


ORIGINAL ARTICLE

# Chemical composition and lipoxygenase activity of soybean (*Glycine max* L. Merrill.) genotypes, specific for human consumption, with different tegument colours

*Composição química e atividade de lipoxigenase em genótipos de soja (Glycine max L. Merrill.), específicos para alimentação humana, com diferentes colorações de tegumento*

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**Cite as:** Ciabotti S., Juhász A. C. P., Mandarino, J. M. G., Costa, L. L., Corrêa, A. D., Simão, A. A., & Santos, E. N. F. (2019). Chemical composition and lipoxygenase activity of soybean (*Glycine max* L. Merrill.) genotypes, specific for human consumption, with different tegument colours. *Brazilian Journal of Food Technology*, 22, e2018003. <https://doi.org/10.1590/1981-6723.00318>

## Abstract

Recently, in Brazil, coloured-tegument soybean cultivars have been developed, such as those with brown and black teguments. Soybeans with black teguments have been widely used for decades due to their health benefits and their use in oriental folk medicine as a result of the presence of phytochemicals. They have been recognized as health-promoting functional food ingredients due to their antioxidant activity, and are also known to have anti-cancer, hypoglycaemic and anti-inflammatory effects and have been used in the treatment of various circulatory disorders. This study aimed to determine the proximate composition, fatty acid levels and lipoxygenase activity of soybean lineages with different tegument colours intended for human consumption. The lineage MGBR10-16601 which has a yellow tegument, presented the highest protein and lowest fat contents, with values of 37.6 g 100 g<sup>-1</sup> and 18.3 g 100 g<sup>-1</sup>, respectively. The lineage MGBR10-16201, which also has a yellow tegument, was identified as free of lipoxygenase isoenzymes. The unsaturated fatty acid levels ranged from 18.48 to 31.37 mg g<sup>-1</sup> and from 47.36 to 58.31 mg g<sup>-1</sup> for oleic and linoleic acids, respectively. The lineage BRN07-50543, which has a black tegument, presented high total isoflavone levels (546 mg 100 g<sup>-1</sup>), with an oleic acid level above and linoleic acid level below the standards established by the Codex Alimentarius for soybean oil, with values of 31.37 mg g<sup>-1</sup> and 47.36 mg g<sup>-1</sup>, respectively. The cultivar BRSMG 790A, which has a yellow tegument, presented the lowest isoflavone



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level (171.4 mg 100 g<sup>-1</sup>). All the genetic materials examined presented crude protein, fat, dietary fibre and ash levels within the commercial parameters established for soybeans.

**Keywords:** Black tegument; Brown tegument; Yellow tegument; Dietary fibre; Fatty acids; Isoflavones.

## Resumo

Recentemente, no Brasil, cultivares de tegumento colorido estão sendo desenvolvidas, como as de tegumentos marrom e preto. A soja com tegumento preto tem sido amplamente utilizada há décadas, devido aos seus benefícios para a saúde e ao uso na medicina popular oriental em razão da presença de fitoquímicos. Estes foram reconhecidos como ingredientes alimentares funcionais promotores da saúde, devido à sua atividade antioxidante, sendo também, conhecidos por terem efeitos anticancerígenos, hipoglicêmicos e anti-inflamatórios, e ainda têm sido usados no tratamento de vários distúrbios circulatórios. Este estudo teve como objetivo determinar a composição centesimal, os níveis de ácidos graxos e a atividade de lipoxygenase de linhagens de soja específicas para a alimentação humana, com diferentes colorações de tegumento. A linhagem MGBR10-16601, de tegumento amarelo, apresentou o teor mais elevado de proteína (37,6 g 100 g<sup>-1</sup>) e menor teor de gorduras (18,3 g 100 g<sup>-1</sup>). A linhagem MGBR10-1620, de tegumento amarelo, foi identificada como livre das isoenzimas de lipoxygenase. Os teores de ácidos graxos insaturados variaram de 18,48 a 31,37 mg g<sup>-1</sup> para o ácido oleico e de 47,36 a 58,31 mg g<sup>-1</sup> para o ácido linoleico. A linhagem de tegumento preto, BRN07-50543, apresentou teores de ácidos oleico (31,37 mg g<sup>-1</sup>) e linoleico (47,36 mg g<sup>-1</sup>) inferiores aos padrões definidos pelo Codex Alimentarius para o óleo de soja, e elevado teor de isoflavonas totais (546 mg 100 g<sup>-1</sup>). A cultivar BRSMG 790A, de tegumento amarelo, apresentou o menor nível de isoflavonas (171,4 mg 100 g<sup>-1</sup>). Todos os materiais genéticos avaliados estão dentro dos parâmetros de soja comercial para os níveis de proteína bruta, gorduras, fibras alimentares e cinzas.

**Palavras-chave:** Tegumento preto; Tegumento marrom; Tegumento amarelo; Fibra dietética; Ácidos graxos; Isoflavonas.

## 1 Introduction

The use of soybeans for human consumption has stood out in recent years, mainly because they are recognized as being functional food, despite not being regularly consumed by Brazilians. The inclusion of soybeans in the daily diet has gained importance due to their high protein content and the presence of phenolic compounds such as isoflavones, which exert antioxidant activities, with potential roles in cancer prevention, heart disease, osteoporosis and a reduction in the menopause symptoms (Levis et al., 2010; Taku et al., 2011; Slavin et al., 2013; He & Chen, 2013; Bolca, 2014). According to the functional claims for soybeans established by the National Agency for Sanitary Vigilance in Brazil, 25 g of soy protein per day is the threshold intake required for cholesterol reduction, and its consumption should be associated with a balanced diet and healthy eating habits (Brasil, 1999).

Although soybeans are rich in fibre, consumers are not aware of the soybean health claims, only that they contain oil, and triacylglycerols are the main component of this fraction. Soybean oil is characterized by relatively high amounts of polyunsaturated fatty acids and these exert important nutritional and physiological functions. However, due to the presence of lipoxygenases in soybeans, the substrates of these enzymes, linoleic and  $\alpha$ -linolenic acids, can make soybean oil prone to rancidity (Dixit et al., 2011). Thus, it is important to evaluate the fatty acid profile for the genetic improvement of soybeans, not only for the genotypes intended for human consumption but also for other purposes.

Several authors have already investigated the characteristics of soybean oil with the objective of mapping the properties of this raw material for various applications (Froehner et al., 2007; Suarez et al., 2007; Brock et al., 2008; Borsato et al., 2010; Luz et al., 2011). However, there is still little information on its physicochemical properties, focusing on the quality of this raw material for use in various products in the chemical industry and in biodiesel.

The benefits of soybeans for human consumption have been demonstrated, but many consumers avoid its dietary use mainly due to the presence of off-odours and off-flavours generated by the action of the enzyme lipoxygenase (Santos et al., 2017), evidence of improper processing or the use of cultivars with unsuitable characteristics for human consumption. Research has confirmed that the chemical composition of soybeans can be influenced by several factors including the cultivar, growing conditions, genetic improvement and processing technology (Bhardwaj et al., 1999).

Commercial soybeans for use in the oil industry have yellow teguments. Recently, in Brazil, coloured-tegument cultivars have been developed, such as the lineage BRSMG 800A, which has a brown tegument and BRSMG 715A, which has a black tegument. The colours of these lineages are due to the presence of anthocyanins, chlorophyll and a combination of many other pigments. Soybeans with black teguments have been widely used for decades due to both their health benefits and their use in Oriental folk medicine due to the presence of phytochemicals including isoflavones, flavonoids, flavones, anthocyanin and saponin (Lee & Cho, 2012). In the soybean genetic improvement program for human consumption, the evaluation of the chemical composition, such as the protein content, fatty acid profile, isoflavone content and lipoxygenase activity, can contribute to the selection of special new lineages for human consumption, in line with the demands of the food industry for healthy products. Considering the above, this study aimed to determine the proximate composition, fatty acid levels and the lipoxygenase activity of soybean lineages with different tegument colours intended for human consumption.

## 2 Material and methods

This study was carried out in the Food Analysis Laboratory of the Federal Institute of Education, Science and Technology of the *Triangulo Mineiro* (IFTM) – Uberaba Campus - MG, the Department of Biochemistry and Biophysics of the Federal University of the *Triangulo Mineiro* (UFTM), Uberaba - MG, and the Laboratory of Physicochemical and Chromatographic Analyses of Embrapa Soja, Londrina - PR. Soybean grains [*Glycine max* (L.) Merrill] with different coloured teguments from four cultivars were studied, including the lipoxygenase free cultivar BRS 213 and six genotypes intended for human consumption, developed in the genetic breeding program of the Embrapa/Epamig/Triangulo Foundation partnership (Table 1).

**Table 1.** Soybean cultivars and lineages from the genetic breeding program of the Embrapa/Epamig/Triângulo Foundation partnership. 2012/2013 crop year, Uberaba-MG.

	Genotypes	Characteristics
Cultivars	Conquista	Yellow tegument and black hilum. Used in the food industry.
	BRS 213	
	BRSMG 790A	Special for human consumption, Yellow tegument.
	BRSMG 800A	
Lineages	MGBR10-16601	Special for human consumption, Yellow tegument and hilum
	MGBR10-16301	
	MGBR10-16201	
	MGBR07-7043	Special for human consumption, brown tegument
	MGBR09-9161	Special for human consumption, black tegument and large grain
	BRN07-50543	Special for human consumption, black tegument and small grain

The grains were ground in a mill, and characterized for their moisture, lipid, crude protein, dietary fibre, ash and carbohydrate contents, according to the following AOAC methodologies (AOAC, 2000): Moisture - gravimetric method, based on the weight loss of the material subjected to heating at 105 °C, to constant weight; Crude protein - Kjeldahl method to determine the nitrogen content of the food, using a conversion factor of 6.25; Fibre, determining the total dietary fibre, soluble dietary fibre and insoluble dietary fibre using the total dietary fibre kit/Sigma; Ash - the mineral residue was determined by incineration of the sample in a

muffle furnace at 550 °C, and the carbohydrates were calculated by difference [100 - (sum of moisture, lipid, crude protein, total fibre and ash)].

The Lipoxygenase I and lipoxygenase II + III activities were determined using a spectrophotometric method. Raw soybeans were ground and defatted with hexane. The samples were sieved (150 mesh) and 1 gram homogenized with 100 mL phosphate buffer (0.2 mol L<sup>-1</sup>, pH 6.8) for 20 minutes at 0 to 4 °C. The homogenized solution was centrifuged at 1050 rpm for 10 minutes at 4 °C (Kumar et al., 2003) and the supernatant used as the crude extract to determine the isoenzyme activity, according to the standard method reported by Axelrod et al. (1981). The reaction mixture for lipoxygenase I consisted of the crude extract as the enzyme source (25 µL), boric acid-borax buffer (0.2 mol L<sup>-1</sup>, pH 9.0) and 10 mol L<sup>-1</sup> sodium linoleate as the substrate. Absorbance readings were taken every minute at 234 nm. The reaction mixture for lipoxygenase II and III consisted of the crude extract as the enzyme source (50 µL), phosphate buffer (0.2 mol L<sup>-1</sup>, pH 6.8) and 10 mmol L<sup>-1</sup> sodium linoleate as the substrate, followed by absorbance readings at 234 nm. One unit of enzyme was equivalent to the amount of enzyme that generated an increase in absorbance of 1.0 per minute.

The fatty acids were analysed by gas chromatography (GC) as described by Abidi et al. (1999), Bannon et al. (1982), Christie (1989) and Rayford et al. (1994). The chromatographic run was carried out in a gas chromatograph with an auto-injector (Supelco, SP 2340), using isothermal chromatography at 170 °C from zero to 10 min, followed by an increase to 220 °C with increments of 3 °C min<sup>-1</sup> and a final increase to 250 °C (end of the run), returning to 170 °C after 3 min (thermal balance). A flame ionization detector (FID) was used at 300 °C and the injector temperature was set at 250 °C. The gas flow rate was set at 40 mL min<sup>-1</sup> of nitrogen, 40 mL min<sup>-1</sup> of hydrogen and 450 mL min<sup>-1</sup> of synthetic air. The total run time was 30 min for each sample. The fatty acids were identified by comparison of the peaks with those of the fatty acid standards analysed under the same conditions.

The isoflavones were determined by high-performance liquid chromatography (HPLC – Waters, USA) after extraction according to the methodology recommended by Carrão-Panizzi et al. (2002). The samples were first defatted with n-hexane by stirring at room temperature for 16 h and then filtered under vacuum with black band filter paper. The material retained on the paper was dried at room temperature for 4 h to evaporate the residual hexane. After evaporation of the solvent, the isoflavones were extracted with 0.1% acetic acid in 70% ethanol at room temperature. For this procedure, 100 mg of defatted samples were weighed and transferred to 10 mL Falcon tubes, and 4 mL of extraction solution added. The mixture in the tubes was vortexed for 5 seconds every 15 min for 1 h and the tubes then placed in an ultrasonic bath for 30 min. After ultrasonication, an aliquot of the supernatant was transferred to an *Eppendorf* tube and centrifuged at 21,000 x g at 4 °C for 15 min. The supernatant was filtered with the aid of a glass syringe coupled to a 0.45 µm membrane filter, and the filtrate collected in a microtube for later quantification by HPLC according to Berhow (2002). The samples were injected into the equipment (Waters, model 2690) by automatic sample injection. An ODS reverse phase C18 column (YMC-Pack ODS-AM Column) with a length of 250 mm x internal diameter of 0.4 mm and 5 µm particles was used to separate the isoflavones. 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% after 40 min, then 20% at 41 min, remaining in this condition up to 60 min. The total run time for each sample was 60 min. The mobile phase flow rate was 1 mL min<sup>-1</sup> and the running temperature was 25 °C. A Waters 996 photodiode array detector (PDA) adjusted to a wavelength of 260 nm was used to detect the isoflavones. Twelve isoflavone standards purchased from Sigma Co and Fuji Co were used to identify the peaks, corresponding to the isoflavones daidzin, genistin, glycitin, daidzein, genistein, glycitein, malonyl daidzin, malonyl genistin, malonyl glycitin, acetyl daidzin, acetyl genistin and acetyl glycitin. The standards were dissolved in methanol (HPLC grade) and used in the

following concentrations: 0.00625 mg mL<sup>-1</sup>; 0.0125 mg mL<sup>-1</sup>; 0.0250 mg mL<sup>-1</sup>; 0.0500 mg mL<sup>-1</sup>; and 0.1000 mg mL<sup>-1</sup>. To quantify the 12 isoflavone forms by external standardization (peak area), the standards were used as references together with the molar extinction coefficient of each isoflavone form.

A completely randomized design was used with three replications. The Scott-Knott test was used to analyse the significant differences at 5% probability. The analysis of variance and the means comparison test were carried out using the SISVAR software (Ferreira, 2014).

### 3 Results and discussion

Significant differences were observed in the proximate composition, except for the insoluble fibre content (Table 2). The moisture content of the grains ranged from 7.46 to 8.40 g 100 g<sup>-1</sup>. Ciabotti et al. (2016) found moisture contents of from 4.9 to 7.1 g 100 g<sup>-1</sup> in genotypes with different seed coat colours, while Esteves et al. (2010) reported moisture levels ranging from 8.09 to 8.13 g 100 g<sup>-1</sup> in lipoxygenase-free and conventional soybeans. The moisture contents of grains are variable since they depend on the drying and storage conditions and the moisture losses of the grains. The lipid levels ranged from 18.2 to 21.4 g 100 g<sup>-1</sup>. Higher protein and lower lipid levels were observed for the lineage MGBR10-16601, with values of 37.6 g 100 g<sup>-1</sup> and 18.3 g 100 g<sup>-1</sup>, respectively, which is desirable in soybeans intended for human consumption. The lineage MGBR10-16601 with a black tegument and the cultivar Conquista with a yellow tegument had significantly lower protein contents when compared to the other samples. Lee & Cho (2012) studied five soybean cultivars with black teguments during two years of storage and obtained higher protein levels, which varied from 33.9 to 44.3 g 100 g<sup>-1</sup>, while the fat levels ranged from 10.0 to 21.6 g 100 g<sup>-1</sup>.

The lineage MGBR10-16201 presented the highest total fibre content of 23.8 g 100 g<sup>-1</sup> while the cultivar BRSMG 800A presented 19.2 g 100 g<sup>-1</sup>. The cultivar BRSMG 790A presented the highest soluble fibre content of 2.26 g 100 g<sup>-1</sup>, and the insoluble fibre contents ranged from 17.7 to 22.5 g 100 g<sup>-1</sup>. Toledo et al. (2007) found soluble fibre contents ranging from 1.37 to 3.81 g 100 g<sup>-1</sup> and insoluble fibre contents ranging from 16 to 18.87 g 100 g<sup>-1</sup> in grains from different cultivars subjected to irradiation with doses of 2, 4 and 8 kGy, when compared to the control. These constituents are important in the human diet for regulation of the intestinal tract, which in turn has numerous physiological functions in maintaining health and preventing chronic non-communicable diseases.

The cultivar BRSMG 800A presented the highest ash contents (5.59 100 g<sup>-1</sup>). Ciabotti et al. (2016) reported high ash level genotypes with different seed coat colours, with values of 4.0 g 100 g<sup>-1</sup> and 4.5 g 100 g<sup>-1</sup>. The carbohydrate level of the lineage MGBR07-7043, which had a brown tegument, was 11.9 g 100 g<sup>-1</sup>, which was much higher than the levels found in the cultivars with yellow teguments intended for human consumption (BRSMG 790A and BRSMG 213), and this can positively affect the flavour of the lineage. Carbohydrates provide a softer and sweeter flavour in vegetable-type soybeans, due to the presence of sugars and even starch, which is practically not found in mature soybeans. The variability in the proximate composition of the lineages may be due to the influence of different planting locations, crops, variations in regional temperature, and the latitude and altitude, as well as the genotype (Callegari et al., 2013).



**Table 2.** Proximate composition (g 100 g<sup>-1</sup>) of soybean cultivars and lineages with characteristics intended for human consumption. 2012/2013 crop year, Uberaba-MG.

Genotypes	Moisture	Lipid	Protein	Soluble Dietary fibre	Insoluble dietary fibre	Total fibre	Ash	Carbohydrate
Conquista	7.60 b	18.8 c	36.0 e	1.20 c	20.5	21.7 c	5.39 b	10.4 b
BRS 213	8.0 a	21.0 a	36.4 d	1.28 c	19.7	21.0 d	5.30 b	8.29 c
BRSMG 790A	7.90 a	21.1 a	36.4 d	2.26 a	19.2	21.5 c	4.96 d	8.33 c
BRSMG 800A	7.60 b	20.9 a	36.2 d	1.52 b	17.7	19.2 e	5.59 a	10.4 b
MGBR10-16601	8.20 a	18.3 d	37.6 a	0.51 d	20.4	20.9 d	5.15 c	9.74 b
MGBR10-16301	7.60 b	20.2 b	36.8 c	1.73 b	18.7	20.4 d	4.92 d	9.93 b
MGBR10-16201	7.66 b	19.2 c	37.3 b	1.28 c	22.5	23.8 a	5.11 c	6.90 d
MGBR07-7043	8.20 a	18.2 d	34.5 f	1.16 c	20.5	21.7 c	5.39 b	11.9 a
MGBR09-9161	8.40 a	21.4 a	37.1 b	1.07 c	20.3	21.9 c	5.02 c	6.20 d
BRN07-50543	7.46 b	20.7 a	35.7 e	0.87 d	21.7	22.6 b	4.84 d	8.69 c

Means with the same letters in the same column do not differ significantly ( $p < 0.05$ ) according to the Scott-Knott test. Data obtained from whole beans.

The lipoxygenase I activity (LOX I) in units g<sup>-1</sup> of soy flour, varied from 127 to 1880 units per gram of soy flour, while the activity of lipoxygenases II + III (LOX II + III) ranged from 85 to 246 units (Table 3). Kumar et al. (2003) studied the activity of eight cultivars from four different locations and reported lipoxygenase I activity of from 450 to 2042 units g<sup>-1</sup> of soy flour and 118 to 600 units g<sup>-1</sup> of soy flour for lipoxygenases II + III. Several factors may have influenced the variability in these results, such as the planting location, biotic and abiotic stress suffered by the plant and the storage conditions, amongst others. The cultivar BRS 213, used as the standard in the present study, is free of lipoxygenase isoenzymes, as also the lineage MGBR10-16201, which can be classified as lipoxygenase free.

**Table 3.** Lipoxygenase activity (units g<sup>-1</sup> soy flour)\* of soybean cultivars and lineages with characteristics intended for human consumption. 2012/2013 crop year, Uberaba-MG.

Genotypes	LOX I	LOX II + III
Conquista	1645 f	162 d
BRS 213	195 b	85 a
BRSMG 790A	1339 c	136 c
BRSMG 800A	1543 e	127 c
MGBR10-16601	1482 d	246 g
MGBR10-16301	188 h	218 f
MGBR10-16201	127 a	102 b
MGBR07-7043	1795 g	150 d
MGBR09-9161	1626 f	151 d
BRN07-50543	1797 g	182 e

Means followed by the same letter do not differ from each other according to the Scott-Knott test at 5% probability; \*One unit of enzyme was taken as equivalent to the amount of enzyme that generated an increase in absorbance of 1.0 per minute at 234 nm (LOX I) or 280 nm (LOX II + III).

The absence of the isoenzymes should provide a milder taste, which can be confirmed by the sensory evaluation (Torres-Penaranda & Reitmeier, 2001; Ciabotti et al., 2006; Boatto et al., 2010). Although the other cultivars and lineages had high isoenzyme activities, the cultivar Conquista exhibited significantly higher LOX I activity, which conferred low flavour acceptance scores, since it is a cultivar not intended for human consumption. Felix et al. (2011) reported that the restrictions of Western consumers as a result of the characteristic flavour called a beany flavour, are due to the association between short-chain carbonyl compounds and protein fractions. These compounds are the end products of a series of reactions that begin with the hydro-peroxidation of polyunsaturated fatty acids, catalysed by lipoxygenases.

Soybeans contain around 20% fat, constituted of high levels of unsaturated fatty acids and reduced levels of saturated fatty acids (Table 4). The saturated fatty acid contents (palmitic and stearic acids) were proportionally lower than the others. In the genotypes studied here, the linoleic acid content ranged from 47.36 to 58.31 mg g<sup>-1</sup>, close to the levels reported by Bellaloui et al. (2013) who evaluated soybean cultivars altered by potassium fertilizer, which gave values ranging from 50.2 to 53.0 mg g<sup>-1</sup>. The lineage BRN07-50543, which had a black tegument, had the lowest linoleic acid level and the highest oleic acid level, with values of 47.36 mg g<sup>-1</sup> and 31.37 mg g<sup>-1</sup>, respectively. Lower oleic acid levels (21.77 mg g<sup>-1</sup>) were found by Esteves et al. (2010) in two lipoxygenase-free cultivars (LOX 2 and LOX 3).

**Table 4.** Fatty acid profiles (mg g<sup>-1</sup>) of the soybean cultivars and lineages with characteristics intended for human consumption. 2012/2013 crop year, Uberaba-MG.

Genotypes	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	$\alpha$ -Linolenic (C18:3)	Arachidonic (C20:4)
Conquista	11.64 b	3.86 d	21.61d	55.02 d	7.44 c	0.42 b
BRS 213	11.74 b	3.68 f	21.53 e	54.97 d	7.68 b	0.39 b
BRSMG 790A	11.39 c	4.13 a	24.91 b	52.92 g	6.17 e	0.46 a
BRSMG 800A	12.18 a	3.88 d	18.48 g	57.38 c	7.65 b	0.41 b
MGBR10-16601	11.69 b	4.07 b	24.01 c	52.14 h	7.64 b	0.43 a
MGBR10-16301	11.83 b	3.59 g	21.81e	54.58 e	7.80 a	0.38 c
MGBR10-16201	12.30 a	3.58 g	22.79 d	53.40 f	7.52 c	0.40 b
MGBR07-7043	11.90 b	3.82 e	17.89 h	58.31 a	7.67 b	0.40 b
MGBR09-9161	11.14 d	4.06 b	20.50 f	56.50 c	7.43 c	0.43 a
BRN07-50543	10.37 e	4.01 c	31.37 a	47.36 i	6.55 d	0.35 d

Means with the same letters in the same column did not differ significantly ( $p < 0.05$ ) according to the Scott-Knott test.

The results obtained for the fatty acid contents of these genotypes showed that the oil extracted could be used for other applications since all the fatty acids were suitable for the production of biofuel. The fatty acid composition of the soybean oil determines the final properties of the biodiesel, since they present differences in the size of the hydrocarbon chain, and in the number and position of the double bonds (unsaturation). Palmitic acid (C16:0) predominates amongst the saturated fatty acids, followed by stearic acid (C18:0), and linoleic acid (C18:2<sup>A9,12</sup>) amongst the unsaturated fatty acids, followed by oleic acid (C18:1<sup>A9</sup>) and linolenic acid (C18:3<sup>A9,12,15</sup>) (Mandarino et al., 2005).

Codex Alimentarius Commission (1993) has established the minimum and maximum parameters for the oleic acid profile of refined soybean oil (17 to 30 mg g<sup>-1</sup>). The lineage BRN07-50543, which has a black tegument, showed an oleic acid level (31.37 mg g<sup>-1</sup>) above the standards established by law and of the other genotypes evaluated, and a linoleic acid level (47.36 mg g<sup>-1</sup>) slightly below the Codex Alimentarius standards (48 to 59 mg g<sup>-1</sup>) and well below the values of the other lineages. The  $\alpha$ -linolenic (C18:3) fatty acid values were within the standards established by CODEX Alimentarius (4.5 to 11 mg g<sup>-1</sup>).

Oleic acid, known as the omega 9 fatty acid, is a monounsaturated fatty acid, and is a constituent of the healthier triacylglycerols, which help to reduce low-density blood cholesterol levels (LDL - bad cholesterol) and increase the high-density cholesterol (HDL - good cholesterol). It is an anti-inflammatory agent, providing good amounts of antioxidants to the human body, which can reduce oxidation by inhibiting lipid peroxidation (Sposito et al., 2007; Santos et al., 2013; Pinheiro et al., 2017). This type of fat has been found in olive oil, oilseeds (nuts and almonds), canola oil and avocado oil (Sposito et al., 2007). In general, soybean oil has low oleic acid levels, although the lineage BRN07-50543, which has a black tegument, stood out. It should be mentioned that this lineage was launched as cultivar BRSMG 715A in 2015. The other varieties of this study were within the legislative standards. Codex Alimentarius has not set limits for the arachidonic acid levels.

**Table 5.** Concentrations of isoflavones (mg 100 g<sup>-1</sup>) in the soybean genotypes intended for human consumption. 2012/2013 crop year. Uberaba-MG.

Genotypes	β-glycoside			Malonyl β-glycoside			Aglycones			Total
	Daidzein	Glycitein	Genistein	Daidzein	Glycitein	Genistein	Daidzein	Glycitein	Genistein	
Conquista	54.9 b	7.55 a	34.26 b	119.2 b	20.29 b	172.3 b	4.52 b	6.20 c	3.04 b	422.2 b
BRS 213	35.9 d	7.90 a	24.37 d	75.5 e	20.12 b	132.3 e	1.28 g	0.00	1.14 g	298.5 e
BRSMG 790A	14.9 h	4.87 c	15.09 e	36.7 i	12.73 e	83.7 g	1.21 g	0.00	2.16 d	171.4 i
BRSMG 800A	33.3 e	7.25 a	26.16 c	74.1 e	17.57 c	117.7 f	2.66 d	0.00	2.67 c	281.4 f
MGBR10-16601	28.0 f	6.16 b	12.89 f	62.1 f	14.63 d	73.8 h	1.21 g	0.00	0.87 h	199.7 h
MGBR10-16301	25.9 g	6.67 a	14.28 e	54.4 h	18.23 c	69.2 i	1.63 f	3.21 d	1.34 f	194.9 h
MGBR10-16201	46.0 c	5.52 b	24.85 d	101.9 c	16.87 c	140.4 d	1.79 e	0.00	1.07 g	338.3 c
MGBR07-7043	35.9 e	7.08 a	23.73 d	86.6 d	13.14 e	147.2 c	3.44 c	6.86 b	1.96 e	326.1 d
MGBR09-9161	34.3 e	6.78 a	15.10 e	57.9 g	15.38 d	80.8 g	1.59 f	0.00	1.12 g	213.1 g
BRN07-50543	77.19 a	1.94 d	46.22 a	145.9 a	31.85 a	213.2 a	5.96 a	9.69 a	4.07 a	546.0 a

Means with the same letters in the same column do not differ significantly ( $p < 0.05$ ) according to the Scott-Knott test.

In relation to the total isoflavones, significant differences were observed amongst the cultivars and lineages evaluated (Table 5). The lineage BRN07-50543, which has a black tegument, showed a high isoflavone content (546 mg 100 g<sup>-1</sup>), while BRSMG 790A showed the lowest content of the glycosylated forms and aglycones. Glycosylated malonyl forms are commonly found in greater amounts in soybeans and defatted soy flour, while the non-conjugated forms (daidzein, genistein, and glycitein) are found in processed foods, thus explaining the low aglycone levels.

Studies have shown that aglycone isoflavones are absent or present at very low levels in whole, recently-harvested soybeans (Ciabotti et al., 2016). However, damage to the soybeans with a consequent increase in moisture content, may provide propitious conditions for the formation of aglycones due to enzyme activity, but in *in natura* soybeans, aglycones are always found in reduced amounts (Silva et al., 2012). The contents and distribution of the isoflavones are influenced by several factors including the region and planting location, crop, growing conditions, temperature, soil nutrition and storage time, rather than the genotype (Kim et al., 2014).

## 4 Conclusions

All the genetic materials evaluated in the present study were within the commercial soybean parameters for the protein, fat, dietary fibre and ash contents. The lineage MGBR10-16201, which has a yellow tegument and hilum, was identified as a lipoxygenase-free lineage. The lineage BRN07-50543, which has a black tegument, presented higher oleic acid and lower linoleic acid contents than the parameters defined by Codex Alimentarius, and had a high total isoflavone level, which was much higher than those of the other genotypes studied.

## Acknowledgements

The authors are grateful to the Foundation for Research Support of the State of Minas Gerais - FAPEMIG for the scholarship awarded to the visiting researcher.



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Funding: None.

Received: Jan. 09 2018; Accepted: Sept. 04, 2018