were added 40 mL of 95% ethanol, remaining under orbital magnetic shaking for 2 hours. After this period of time, samples were allowed to rest for 48 hours, according to the method described by Bertoldi (2006). The extraction of phenolic content was run in triplicate for each corn variety.

2.4 Determination of total phenolic content:

Phenolic content of corn seeds extracts was determined using the Folin-Ciocalteu colorimetric method, based in the formation of a blue complex produced by the reduction of the reagent caused by phenolic compounds present in samples.

Determination of total phenolic content adopted the procedure previously described by Nascimento *et al*. (2007). Briefly, 0.5 mL of each extract prepared in ethanol 95% was collected and placed in test tubes, to which were added 2.5 mL of 10% (v/v) Folin–Ciocalteu solution plus and 2 mL 4% (w/v) Na2CO3.

Then, the mixture was kept in water bath at a temperature of 50 C° for 5 minutes. Samples were allowed to rest at room temperature, and then the absorbance was measured at 760 nm in a spectrophotometer. Total phenolic content of extracts was carried out in triplicate and results were expressed as mean of mg of gallic acid equivalents (GA) per 100 g of dry sample, using a calibration curve prepared with gallic acid.

For the calibration curve, a stock solution of 100 mg.L-1 gallic acid was prepared.

From the stock solution, standard curve points were prepared (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg / L). Aliquots of 0.5 mL from each point were withdrawn and 2.5 mL 10% (v/v) Folin-Ciocalteu solution plus 2 mL of 4% (w/v) Na2CO3 were added. The mixture was kept in a water bath at a temperature of 50 C° for 5 minutes. Samples were allowed to rest at room temperature and then the absorbance was measured at 760 nm in a spectrophotometer.

2.5 Bromatological profile

To evaluate the bromatological composition of corn seeds dry matter (DM), crude protein (CP), ether extract (EE) and ash (A) were analyzed according to AOAC protocols (1984).

2.5.1 Dry matter (DM)

Dry matter was determined by using an oven at 105 C° for 24 hours. Briefly, Petri dishes were previously identified and dried in an oven at 105 C° for 2 hours. Then, dishes were placed in a desiccator until reaching room temperature. Dishes weights were registered. About 5 g of grounded corn seeds were added to Petri dishes and their weights were registered. After that, dishes containing the samples were placed into the oven at 105 C° for 24 hours to ensure the total drying of the seeds. After this period of time, plates with samples were placed in the desiccator until reaching room temperature. Samples were weighed again. DM analysis was performed in triplicate for each corn variety.

2.5.2 Crude Protein (CP)

Kjeldahl method (AOAC, 1984) was used to determine total nitrogen in the organic matter, including protein nitrogen. About 0.5 g of dry samples were placed into a digestion tube. Around 2 g of a digester mixture (Na2SO4 e CuSO4 (1:10)) plus 5 mL 0.2 N H2SO4 concentrated was used to start the digestion process at low temperature. Maximal temperature of 350 Cº was reached and samples were let to rest for 30 minutes until the lightening of the solution. After cooling the samples, a little portion of distilled water was added until complete dissolution. Digester tubes containing the samples were transferred to the distillation apparatus to which were added 5 mL 0.2 N NaOH. Fifty milliliters of distilled water plus 50 mL of 4% H3BO3 and the mixed indicator were added to an Erlenmeyer. Distillation was performed by samples dragging, keeping the condenser terminal inside the receptor solution that was present in the Erlenmeyer flask until all amonia was released in a volume of approximately 100 mL. By withdrawing the flask, the tip of the condenser was washed with distilled water and titrated with standard 0.1N HCl to turn the mixed indicator. A blank test was conducted in order to eliminate interference of reagents.

2.5.3 Ether Extract (EE)

About 2 g of each samples were weighed and submitted to dry process in an oven at 100 C° for 5 hours. After the dry time, all samples were cooled in a desiccator and had their weights registered. A type of Soxhlet apparatus with five units of extraction was used to perform EE extraction. Around 250 mL of petroleum ether were added to each round bottomed flask that were aligned in front of the extractors. To finally start the EE extraction, the heating was set to a mean temperature

(condensation rate of 5 to 6 drops/ second). Flasks containing the distilled products were taken to an occupational chapel for ether evaporation. A heating blanket was used to speed the process. The flasks were then placed into a dryness by convection oven at 105 C° for 1 hour. When cooled to a room temperature in a desiccator, the weight was recorded. The difference between the latter weight and the weight of the empty round bottomed flask corresponded to the weight extracted fat.

2.5.4 Ash (A)

Porcelain crucibles were placed in a drying oven at 105 C° for 2 hours and cooled sequentially in a desiccator for 1 hour. Two grams of each corn sample were weighed and subjected to burning in a muffle furnace for 6 hours after the temperature reached 600 C° to obtain a light ash. After this period, the furnace was turned off and the crucibles were cooled at a temperature lower than 250 C°. At the end of the process, the crucibles with the samples were cooled to room temperature in a desiccator and the weight was recorded.

2.6 Statistical Analysis:

Means and standard deviations were calculated for each of the measures performed and for each of the groups analyzed. Analysis of variance (ANOVA) followed by Bonferroni's post-hoc test was applied for comparison between groups. Differences were considered significant when the statistical analysis showed $p<0.05$. The GraphPad Prism software was used as a computational tool for statistical analysis.

3. Results & Discussion

3.1 Phenolic Content

Phenolic compounds present in different varieties of corn seeds were quantified by means of Folin-Ciocalteu method, using gallic acid as a standard. Results are presented in Table C1, showing around 109 to 180 mg eq GA/ 100 g of sample. Rios *et al*. (2014) reported that corn grain color was directly influenced by the concentration of carotenoids in different corn varieties, according to their genotype. Data of this study show that there were no significant differences in the total phenolic content among the corn varieties evaluated ($p<0, 05$), indicating that there was no positive correlation between seed color and phenolic content ($R=0,48$). However, it's possible that the total phenolic content may be correlated to the genotype profile of each corn variety and not directly to the color phenotype of corn seeds.

Kuhnen (2007) found about 32 to 114 mg/ 100 g of total (poli) phenols in samples of different varieties of local and comercial corn, showing that this parameter may fluctuate greatly between the analyzed corn seeds. Bacchett *et al*. (2013), for example, found total phenolic content around 115 to 175 mg/ 100 g for different Italian local corn varieties. In the present study, we found results from 109 to 180 mg/ 100 g of total phenolic compounds in the varieties evaluated. Thus, the variety V1 (BA125-BRA031194) red color is probably the more suitable for future investigations since the higher phenol content found may indicate a greater antioxidant potential, which may be related to a greater potential in treating pathologies, when compared to the other seeds evaluated.

3.2 Dry matter (DM)

The water content present in corn seeds was determined in order to evaluate the quality of the grains in comparison to standard values found in the literature. The values obtained in the present study were around 6.5 – 7.5% and were significantly lower than the standards already described in the literature (Figure A2). Data from *Ministério da Agricultura, Pecuária e Abastecimento* (MAPA) presented by Ascheri & Germani (2004), show that the moisture content after drying corn grain is around 11.5 – 12.5%. Water percentage in the grain directly influences the germination and deterioration processes, therefore, keeping the water content at low levels, both difficults the embryo germination as well as blunts seed attack by fungi and other microorganisms [22].

The results obtained in this work show that moisture is significantly decreased in all corn seeds varieties studied, except V2, when compared to literature standards. However, the low water content found does not represent any limitation for this work since the planting of these grains is not a goal of the present study, but rather their chemical analysis.

3.3 Crude Protein (CP)

The method of Kjeldahl was adopted to determine the amount of crude protein (CP) in corn varieties, which considers the fact that proteins present about 16% of nitrogen in their composition [1]. According to this method, nitrogen is converted to ammonium throughout acid digestion. After that, ammonium is separated by means of distillation and recovered into a receptor solution to be further titrated with HCl [21]. Thus, considering the correction factor Cf = 1,013 and the molarity M = 0.1, the results of CP (Figure A3) were obtained through Equation B1.

As shown in Figure A3, V1 (15%) and V4 (13.5%) varieties exhibited higher concentration than the literature standards. According to Paes (2008), the amount of protein in dry corn grains is around 8 to 10%. Bjarnasan & Vasal (1992) reported that protein amount present in corn may vary according to seed type, being inconstant due to alterations caused by the presence of mutant genes in seed endosperm. Thus, genotypic analyses could explain the high protein concentration found in V1 and V4.

3.4 Ether Extract (EE)

Ether extract or crude vegetal lipids were extracted by using petroleum ether as solvent, as described before. Results are presented in Figure A4 and reached means around 3.5 to 4.5%.

In accordance to Alexander (1986), corn seeds have around 3.5 to 5% of ether extract, which is located mainly in the embryo. Data found in this study confirmed these values. The determination of EE in corn is necessary to drive its utilization in oil production or as animal feed, since corn oil is more caloric than starch [16].

3.5 Ash (A)

Mineral matter of corn varieties was determined from ash. Samples were submitted to high temperature in a muffle-furnace until total combustion of organic matter. Results show that ash average in all corn varieties analysed varied from 1.5 to 2.0% (Figure A5).

The general minerals found in corn ash are calcium, potassium, magnesium, iron, copper, cobalt, sodium, aluminum and others in different concentrations [21]. Total content of ash in a food is important to the general classification of the food product. For example, in food that are rich in specific minerals, ash analysis is essential to qualify the final product of interest [25]. Additionally, as ash is the non-organic matter of a food, the

higher is the ash content, the lower is the energetic potencial. However, for vegetal products, the ash determination is less informative since mineral components in vegetal are greatly variable [21]. Data from MAPA described by Ascheri & Germani (2004) point out that ash in corn may vary from 1 to 2%, which was corroborated in the present study.

4. Tables and Figures

Fig 1: Identification of corn varieties according to their color. V1 – BA125-BRA031194 red color, V2 – BRS4104 yellow color, precursor of vitamin A, V3 – MG020-BRA52299 yellow color, V4 – MG089-BRA052825 white color, V5 – MG069-BRA052612 white color.

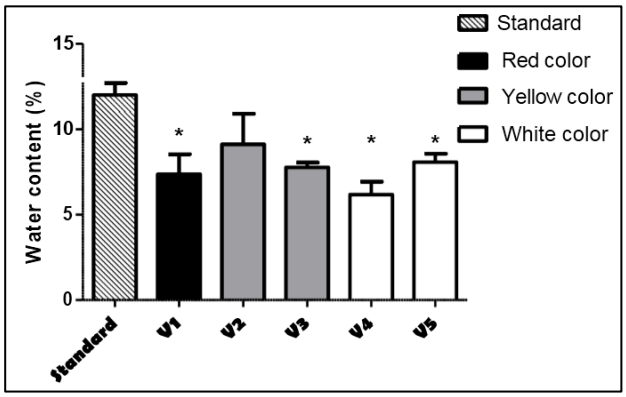

Fig 2: Water content in grains of different varieties of corn seeds (*Zea mays*, L.). V1 - BA125-BRA031194 red color, V2 – BRS4104 yellow color, vitamin A precursor, V3 – MG020-BRA52299 yellow color, V4 – MG089-BRA052825 white color, V5 – MG069- BRA052612 white color. *represents significantly statistical difference from literature standard ($p<0,05$), n=3.

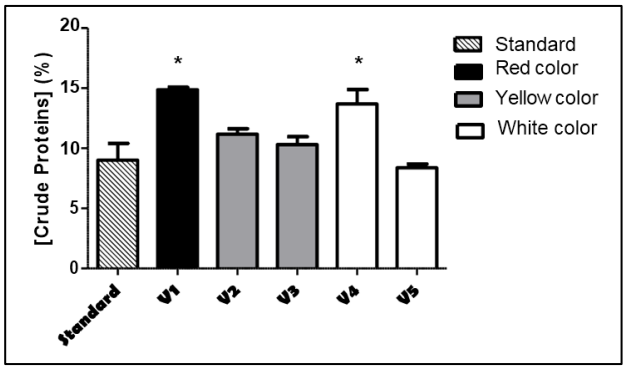

Fig 3: Concentration of crude protein (CP) in different corn seeds varieties (*Zea mays*, L.). V1 - BA125-BRA031194 red color, V2 – BRS4104 yellow color, vitamin A precursor, V3 – MG020-BRA52299 yellow color, V4 – MG089-BRA052825 white color, V5 – MG069- BRA052612 white color. *represents significantly statistical difference from literature standards ($p<0, 05$), n=3.

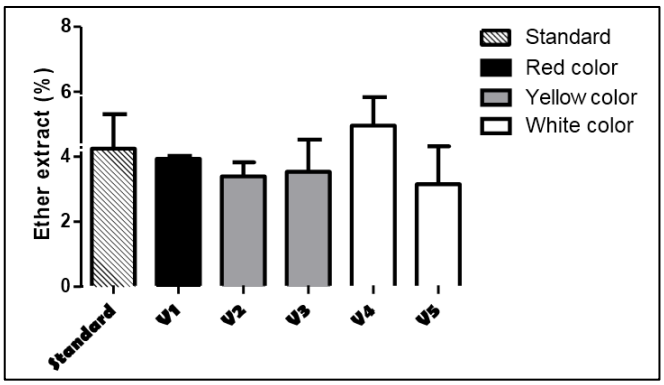

Fig 4: Percentage of ether extract (EE) in different corn seeds (*Zea mays*, L.). V1 - BA125-BRA031194 red color, V2 – BRS4104 yellow color, vitamin A precursor, V3 – MG020-BRA52299 yellow color, V4 – MG089-BRA052825 white color, V5 – MG069- BRA052612 white color, n=3.

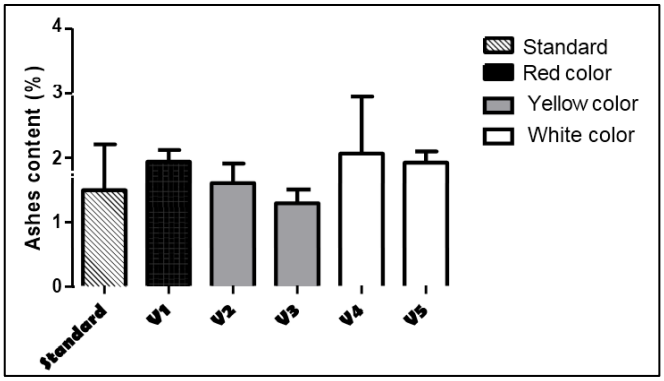

Fig 5: Ash (A) in different corn varieties (*Zea mays*, L.). V1 - BA125-BRA031194 red color, V2 – BRS4104 yellow color, vitamin A precursor, V3 – MG020-BRA52299 yellow color, V4 – MG089-BRA052825 white color, V5 – MG069-BRA052612 white color, n=3.

5. Equations

$$\% \text{ Crude Protein} = \frac{[(V' - V) \times Fc \times N \times 6{,}25 \times 0{,}014]}{W} \times 100$$

V' = volume of 0,1N HCl spent in titration; V = volume of 0.1 N HCl spent in blank test; Cf = correction factor of 0.1N HCl;
N = normality;
W = sample weight in grams;
6, 25 = conversion factor of nitrogen in proteins (16% = 16 g/100 g = 6, 25); 0,014 = mill equivalent-gram of nitrogen.
(1)

6. Conclusions

Chemico-bromatological profile of corn varieties (*Zea mays*, L.) provided by native germplasm, V1 - BA125-BRA031194 red color, V2 – BRS4104 yellow color, vitamin A precursor, V3 – MG020-BRA52299 yellow color, V4 – MG089-BRA052825 white color, V5 – MG069-BRA052612 white color, was analyzed and characterized for the first time, which may help in further studies, driving the right use for each one of the tested seeds in food industry, as animal feed or for direct consume in human meals. As a global result, color of seeds did not

significantly influence their chemical and bromatological characteristics.

Also, the analyses made in this work helped to select the variety to be used in a further study which aims to elucidate the contribution of corn as adjuvant in systemic arterial hypertension treatment. The choice will be made as a function of phenolic compounds quantity in grains. Thus, the V1 - BA125-BRA031194 red color variety seems to be the more indicated to test its potential in the treatment of several pathologies, including cardiovascular pathologies, as it presents the higher content of phenolic compounds and, probably, a higher content of antioxidant substances. In this sense, the potential of V1 seeds in modifying vascular tonus will be tested in smooth muscle cells culture in future.

7. Acknowledgments

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