

## Chemical composition, protein profile, and isoflavones content in soybean genotypes with different seed coat colors

<sup>1</sup>\*Ciabotti, S., <sup>2</sup>Silva, A. C. B. B., <sup>1</sup>Juhasz, A. C. P., <sup>2</sup>Mendonça, C. D., <sup>4</sup>Tavano, O. L., <sup>4</sup>Mandarino, J. M. G. and <sup>2</sup>Gonçalves, C. A. A.

<sup>1</sup>Empresa de Pesquisa Agropecuária de Minas Gerais, CEP 38001-970, Uberaba, Minas Gerais, Brazil

<sup>2</sup>Instituto Federal do Triângulo Mineiro, CEP 38020-300, Uberaba, Minas Gerais, Brazil

<sup>3</sup>Universidade Federal do Triângulo Mineiro, CEP 38025-180, Uberaba, Minas Gerais, Brazil

<sup>4</sup>Empresa Brasileira de Pesquisa Agropecuária - Embrapa Soja, CEP 86001-970, Londrina, Paraná, Brazil

### Article history

Received: 2 December 2014

Received in revised form:

8 July 2015

Accepted: 7 August 2015

### Abstract

This study aimed to evaluate the chemical composition, protein profile, and isoflavones content of soybean genotypes with different seed coat colors, intended for human consumption, for selection purposes regarding their nutritional properties. The cultivars BRSMG 800A, BRSMG 790A, and Conquista and the lineage MGBR07-7141 with matte brown, light yellow hilum, dark yellow hilum, and glossy black seed coats, respectively, were evaluated. Significant differences were observed between the genotypes in relation to moisture content, lipids, protein and crude fiber. Although the fractions albumin and globulin are the major protein fractions in leguminous plants, both the cultivar Conquista and BRSMG 800A showed albumin and glutelin as the main protein fractions. In all samples, the albumin fraction showed a great variety of proteins, with molecular weights in the range of 100 to 25 kDa. All globulins exhibited weights between 35 and 55 kDa. Considering the total isoflavones content, the cultivar BRSMG 790A with yellow seed coat had the highest isoflavones content (415.98 mg.100 g<sup>-1</sup>), while the cultivar Conquista presented the lowest content (220.88 g mg.100<sup>-1</sup>). All cultivars and lineage of the current study constituted high quality raw materials for food production and human consumption.

### Keywords

Brown soybeans

Black soybeans

Electrophoresis

Protein fractionation

Albumin

Globulin

© All Rights Reserved

### Introduction

Soybean is the basis of human diets in many Eastern countries, due to its high nutritional value and low cost. The relationship between soybean intake and human health has been widely investigated due to the nutritional characteristics of this grain, including its high quality protein, significant content of minerals and fibers, small amounts of saturated fat, and absence of cholesterol (Silva *et al.*, 2006). Numerous studies have been performed on the nutritional and functional value of genetically improved soybean with different seed coat colors, standing out the black soybeans. They are associated with a wide range of health benefits such as antimutagenic effect, anti-inflammatory properties, reduction in synthesis of low-density lipoprotein, antioxidant properties, and reduced effects of DNA damage (Astadi *et al.*, 2009; Wang *et al.*, 2010).

Researches have employed great efforts and investments in obtaining cultivars with more acceptable sensory characteristics in order to

introduce this leguminous plant in the Brazilian diet. As occurring with other vegetables, it is fundamental to investigate the nutritional quality of new cultivars in addition to its agronomic characteristics.

Recently, in Brazil, a soybean cultivar with brown seed coat has been developed and improved genetically through a partnership between Empresa Brasileira de Pesquisa Agropecuária/Epamig/ Empresa de Pesquisa Agropecuária de Minas Gerais /Triangle Foundation in Minas Gerais state, Brazil. This cultivar was named BRSMG 800A. This cultivar has brown seed coat color and favorable sensory characteristics, and may be mixed with beans, increasing protein content (30%) without modifying visual appearance or taste. This mixture soybeans + beans improves the acceptance of soybeans in Brazil, once it can be consumed together with the most popular dish in the country that is the cooked beans (Embrapa Soja, 2010).

Studies on the chemical composition, protein fraction, and isoflavones are of great importance to establish the differences between the characteristics

\*Corresponding author.

Email: [sueliciabotti@ifm.edu.br](mailto:sueliciabotti@ifm.edu.br)

Tel: +55-34-33128812; Fax: +55-34-3326-1101

of soybean cultivars and breeding lines. These characteristics are relevant for the consumer market, considering the nutritional value and functional aspects. From the point of view nutritional composition, soybeans contain essential components for human consumption, thus becoming excellent source of protein and lipids. However, the levels of these constituents are influenced by a number of environmental and genetic factors, which may vary according to the crop (Bhardwaj *et al.*, 1999).

The total protein fraction of the seeds is a complex mixture of globulins, albumins, prolamins and glutelins, the first two being the main components. The dicotyledons, such as soybeans, have globulins and albumin as major storage proteins (Drzewiecki *et al.*, 2003). Globulin is the main protein fraction representing 50-70% of the total protein (Neves *et al.*, 2006). The proportion of these fractions in the total protein differs between species, varieties and / or cultivars, which explains the differences in the functional properties and nutritional quality of soybeans (Neves *et al.*, 2006). The solubility of the seed proteins varies with species, variety, cultivar, and experimental conditions such as type and salt concentration, pH and temperature (Sgarbieri, 1980; Sathe and Salunkhe, 1981; Silva, 1997; Neves *et al.*, 1998).

Studies have shown that isoflavones, compounds present in soybeans, have estrogen and antiestrogen action, inhibit cell proliferation, and reduce serum cholesterol levels, which are relevant attributes to reduce the risks of non-communicable chronic diseases such as cardiovascular diseases, cancer, osteoporosis, and symptoms of menopause (Penha *et al.*, 2007). Moreover, their antioxidant properties prevent the excessive amount of free radicals, retarding premature aging (Souza *et al.*, 2000).

The soybean protein functionality was also recognized in 1999 by the FDA, food control agency of the United States of America. It was reported that, for purposes of nutrition labeling, diets low in saturated fat and cholesterol that include daily intake of 25 grams of soybean protein may reduce the risk of heart disease. The American Heart Association recommends the intake of soybean-based products for patients with high cholesterol (Messina *et al.*, 2002). In Brazil, The National Health Surveillance Agency (ANVISA) recognizes the following health claim for soybeans and soybean products: "the daily intake of at least 25 g of soybean protein may help reduce cholesterol. Consumption must be associated with a balanced diet and healthy lifestyle habits" (ANVISA, 2013). Since Brazil is the second worldwide producer of soybeans it is feasible to encourage consumption

and evaluate its properties. Thus, the present study aimed to evaluate the chemical composition, protein profile, and the isoflavones content of soybean genotypes with different seed coat colors for human consumption and for selection purposes regarding their nutritional properties.

## Material and Methods

Soybean [*Glycine max* (L.) Merrill] from three cultivars, suitable for human consumption, and a soybean breeding lines from the breeding program of the partnership Embrapa/Epamig/Triangle Foundation were used. The cultivars were BRSMG 800A, BRSMG 790A, and Conquista, and the lineage was MGBR07-7141. The cultivar BRSMG 800A has matt brown seed coats. The BRSMG 790A has yellow seed coat and hilum. The cultivar Conquista has yellow seed coat and black hilum. The lineage MGBR07-7141 has glossy black seed coats. The grains were analyzed for their chemical composition, protein fractions, and isoflavones content.

### Sampling

The plants were cultivated in experimental field, in the growing season of 2012/2013. When the seeds were complete matured, they were harvest and 0,2 kg of seeds were sampled to the analysis. The soybean seeds of the cultivars and breeding lines ground in a refrigerated mill model MA-345/T, Marconi (Brazil). The obtained flour was placed in glass flasks with caps and store under refrigeration

### Chemical composition

Chemical composition was accomplished in the flour samples Moisture, lipids, protein and ash contents were determined according to the methods recommended by AOAC (1990). Crude fiber was determined according to the gravimetric method described in IAL (2005). The carbohydrate content was calculated by the difference: [100- (protein + lipid + ash + fiber + moisture)].

### Protein fractions

The protein fractions were determined in the defatted soybean flour. The flours were defatted using n-hexane as solvent for lipid extraction. A ratio of 1:10 was used (solvent: sample), under constant agitation for 30 minutes at room temperature. After this step, the mixture was vacuum filtered, and the defatted flour was left in a chapel of exhaust gases at room temperature for solvent evaporation. The separation of protein fractions was based on the protein solubility at different extraction solutions.

The extraction of albumin, globulin, glutelin, and prolamin was performed according to the methodology described by Sathe and Salunkhe (1981), with some modifications.

#### *Extraction of total albumin and globulin*

Aliquots of the defatted flour were dispersed in 0.5 M NaCl 1:10 (w / v) and subjected to mechanical stirring for 30 minutes. Then, the suspension was centrifuged at 6,500 g for 15 minutes at 5°C, obtaining the supernatant S1 and the residue R1. The residue (R1) was dispersed in 0.5 M NaCl 1:10 (w / v) and centrifuged twice more, yielding supernatants S2 and S3 and the residue R3. The supernatants S1, S2 and S3 were dialyzed against distilled water for 24 hours at 4°C with several water exchanges during the period. During dialysis, the globulins precipitated from the extracts. The dialyzed material was centrifuged at 6,500 g for 15 minutes at 5°C. The precipitates containing globulins (G1, G2 and G3) were separated and the supernatants containing the albumins (A1, A2 and A3) were stored. The precipitated globulin were again dispersed in water to remove albumin which may have been dragged with the precipitate, and again centrifuged at 6,500 g at 5°C for 15 minutes and then stored.

#### *Extraction of prolamin*

The residue R3 from the above procedure was dispersed in 70% ethanol solution at a ratio of 1:10 (w / v) and subjected to constant mechanical stirring for 30 minutes. Then the suspension was centrifuged at 6,500 g at 5°C for 15 minutes. The residue (R4) obtained was separated and the supernatant containing the prolamin fraction was stored.

#### *Extraction of glutelin*

The residue R4 was dried in an oven with air circulation at 25°C for evaporation of ethanol. Then, it was solubilized in 0.1 N NaOH (1:20 w / v) and subjected to constant mechanical stirring for 30 minutes. This suspension was centrifuged at 6,500 g for 15 minutes at 5°C. After centrifugation, the supernatant (Glut1) was stored. The residue obtained (R5) was solubilized in 0.1 N NaOH (1:10 w / v), and subjected to constant mechanical stirring for 30 minutes followed by centrifugation under the same conditions, thus producing the final residue containing the insoluble protein fraction and the supernatant (Glut2).

#### *Separation of proteins by polyacrylamide gel electrophoresis*

The separation of the isolated protein subunits

and their estimated molecular weights were performed according to the electrophoretic profile of the proteins in polyacrylamide gel. Sodium dodecyl sulfate (SDS) - PAGE was carried out as described by Laemmli (1970), using a monomer concentration of 75 g/L. MW markers ranging from 25.0 to 100.0 kDa (AMRESCO LLC, OH, USA) were used. Samples were injected in a concentration zone of 5% polyacrylamide, and the gels were stained with Coomassie brilliant blue.

#### *Determination of isoflavones*

Isoflavones were determined by high performance liquid chromatography (HPLC – Waters, USA). The extraction of isoflavones was performed according to the methodology recommended by Carrão-Panizzi *et al.* (2002). Initially, the samples were defatted with n-hexane under stirring at room temperature for 16 hours. Then, they were filtered under vacuum, with black band filter paper. The samples retained on the paper were dried at room temperature for 4 hours to evaporate the residual hexane. After evaporation of the solvent present in the defatted samples, the isoflavones were extracted with 0.1% acetic acid in 70% ethanol at room temperature. For that, 100 mg of defatted samples were weighed, and transferred to Falcon tubes of 10 mL, where 4 mL of extraction solution were added. The extraction preceded by vortexing the tubes (5 seconds) every 15 minutes for 1 hour. Then, the tubes were transferred to the ultrasonic bath for 30 minutes. After ultra-sonication, an aliquot of the supernatant was transferred to Eppendorf micro tubes, which were centrifuged at 21,000 G and 4°C for 15 minutes. The supernatant was filtered with the aid of a glass syringe coupled to 0.45 µm membrane filter and the filtrate was collected in a microtube for later quantification by HPLC, according to Berhow (2002). The samples were injected in the equipment (Waters, model 2690), with automatic sample injection. An ODS reverse phase C18 column (YMC-Pack ODS-AM Column) with 250 mm length x 0.4 mm internal diameter and 5 µm particles was used for isoflavones separation. A linear binary gradient system was adopted, using the following mobile phases: 1) methanol containing 0.025% trifluoroacetic acid (TFA) (Solvent A), and 2) distilled deionized ultrapure water containing 0.025% TFA (solvent B). The initial gradient was 20% solvent A reaching 100% at 40 minutes, then to 20% at 41 minutes, remaining in this condition until 60 minutes. Therefore, the total running time for each sample was 60 minutes. The mobile phase flow rate was 1 mL / min. and the running temperature was 25°C.

Table 1. Chemical composition (g.100g<sup>-1</sup>) of soybeans from the cultivars and lineage with different seed coat colors

| Genotype             | Conquista <sup>1</sup> | BRSMG 800A <sup>2</sup> | BRSMG 790A <sup>3</sup> | MGBR07-7141 <sup>4</sup> |
|----------------------|------------------------|-------------------------|-------------------------|--------------------------|
| <b>Moisture</b>      | 4.90 <sup>b</sup>      | 7.05 <sup>a</sup>       | 6.95 <sup>a</sup>       | 7.10 <sup>a</sup>        |
| <b>Lipids</b>        | 19.40 <sup>a</sup>     | 19.15 <sup>a</sup>      | 19.50 <sup>a</sup>      | 18.15 <sup>b</sup>       |
| <b>Proteins</b>      | 39.80 <sup>a</sup>     | 38.85 <sup>a</sup>      | 37.05 <sup>b</sup>      | 35.35 <sup>b</sup>       |
| <b>Ash</b>           | 4.05 <sup>a</sup>      | 4.50 <sup>a</sup>       | 4.00 <sup>a</sup>       | 4.25 <sup>a</sup>        |
| <b>Fiber crude</b>   | 6.70 <sup>c</sup>      | 6.95 <sup>c</sup>       | 6.85 <sup>b,c</sup>     | 10.70 <sup>a</sup>       |
| <b>Carbohydrates</b> | 25.15                  | 23.50                   | 25.65                   | 24.45                    |

Means with the same letters in the same column did not differ significantly ( $p < 0.05$ ) in the Tukey's test. <sup>1</sup>Cultivar with dark yellow hilum seed coat, <sup>2</sup>Cultivar with matte brown seed coat, <sup>3</sup>Cultivar with light yellow hilum seed coat, <sup>4</sup>Lineage with glossy black seed coat, <sup>c</sup>Data expressed as whole matter.

For detection of isoflavones, a Waters 996 photodiode array detector (PDA) adjusted to a 260 nm wavelength was used. To identify the peaks corresponding to each isoflavone: daidzin, genistin, glycitin, daidzein, genistein, glycitein, malonyl daidzin, malonyl genistin, malonyl glycitin, acetyl daidzin, acetyl genistin, acetyl glycitin, the 12 isoflavones standards were purchased from Sigma Co and Fuji Co. The standards were solubilized in methanol (HPLC grade) and used at the following concentrations: 0.00625 mg / mL ; 0.0125 mg / mL; 0.0250 mg / mL; 0.0500 mg / mL, and 0.1000 mg / mL. To quantify the 12 forms of isoflavones by external standard (peak area), the standards were used as reference together with the molar extinction coefficient of each isoflavone form.

#### Statistical analysis

The completely randomized design (CRD) was used with 5 replicates for both the chemical composition and determination of isoflavones of soybean cultivars. Tukey's test at 5% significance level was used to identify significant differences between means. Analyses of variance and the mean test were performed according to the standard procedures of the Sisvar software (Ferreira, 2008).

## Results and Discussion

Significant differences were observed between the genotypes with respect to moisture content, lipids, protein and crude fiber (Table 1). The moisture ranged from 4.90 to 7.10 g.100g<sup>-1</sup>, and the cultivar Conquista had the lowest content, below the moisture values found by other authors. Silva *et al.* (2006) investigated the chemical composition of soybeans in the state of Goiás, and found moisture content of 5.60 g.100g<sup>-1</sup>. Ciabotti *et al.* (2006) studied common

soybean (BRS 133) and lipoxygenase-free soybean (BRS 213), and found moisture contents of 9.5 and 7.38 g.100g<sup>-1</sup>, respectively. These differences in moisture content may be due to the conditions of drying the grain after harvest, storage period, ability of the grains to lose moisture, among other factors (Bhardwaj *et al.*, 1999).

During the maturation process, there are changes in moisture content of the grains, which comprise a series of morphological, physiological and functional changes that occur from fertilization, continuing until the moment when the grains reach sufficient moisture for harvest (Henning and França Neto, 1980; Costa, 1995; Santos *et al.*, 2001). With respect to the lipids content, no differences were observed between the cultivars Conquista, BRSMG 800A, and BRSMG 790A. The genotype MGBR07-7141 (black seed coat) presented significantly lower values, 18.15 g.100g<sup>-1</sup>.

Yamada *et al.* (2003) investigated soybeans grown at the Agronomic Institute of Campinas - SP, and found lipids content ranging from 18.45 to 20.07 g.100g<sup>-1</sup>. Lee and Cho (2011) studied five black soybean genotypes and found lipids values ranging from 10.1 to 21.6 g.100g<sup>-1</sup>. High temperatures during seed development are associated with lower lipids contents; however, this effect varies under field conditions depending on other environmental factors such as water stress, which influences the oil production by its effects on growth and grain development (Harris *et al.*, 1978).

The grains from BRSMG 790A and MGBR07-7141 had significantly lower crude protein content, while no significant ( $p < 0.05$ ) difference was observed between the cultivars BRSMG 800A and Conquista, which presented the highest protein contents. Silva *et al.* (2006) studied soybean grains that have yellow seed coat in the state of Goiás for the production of

Table 2. Protein fractions (%) in the soybeans genotypes with different seed coat colors

| Protein fraction | Conquista <sup>1</sup> | BRSMG 800A <sup>2</sup> | BRSMG 790A <sup>3</sup> | MGBR07-7141 <sup>4</sup> |
|------------------|------------------------|-------------------------|-------------------------|--------------------------|
|                  | (% Crude protein)      |                         |                         |                          |
| Albumin          | 22.90                  | 15.70                   | 22.2                    | 31.80                    |
| Globulin         | 46.50                  | 30.20                   | 39.80                   | 41.40                    |
| Glutelin         | 30.40                  | 16.10                   | 38.50                   | 26.00                    |
| Prolamin         | 0.29                   | 0.70                    | 0.20                    | 0.82                     |

<sup>1</sup>Cultivar with dark yellow hilum seed coat, <sup>2</sup>Cultivar with matte brown seed coat, <sup>3</sup>Cultivar with light yellow hilum seed coat, <sup>4</sup>Lineage with glossy black seed coat,

animal feed, and found protein content of 40.4%. According to Lee and Cho (2011), despite the soybean grains with black seed coat are recognized for being an excellent source of high-quality protein, and there are several papers in the literature reporting protein values of 30-40 g.100g<sup>-1</sup> for this grain, there are no conclusive studies relating the color of the seed coat and the protein values of the grain.

Although the protein content in soybeans is genetically defined, environmental factors can lead to changes in the nitrogen availability during grain formation (Hayati *et al.*, 1995). These changes in the nitrogen availability and hence in the protein content may be directly related to the biological nitrogen fixation (Pipolo, 2002). In tropical environments, the biological nitrogen fixation may be affected by high temperatures, water stress, and soil acidity. Pipolo (2002) reported that the rainfall during the grain-filling stage best explains the difference in protein content rather than temperature changes, so this parameter can be linked to water stress. However, these variations may also affect productivity.

Concerning the ash content, there was no significant difference between the genotypes (Table 1). Yamada *et al.* (2003) analyzed soybean grown in São Paulo, and reported ash contents ranging from 4.11 to 5.19 g.100g<sup>-1</sup>, which are close to the values found in this study. Antunes *et al.* (1995) and Ciabotti *et al.* (2006) studied soybean genotypes and obtained values of 4.18 and 3.64 g.100g<sup>-1</sup>, respectively. The black coat MGBR07-7141 genotype had higher fiber content, when compared to the other samples. Probably, the amount of crude fiber found in the grains of this genotype is related to the bark, which is thicker than the other genotypes analyzed. Ciabotti *et al.* (2006) found fiber content of 7.56 g.100g<sup>-1</sup> for conventional soybeans, and 7.09 in g.100g<sup>-1</sup> for lipoxygenase-free soybeans. Vieira *et al.* (1999) analyzed six different soybean cultivars grown in the southern and southeastern regions, and found fiber values ranging from 5.24 to 6.38 g.100g<sup>-1</sup>.

The total carbohydrates content in soybeans is approximately 34 g.100g<sup>-1</sup> (Bordingnon and Mandarino, 1994). Silva *et al.* (2006) studied grains and residues in the Brazilian center-west, and found 17.26% carbohydrates, which is lower than the values observed in this study. Vieira *et al.* (1999) evaluated six cultivars grown in Campinas-SP, and found values ranging from 29.81% to 33.33% carbohydrates, higher than those found in this study, which ranged from 23.5 to 25.65 g .100g<sup>-1</sup>.

#### Protein fractionation

A wide variation was observed among the protein fractions of the genotypes (Table 2). In relation to albumin, MGBR07-7141 genotype showed the highest percentage (31.80%), while the cultivar BRSMG 800A showed the lowest percentage (15.70%). The albumin fraction of leguminous plants corresponds to 8-20% (Neves *et al.*, 2006), but the genotype MGBR07-7141 (black coat) showed higher value than the witness cultivar (Conquista). With respect to the globulin fraction, the cultivar Conquista presented the highest values (46.50%), while the cultivar BRSMG 800A exhibited the lowest values (30.20%). According to Silva *et al.* (2006), globulin corresponds to 40-60% of the protein fraction, but the globulin in the BRSMG 800A genotype of this study was below the witness value (cultivar Conquista). The glutelin fraction varied from 16.1% for the cultivar BRSMG 800A to 38.5% for the cultivar BRSMG 790A. It has been known for some time that glutelin is an important fraction on cereal grains, and comprises 85% of wheat proteins (Orth and Bushuk, 1972), which confers a viscoelastic property to the wheat gluten. For this reason, soybean flour cannot be used to replace no more than 30% of the wheat flour. Once soybeans is a leguminous plant (Osborne, 1924), higher levels of albumin and globulin fractions are expected, and the prolamin and glutelin fractions are more representative in monocots. Regarding the prolamin fraction, the genotypes presented less than

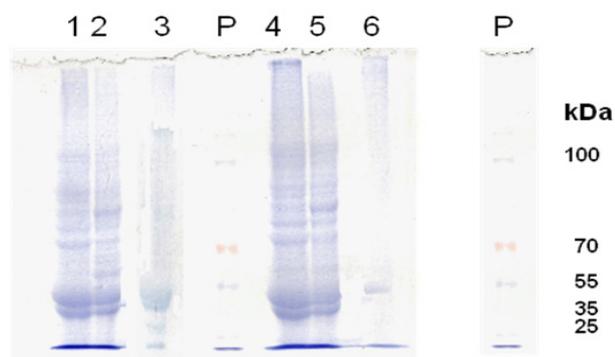


Figure 1. Electrophoretic profile in 7.5% polyacrylamide gel of the protein fractions of the cultivar BRSMG 800A (1-3) and genotype MGBR07-7141 (4-6). P = molecular weight standards; 1 and 4 = salt soluble fractions; 2 and 5 = albumins; 3 and 6 = globulins

1%. According to Sgarbieri (2000), this fraction has not been isolated and characterized in soybeans.

#### Electrophoretic profile of protein fractions

For all the genotypes analyzed, the albumin fraction showed a great variety of proteins, in particular having molecular weights in the range of 100 to 25 kDa, with profile very similar to the salt-soluble fraction (Figures 1 and 2). The globulin fraction of Conquista showed a small band in the range of 25-35 kDa that was not noticed in the cultivar BRSMG 790A. This small band observed in Conquista is very similar to that observed for MGBR07-7141 genotype, which also showed a single protein band of higher concentration, but in the range of 35 kDa. In fact, all globulins were identified in the range from 35 and 55 kDa, with minor variations. In general, the changes in protein fractions of the genotypes did not reflected on the protein profiles of the genotypes studied, since their general characteristics remained very similar. The globulins appeared in the range of 45 kDa, but the denaturing conditions using mercaptoethanol could generate subunits in the range of 20 to 35 kDa, as described by Sadeghi *et al.*, (2006).

Albumins were found to be more complex than the globulin fraction, once they have a greater number of bands and higher variation in the molecular weight of their protein fractions (Figures 1 and 2). Gorinstein *et al.* (2001) studied soybean albumin, and found a higher concentration in the range from 35 to 80 kDa. Once the glutelin fraction consists of a large protein complex, it presented very dense profile, without allowing differentiation of bands (data not shown) and therefore they were excluded from the combined electrophoresis runs.

#### Determination of isoflavones

Table 3 shows the mean isoflavones content

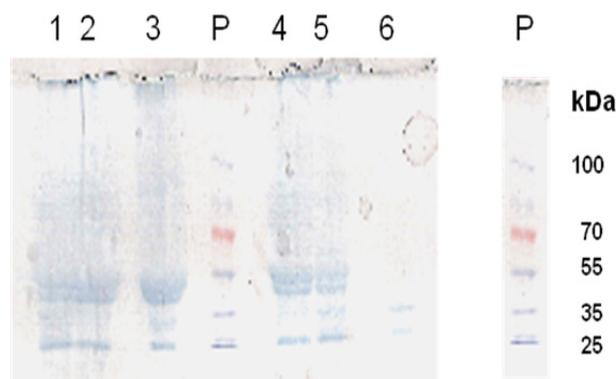


Figure 2. Electrophoretic profile in 7.5% polyacrylamide gel of the protein fractions of the cultivar Conquista (1-3) and BRSMG 790A (4-6). P = molecular weight standards; 1 and 4 = salt soluble fractions; 2 and 5 = albumins; 3 and 6 = globulins

of the genotypes studied. In general, the cultivar BRSMG 790A with yellow seed coat showed the highest isoflavones content (415.98 mg. 100g<sup>-1</sup>), while the cultivar Conquista presented the lowest value (220.88 mg.100 g<sup>-1</sup>). Although the cultivar with brown seed coat (BRSMG 800A) and the lineage with black seed coat (MGBR 07-7141) presented similar isoflavones content (271.30 and 297.77 mg.100g<sup>-1</sup>). All genotypes showed significantly different levels of isoflavone glycosides (glycoside and malonyl), highlighting the malonyl genistin in the cultivar BRSMG 790A (234.06 mg.100g<sup>-1</sup>). The malonyl glycosylated forms are commonly found in larger amounts in soybeans and defatted soybean flour (Kudou *et al.*, 1991), while the unconjugated forms (daidzein, genistein, and glycitein) are found in processed foods, justifying the low aglycone values.

It has been observed that the isoflavone aglycones are absent or in very low levels in soybeans not subjected to storage (Carrão-Panizzi *et al.*, 2004). It is presumed, therefore, that if damage in soybeans with consequent increase in moisture content occurs, so there will be favorable conditions to formation of aglycones by enzymatic action. However, in the grains in natura, the aglycones are always present in small quantities (Silva *et al.*, 2012). Several factors can alter the concentration of isoflavones. Carrão-Panizzi *et al.* (1999) reported that the variability of these concentrations is attributed, besides the genotype, to the effect of various planting places, crops, regional temperature variations, latitude, and altitude.

Park *et al.* (2012) evaluated the total isoflavones in some Brazilian soybean cultivars and found values ranging from 140.5 to 300.8 mg.100g<sup>-1</sup> in non-heat treated soybeans. Silva *et al.* (2012) investigated soybeans from different cultivars and reported values ranging from 165.26 to 336.66 mg.100g<sup>-1</sup>.

Table 3. Concentration of isoflavones (mg.100 g<sup>-1</sup>) in the soybean genotypes with different seed coat colors

| Isoflavones | Concentration of isoflavones (mg. 100 g <sup>-1</sup> ) |                           |                           |                           |                     |
|-------------|---------------------------------------------------------|---------------------------|---------------------------|---------------------------|---------------------|
|             | Conquista <sup>1</sup>                                  | BRSMG 800A <sup>2</sup>   | BRSMG 790A <sup>3</sup>   | MGBR 07-7141 <sup>4</sup> |                     |
| β-glucoside | Daidzin                                                 | 40.88 <sup>c</sup>        | 48.20 <sup>b</sup>        | 91.23 <sup>a</sup>        | 48.34 <sup>b</sup>  |
|             | Glycitin                                                | 04.57 <sup>b</sup>        | 03.65 <sup>d</sup>        | 04.15 <sup>c</sup>        | 05.23 <sup>a</sup>  |
|             | Genistin                                                | 36.27 <sup>d</sup>        | 51.88 <sup>b</sup>        | 65.11 <sup>a</sup>        | 46.80 <sup>c</sup>  |
| Subtotal    | <b>81.72</b>                                            | <b>103.73</b>             | <b>160.49</b>             | <b>100.37</b>             |                     |
| Malonyl     | Daidzin                                                 | 40.18 <sup>c</sup>        | 41.24 <sup>c</sup>        | 87.51 <sup>a</sup>        | 56.02 <sup>b</sup>  |
|             | Glycitin                                                | 08.55 <sup>b</sup>        | 05.69 <sup>c</sup>        | 07.98 <sup>b</sup>        | 09.32 <sup>a</sup>  |
|             | Genistin                                                | 74.98 <sup>d</sup>        | 95.23 <sup>b</sup>        | 138.57 <sup>a</sup>       | 116.29 <sup>c</sup> |
| Subtotal    | <b>123.71</b>                                           | <b>142.16</b>             | <b>234.06</b>             | <b>181.63</b>             |                     |
| Aglycones   | Daidzein                                                | 04.46 <sup>b</sup>        | 06.14 <sup>a</sup>        | 04.59 <sup>b</sup>        | 03.55 <sup>c</sup>  |
|             | Glycitein                                               | 07.65 <sup>c</sup>        | 14.76 <sup>a</sup>        | 14.34 <sup>a</sup>        | 09.57 <sup>b</sup>  |
|             | Genistein                                               | 03.34 <sup>b</sup>        | 04.44 <sup>a</sup>        | 02.47 <sup>d</sup>        | 02.62 <sup>c</sup>  |
| Subtotal    | <b>15.45</b>                                            | <b>25.34</b>              | <b>21.4</b>               | <b>15.74</b>              |                     |
| Total       | <b>220.88<sup>d</sup></b>                               | <b>271.30<sup>c</sup></b> | <b>415.98<sup>a</sup></b> | <b>297.77<sup>b</sup></b> |                     |

Means with the same letters in the same column did not differ significantly ( $p < 0.05$ ) by the Tukey's test. <sup>1</sup>Cultivar with dark yellow hilum seed coat, <sup>2</sup>Cultivar with matte brown seed coat, <sup>3</sup>Cultivar with light yellow hilum seed coat, <sup>4</sup>Lineage with glossy black seed coat,

The choice of raw materials is an important factor, especially when there is genetic variability among soybean cultivars with respect to the isoflavones levels (Carrão-Panizzi *et al.*, 2009a). It is worth noting that the soybean isoflavones levels may increase depending on the place of cultivation, since this increase is favored by low temperatures during the grain-filling stage (Carrão-Panizzi *et al.*, 1999).

## Conclusions

Although the soybeans with black seed coat exhibited lower lipids and protein levels, higher concentration of crude fiber was observed. The chemical composition of the genotypes was within the range found in other studies on soybeans. All three cultivars and the lineage evaluated can be considered as high quality raw material for the product manufacture and human consumption. Despite all grains have shown similar protein contents, slight variations were observed for the protein fractions. The major differences were observed for the cultivar BRSMG 800A when compared to the cultivar Conquista, which was considered the reference sample in this study, and presented the lowest values of glutelins and albumins. With respect to the proteins separated by electrophoresis and their different molecular weights, the differences in the genotypes did not affect the protein profiles, once their overall characteristics were very similar. The cultivar

BRSMG 790A exhibited the highest isoflavones content as compared to all genotypes studied, thus it may be a source of isoflavones in the human diet.

## Acknowledgements

The authors thank the Foundation for Research Support of the State of Minas Gerais - FAPEMIG for the scholarship for the visiting researcher.

## References

- AOAC (Association of official analytical chemists) 1990. Official methods of analysis of the Association of Official Analytical Chemists. Arlington, USA.
- ANVISA (Agência Nacional de Vigilância Sanitária) 2013. Alegação de propriedade funcional. Downloaded from <http://portal/Anvisa/Inicio/Alimentos/Assuntos+de+Interesse/Alimentos+Com+Alegacoes+de+Propriedades+Funcionais+e+ou+de+Saude/Alegacoes+de+propriedade+funcional+aprovadas.html> on 04/12/2013.
- Astadi, I.R., Asturi, M., Santoso, U. and Nugraheni, P.S. 2009. *In vitro* antioxidant activity of anthocyanins of black soybean seed coat in human low density lipoprotein (LDL). Food Chemistry 112: 659-663.
- Berhow, M. A. 2002. Modern analytical techniques for flavonoid determination. In: BUSLIG, B. S., MANTHEY, J. A. (Ed.). Flavonoids in the living cell p. 61-76. New York: Kluwer Academic.
- Bhardwaj, H. L., Bhagsari, A. S., Joshi, J. M., Rangappa, M., Sapra, V. T. and Rao, M. S. S. 1999. Yield and quality of soymilk and tofu made from soybean

- genotypes grown at four locations. *Crop Science* 39(2): 401-405.
- Carrão-Panizzi, M. C., Beléia, A. D. P., Kitamura, K. and Oliveira, M. C. 1999. N. Effects of genetics and environment on isoflavone content fo soybean from different regions of Brazil. *Pesquisa Agropecuária Brasileira* 34(10): 1788-1795.
- Carrão-Panizzi, M. C., Berhow, M., Mandarino, J. M. G. and Oliveira, M. C. N. 2009a. Enviromental and genetic variation of isoflavones content of soybean seeds grown in Brazil. *Pesquisa Agropecuária Brasileira* 44(11): 1444-1451.
- Carrão-Panizzi, M.C., Favoni, S.P.G. and Kikuchi, A. 2002. Extraction time for isoflavone determination. *Brazilian Archives of Biology and Technology* 45(4): 515-518.
- Carrão-Panizzi, M. C., Goés-Favoni, S. P. and Kikuchi, A. 2004. Hydrothermal treatments in the development of isoflavone aglycones in soybean (*Glycine max* (L.) Merrill) grains. *Brazilian Archives of Biology and Technology* 47(2): 225-232.
- Ciabotti, S. Barcelos, M. F. P., Mandarino, J. M. G. and Tarone, A. G. 2006. Avaliações químicas e bioquímicas dos grãos, extratos e tofus de soja comum e de soja livre de lipoxigenase. *Ciência Agrotecnologia* 30(5): 920-929.
- Costa, N.P. and Tavares, L.C.V. 1995. Fatores responsáveis pelos elevados percentuais de perdas de grãos durante a colheita mecânica em soja. *Informativo ABRATES* 5(1): 17-25.
- Drzewiecki, J., Delgado, L. E., Haruenkit R, Pawelzik E, Martin B. O., Park Y. S, Jung S.T, Trakhtenberg S. and Goristein S. 2003. Identification and differences of total proteins an their soluble fractions in some pseudocereals based on electrophoretic patterns. *Journal of Agricultural Food Chemistry* 51: 7798-7804.
- EMBRAPA SOJA - Empresa Brasileira DE Pesquisa Agropecuária. Pesquisa desenvolve soja marrom. 2010. Downloaded from <http://www.embrapa.br/imprensa/noticias/2011/abril/4asemana/pesquisa-desenvolve-soja-marrom/> html on 8/8/2013.
- Ferreira, D. F. 2008. SISVAR: um programa para análises e ensino de estatística. *Revista Symposium* 6: 36-41.
- Goristein, S., Delgado-Licon, E., Pawelzik, E., Permady, H.H., Weisz, M. and Trakhtenberg, S. 2001. Characterization of soluble amaranth ans soybean proteins based on fluorescence, hudrophobicity, eletrophoresis, amino acid analysis, circular dichroism, and differential scanning calorimetry measurements. *Journal of Agricultural Food Chemistry* 49: 5595-5601.
- Harris, H. C., Mc William, J.R. and Mason, W.K. 1978. Influence of Temperature on Oil Content and Composition of Sunflower Seed. *Australian Journal of Agricultural Research* 29(3): 1203-1212.
- Hayati, R., Egli, D.B. and Crafts-Brandner, S.J. 1995. Carbon and nitrogen supply during seed filling and leaf senescence in soybean. *Crop science* 35: 1063-1069.
- Henning, A. A. and França Neto, J. B. 1980. Problemas na avaliação da germinação de semente de soja com alta incidência de *Phomopsis* sp. *Revista Brasileira de Sementes* 2(3): 9-22.
- IAL. Instituto Adolfo Lutz. Normas Analíticas do Instituto Adolfo Lutz. Métodos físico-químicos para análise de alimentos. 2005. 4.ed. 1018p. Brasília: IAL.
- Kudou, S., Fleury, Y., Welti, D., Magnolato, D., Uchida, T., Kitamura, K. and Okubo, K. 1991. Malonyl isoflavone glycosides in soybean seeds (*Glycine max* Merrill). *Agricultural and Biological Chemistry* 55(9): 2227-2233.
- Lee, I. H. and Chou, C. C. 2011. Distribution Profiles of Isoflavon Isomers in Black Bean Kojis Prepared with Various Filamentous Fungi. *Journal of Agricultural Food Chemistry* 54: 1309-1314.
- Messina, M., Gardner, C., and Barnes, S. 2002. Gaining insight into the health effects of soy but a long way still to go: Commentary on the Fourth International Symposium on the Role of Soy in Preventing and Treating Chronic Disease. *Journal of Nutrition* 132(3): 547- 551.
- Neves, V. A., Lourenço, E. J, and Silva, M. A. 1998. Isolation and *in vitro* hydrolysis of lentil protein fractions by trypsin. *Archivos Latinoamericano Nutrition* 46(3): 238-242.
- Neves, V. A., Silva, S. I. and SILVA, M. A. 2006. Isolamento da globulina majoritária, digestibilidade *in vivo* e *in vitro* das proteínas do tremoço-doce (*Lupinus albus* L.), var. multolupa. *Ciência e Tecnologia de Alimentos* 26(4): 832-840.
- Orth, R. A. and Bushuk, W. 1972. A comparative studies of the proteins of weats of diversers baking quality. *Cereal Chemistry* 49: 268-275.
- Osborne, T.B. 1924. *The vegetable proteins*. 2.ed. 154p. London: Longmans Green and Company.
- Park, Y. K., Aguiar, C. L., Alencar, S. M., Mascarenhas, H. A. A. and Scamparini, A. R. P. 2012. Conversão de malonil-β-glicosil isoflavonas em isoflavonas glicosiladas presentes em alguns cultivares de soja brasileira. *Ciência Tecnologia Alimentos*, 22 (2): 130-135.
- Penha, L. A. O., Fonseca I. C. B., Mandarino, J. M. and Benasse, V. T. 2007. A soja como alimento: valor nutricional, benefícios para a saúde e cultivo orgânico. *Boletim do Centro de Pesquisa e Processamento de Alimentos* 25(1): 91-102.
- Pipolo, A. E. 2002. Influência da temperatura sobre as concentrações de proteína e óleo em sementes de soja *Glycine max* (L.) Merrill). Piracicaba, São Paulo - Brasil. Universidade de São Paulo, Dsc Thesis.
- Sadeghi, A. A. and Shawrang. P. 2006. Effects of microwave irradiation on ruminal degradability and *in vitro* digestibility of canola meal. *Animal Feed Science and Technology* 127(1): 45-54.
- Santos, I.C., Silva, A. A., Ferreira, F. A., Miranda, G. V. and Pinheiro, R. A. N. 2001. Eficiência do herbicidaglyphosate no controle de *Commelina benghalensis* e *Commelina diffusa*. *Planta Daninha*, 19(1): 135-143.

- Sathe, S. K. and Salunkhe, D. K. 1981. Solubilization and electrophoretic characterization of the Great Northern Bean (*Phaseolus vulgaris* L.) proteins. *Journal Food Science* 46: 82-89.
- Sgarbieri, V. C. 1980. Estudo do conteúdo e de algumas características das proteínas em sementes de plantas da família Leguminosa. *Ciência e Cultura* 32: 78-84.
- Silva, M. S., Naves, M. M. V., Oliveira, R.B. and Leite, O. S. M. 2006. Composição química e valor protéico do resíduo de soja em relação ao grão de soja. *Ciência e Tecnologia de Alimentos* 26 (3): 571-576.
- Silva, M. A. R. E., Hanada, H. S. A. SILVA; R. D., LIMA, R.D. and VIEIRA, R. F. 1997. Reprodução de *Heterodera glycines* em algumas leguminosas. *Fitopatologia brasileira* 22: 324.
- Silva, C.E., Carrão-Panizzi, M. C., Mandarino, J. M. G., Leite, R. S. and Mônico, A. P. A. 2012. Teores de isoflavonas em grãos inteiros e nos componentes dos grãos de diferentes cultivares de soja (*Glycine max* (L.) Merrill). *Brazilian Journal of food technology* 15(2): 150-156.
- Vieira, C. R., Cabral, L. C. and Paula, A. C. O. 1999. Composição centesimal e conteúdo de aminoácidos, ácidos graxos e minerais de seis cultivares de soja destinada à alimentação humana. *Pesquisa Agropecuária Brasileira* 34(7): 1277-1283.
- Wang, Y. I., Sheen, L. Y., CHOU, C. C. 2010. Storage effects on the content of anthocyanin, mutagenicity, and antimutagenicity of black soybean koji. *LWT Food Science and Technology* 43: 702-707.
- Yamada, L.T., Barcelos, M.F.P., Sousa, R.V. and Lima, A.L. 2003. Composição química e conteúdo de ferro solúvel em soja [*Glycine max* (L.) Merrill]. *Ciência e Agrotecnologia* 27: 406-413.