BRAZILIAN JOURNAL OF FOOD TECHNOLOGY

ISSN 1981-6723
Online version

ORIGINAL ARTICLE

Phytochemical evaluation of lipoxygenase-free soybean genotypes for human consumption

Avaliação fitoquímica de genótipos de soja sem lipoxigenases para consumo humano

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Cite as: Gondim-Tomaz, R. M. A., Braga, N. R., Carvalho, C. R. L., Gallo, P. B., & Erismann, N. M. (2022). Phytochemical evaluation of lipoxygenase-free soybean genotypes for human consumption. *Brazilian Journal of Food Technology*, *25*, e2021032. https://doi.org/10.1590/1981-6723.03221

Abstract

The present study aimed to screen genotypes for the presence/absence of lipoxygenases and to evaluate the phytochemical characteristics of lipoxygenase-free genotypes. Progenies derived from the cross between the cultivars IAC PL-2 (with lipoxygenases) and BRS 213 (lipoxygenase-free) generated 114 inbred lines in the F6/7 generation, evaluated by the qualitative colorimetric method, to verify the presence/absence of lipoxygenase isoenzymes L-1, L-2 and L-3. Eight triple-null (lipoxygenase-free) lines were confirmed by visible spectrophotometry. Progenitors and triple-null lines IAC 10-0003-1, IAC 10-0010-1, IAC 10-0010-2, IAC 10-0012, IAC 10-0043, IAC 10-0046, IAC 10-0107-1 and IAC 10-0115-2 were analyzed to assess their contents of protein, oil, phytic acid, tannins, total phenols and total flavonoids. Analyses showed significant differences in protein (from 38.80% to 41.89%) and oil (from 18.86% to 22.88%), consistent with the values observed in commercial soybean cultivars. The new triple-null lines, compared to the BRS 213 standard, showed lower contents of soluble condensed tannins; lower phytic acid in line IAC 10-0043 and greater amount of total phenols in line IAC 10-0115-2; whereas, the lines IAC 10-0010-1, IAC 10-0010-2, IAC 10-0043, IAC 10-0046 e IAC 10-0107-1 stood out with higher values of total flavonoids. The new lines will be potentially useful parents for genetic breeding and for a multiline strategy, aiming at greater phenotypic stability of lipoxygenase-free soybeans for human consumption.

Keywords: *Glycine max* (L.) merrill; Lipoxygenases; Protein; Phytates; Phenols; Human food.

Resumo

O presente estudo teve como objetivo selecionar genótipos quanto à presença/ausência das lipoxigenases, além de avaliar as características fitoquímicas dos genótipos com ausência das lipoxigenases**.** Progênies oriundas do

cruzamento entre as cultivares IAC PL-2 (com lipoxigenases) e BRS 213 (sem lipoxigenases) geraram 114 linhagens na geração F6/7, avaliadas pelo método qualitativo colorimétrico, para constatar a presença/ausência das isoenzimas lipoxigenases L-1, L-2 e L-3. Foram confirmadas por espectrofotometria na região do visível oito linhagens triplo-nulas (sem lipoxigenases). Os progenitores e as linhagens triplo-nulas IAC 10-0003-1, IAC 10-0010-1, IAC 10-0010-2, IAC 10-0012, IAC 10-0043, IAC 10-0046, IAC 10-0107-1 e IAC 10-0115-2 foram analisados para aferir seus teores de proteína, óleo, ácido fítico, taninos condensados solúveis, fenóis totais e flavonoides totais. As análises apontaram diferenças significativas nos teores de proteínas (38,80% a 41,89%) e de óleo (18,86% a 22,88%), coerentes com os valores observados em cultivares comercializados de soja. As novas linhagens triplo-nulas, comparativamente ao padrão BRS 213, apresentam menores teores de taninos condensados, menor presença de ácido fítico na linhagem IAC 10-0043 e maior presença de fenóis totais na linhagem IAC 10-0115-2, enquanto as linhagens IAC 10-0010-1, IAC 10-0010-2, IAC 10-0043, IAC 10-0046 e IAC 10-0107-1 se destacam com valores superiores de flavonoides totais. As novas linhagens serão parentais potencialmente úteis para melhoramento genético e para uma estratégia de multilinhas, visando à maior estabilidade fenotípica de soja sem lipoxigenases, para alimentação humana.

Palavras-chave: *Glycine max* (L.) merrill; Lipoxigenases; Proteína; Fitatos; Fenóis; Alimentação humana.

1 Introduction

The growing world production of soybean (*Glycine max* (L.) Merrill) stems from its nutritional characteristics for food and feed, and by the evolution of the technological support developed for its production. Brazil is the largest world producer of soybeans with its production exceeding 120 million tons of grains, reached in the 2019/20 harvest, occupying almost 60% of the country's cultivated area (Companhia Nacional de Abastecimento, 2020). The Brazilian inland has favorable climatic characteristics and availability of soil suitable for soybeans in all its latitudes, despite restrictions on its expansion in priority areas for the environmental preservation of tropical biomes in the Cerrado and Amazonian regions. Its production is primarily intended for export as non-industrialized grains, bran and oil. It meets the demands of the domestic market to produce seeds for successive plantings, and industrial processing for the extraction of oil for human consumption and even as a raw material for biodiesel production. The bran by-product is used in animal feed formulation. The human consumption of industrialized soy as a morning drink, based on soluble protein extract, with the addition of different flavors, has evolved significantly, among other uses of its derivatives, aiming at a healthier diet (Carrão-Panizzi & Silva, 2011). The consumption of *in natura* soybean in Brazil is still not very expressive due to the cultural habits of its population, except in communities of Asian origin where its daily consumption is traditional. The so-called special, non-transgenic soybeans, identified by their superior flavor (light and sweet), are also used as vegetables, in the form of sprouts (‘moyash’) or green grains at the R-6 stage of grain-filling (Rigo et al., 2015).

Soybean seeds are excellent sources of protein, oil, polyunsaturated fatty acids, and isoflavones (subclass of flavonoids; Mozaffarian & Wu, 2018); however, they also contain anti-nutritional factors (like phytic acid, tannins and protease inhibitors) which decrease their nutritional qualities (Silva & Silva, 1999; Silva et al., 2020; Salgado & Donado-Pestana, 2011; Benevides et al., 2011).

One of the anti-nutritional factors is phytic acid, which in high quantities decreases the availability of minerals by interacting with multivalent cations, and forming insoluble complexes. On the other hand, in smaller amounts, it can block lipid oxidation by chelating divalent minerals such as copper, zinc, iron and calcium, which could explain its anti-cancer effects in the body (Silva & Silva, 1999). Tannins, high molecular weight polyphenols, are antioxidants, although they also act as inhibitors of certain enzymes, and can complex proteins, negatively influencing their digestibility and absorption (Silva & Silva, 1999).

Several studies have shown that regular soybean consumption is associated with a reduced risk of chronic western diseases, such as hormonal dependent cancers, cardiovascular diseases, atherosclerosis, diabetes,

osteoporosis and probable relief from symptoms related to menopause. Its beneficial effect is mainly due to the presence of high concentrations of phenolic and flavonoid compounds, such as isoflavones, which have estrogen agonist-antagonist properties, and antioxidant activity (Silva & Silva, 1999; Ramdath et al., 2017; Salgado & Donado-Pestana, 2011).

The unpleasant taste of soybeans, similar to beany flavor ('raw beans'), results from hydroperoxidation reactions of polyunsaturated fatty acids (linoleic, linolenic and arachidonic acids), catalyzed by certain enzymes called lipoxygenases. Heat treatment has been used to inactivate these enzymes in industrial processes, seeking to promote nutritional and organoleptic qualities of soybeans. The genetic elimination of lipoxygenases can improve the organoleptic characteristics of the soybean grains and their bioproducts, due to the lower production of hydroperoxides, associated with the bitter and beany flavors. The absence of these isoenzymes contributes to the 'light' and sweet taste in soybeans, making it more palatable (Suda et al., 1995; Rigo et al., 2015). The availability of these cultivars is interesting for their introduction in agricultural regions with predominance of family farming, with no tradition of conventional soybean production and without risks of contamination by transgenic soybeans.

The program for the genetic improvement of soybean at the *Instituto Agronômico* (IAC) had among its goals the achievement of cultivars with better organoleptic and nutritional quality. There have been attempts to incorporate genes to eliminate the presence of lipoxygenases in the seeds of cultivar IAC-8, using soybean mutations PI 408251 (absence of L-1), PI86023 (absence of L-2) and Tohoku (absence of L-3) (Sawazaki et al., 1987). Although it was not successful in obtaining a triple-null line, this effort resulted in lines essentially derived from IAC-8 with partial removal of lipoxygenases (Tavares et al., 1993; Silva, 2004).

In 2003, new hybridizations were performed at IAC, involving the parental IAC PL-2 with the presence of the three isoenzymes L-1, L-2 and L-3, triple-positive, and BRS 213, lacking the three isoenzymes, triple-null, aimed at genotypes with better organoleptic and nutritional characteristics, thus inducing greater acceptance of soya flavor. Therefore, it was intended to obtain genotypes lacking lipoxygenases, with high protein content and with a lower incidence of anti-nutritional factors. These genotypes should also present a larger size and a better appearance of the seeds with yellow integument and hilum. The new cultivars should also have positive agronomic characteristics, such as precocious maturation, with a life cycle of 120-125 days, greater resistance to phytoparasites, greater amplitude of the favorable sowing period due to their photoperiodic response, better adaptation of plant height to mechanical harvesting and higher grain productivity in the corresponding latitudes to the State of São Paulo (SP).

Thus, the present study aimed to investigate the presence/absence of lipoxygenases activities in 114 inbred lines from the afore mentioned crossing and to evaluate the phytochemical characteristics of those that stood out as triple-null. In this approach, the contents of protein, oil, phytic acid, condensed tannins, total phenols and total flavonoids were determined.

2 Material and methods

Samples of soybean seeds produced under greenhouse conditions in the Leguminous plants Sector, located at the *Centro Experimental de Campinas*, IAC, in Campinas (SP) (22°54'20" S, 47°03'39" W at 674 m asl.) were evaluated. The 114 analyzed inbred lines were obtained from the soybean breeding program, selected from 40 progenies in the F-6/7 generation, from the cross between the parental IAC PL-2 (with lipoxygenases) and BRS 213 (lipoxygenase-free). The first parental was essentially derived from the cultivar IAC PL 1 with high protein content with high digestibility and less inhibition of trypsin (Mendes et al., 2009), traditional standard in the IAC for human consumption, a probable mutation for late flowering of native cultivar from Japan. The second parental, generated by CNP-Soja from Embrapa, Londrina – in the state of Paraná (PR), was the result of crossing BR 94-23354 x BR 94-23321, triple-null, regarding the presence of

the 3 lipoxygenases responsible for the beany flavor, with favorable agronomic characteristics (Carrão-Panizzi et al., 2002).

The lines were classified according to the activity of lipoxygenases, by the visual colorimetric method, described by Kikuchi & Carrão-Panizzi (2001). The determination of the lipoxygenase isoenzymes L-1, L-2 and L-3, by this method, is faster and simpler than by spectrophotometry. Colorimetric tests were performed based on the isoenzyme bleaching activity on the linoleic acid substrate and co-oxidation with methylene blue (L-1 and L-2) and β - carotene (L-3). To enhance the reactivity of the L-3 isoenzyme, the synergistic effect that occurs when adding the extract of the lipoxygenase L-2 (Kanto 102) was used, increasing the bleaching activity of β-carotene. The detected triple-null lines were subjected to the spectrophotometric (Hitachi U-2000, Chiyoda-ku, Tokyo, Japan) method (Suda et al., 1995), confirming the absence of the three lipoxygenases. The eight triple-null lines were analyzed to evaluate their contents of protein, oil, phytic acid, tannins, total phenols and total flavonoids.

For evaluations, seed samples were ground in an analytical mill (Ika A11, Staufen, Germany) and moisture was determined by the gravimetric method according to Araújo et al. (2006). All results of the chemical analyses were expressed on a dry matter (DM) base. The protein content was determined by the micro-Kjeldahl method in a 0.2 g sample of ground soybeans, using copper sulfate as a catalyst (Bataglia et al., 1983). The oil content was determined in 3 g of ground soybeans in Butt-type extraction tube (Firestone, 2009), using hexane as a solvent.

The analyses to assess the contents of phytic acid, tannins, total phenols and total flavonoids were performed after extracting the oil from the samples. The soybeans grains of each line and of the standards were ground, extracting the oil with hexane to avoid its interference in the results (Frühbeck et al., 1995). Phytic acid contents were determined by anion exchange chromatography, followed by reaction with Wade's solution and spectrophotometric reading at 500 nm. The extraction was carried out with 2 g of soybean flour in 2.4% HCL (0.78 M; 37% purity) for 2 h on an orbital shaker; the elution of the extract was performed by ion exchange chromatography with Dowex 1x4-200 ion-exchange resin (Sigma-Aldrich) and 0.7 M NaCL as eluent (Latta & Eskin, 1980; Frühbeck et al., 1995). Phytate contents, expressed as amounts of phytic acid, in mg 100 g^{-1} DM, were determined using a standard phytic acid curve (0 to 240 μg).

For the determination of soluble condensed tannins, 0.5 g of ground soybeans was extracted with 70% (v/v) acetone and 0.1% (w/v) ascorbic acid. The extraction was repeated three times (2 x 30 min and once overnight) in a 25 kHz ultrasonic bath (Ultronique Eco-sonics Q3.0/25A, Brazil) and centrifuged at 10,595 x g. The pooled extract was separated for the determination of the soluble condensed tannins by the 4-dimethylaminocinnamaldehyde (DMACA) method (Li et al., 1996). The DMACA reagent was prepared immediately before use, by adding 2% (w/v) DMACA in a cold solution of methanol-HCL 6 M (1:1, v/v). The tannin contents were determined from a standard grape leucoanthocyanidin curve (3 to 50 μg) and expressed in mg leucoanthocyanidin equivalent (LE) 100 g^{-1} DM.

The extraction for total phenol and flavonoids analyses was carried out in 2 g sample of ground soybeans with 10 mL of the extraction solution, 60% (v/v) ethanol containing 20 mM dimethyl sulfoxide acid (w/v). The sample was taken in a 25 kHz ultrasonic bath for 10 min, and then it was left for overnight extraction at 4 °C. After that, it was sonicated for 30 min and centrifuged at 10,595 x g for 10 min, at 4 °C. The extraction was repeated three times (in the second and third extraction, 5 mL of the extraction solution was added). The extracts were kept in the -80 °C freezer for further analysis.

Total phenols were determined by the Folin-Ciocalteu method (Singleton et al., 1999). For quantification, 0.04 mL of the extract was mixed with 2.4 mL of distilled water and 0.20 mL of Folin-Ciocalteu reagent. After 5 min; 0.6 mL of 20% (w/v) Na_2CO_3 and 0.76 mL of distilled water were added, allowing to react for 50 min and then measuring the absorbance at 760 nm. Gallic acid was used as a standard, and the contents of total phenolic compounds expressed in mg of gallic acid equivalents per 100 g of DM soybean flour (mg GAE 100 g^{-1} DM). Linearity range of the calibration curve was 2.5 to 50 μg gallic acid.

The total flavonoid content was determined using a spectrophotometric method (Xu & Chang, 2008) in which 0.25 mL of the extract was mixed with 1.25 mL of distilled water and 0.075 mL of 5% (w/v) $NaNO_2$. After 6 min, 0.15 mL of 10% (w/v) $AlCl_3$ was added, allowing to react for 5 min before adding 0.5 mL of 1 M NaOH and 0.275 mL of distilled water, to a final volume of 2.5 mL. The absorbance at 510 nm was immediately measured, against a blanc consisting of the same mixture without extract. The total flavonoid content was expressed in mg of catechin equivalents per 100 g of DM sample (mg CE 100 g^{-1} DM). The linearity range of the calibration curve was 3 to 60 µg catechin.

Data were subjected to analysis of variance in a completely randomized design with three replications per soybean genotype, and when significant ($p \leq 0.05$) means were grouped by the Scott-Knott's test at 5% probability. Analyses were performed using the SISVAR statistical program (Ferreira, 2011).

3 Results and discussion

The results of the colorimetric screening of the 114 lines for the presence/absence of the three lipoxygenases (L-1, L-2, L-3) showed a wide segregation (Table 1). It is worth mentioning that, in only 19 lines, the L-3 isoenzyme was absent, while L-1 and L-2 were absent in 52 and 53 of the analyzed lines, respectively, suggesting an association between the last two isoenzymes. The triple-positive lines for the three lipoxygenases were dominant. The results indicate greater difficulty in removing the L-3 isoenzyme by means of genetic improvement. Another 11 lines lacking this isoenzyme, in addition to the 8 triple-null, could be indicated for eventual hybridizations, as parents with greater probability of obtaining triple-null cultivars. The absence of the three lipoxygenase isoenzymes (L-1, L-2, L-3) and seed purity were confirmed by the spectrophotometric method in the lines: IAC 10 0003-1, IAC 10 0010-1, IAC 10 0010-2, IAC 10 0012, IAC 10-0043, IAC 10-0046, IAC 10 0107-1, and IAC 10 0115-2, in addition to the standard cultivar BRS 213, while their presence was confirmed for the standard IAC PL-2.

Table 1. Presence of L-1, L-2 and L-3 lipoxygenase isoenzymes or absence (L-0, triple-null), evaluated by colorimetry, in seeds of 114 soybean lines.

	Lipoxygenases (L)							
	L-0	L-1	L-2	L-3	L-1,2	L-1,3	L-2,3	L-1,2,3
Lines (n°)	8	4	2	22	5	19	20	34

Soybean is a rich source of protein with a high nutritional value, favoring its intake in situations of insufficient food consumption, healthy eating and suitable as a plant-based meat in vegan and sustainable diets (Silva et al., 2006). The protein contents of these eight (8) lines identified as triple-null and their parents showed significant differences by the Scott-Knott's test at 5% probability (Table 2), varying between 38.80% and 41.89%. The triple-null lines IAC 10-0046, IAC 10-0010-2 and IAC 10-0043 showed values similar to those obtained by the standard cultivar BRS 213. These results were similar to those reported by Sales et al. (2016), from 33.38% to 42.53% for transgenic and conventional cultivars, and are within the range cited by Greggio & Bonini (2014), from 25.64% to 43.23% for Brazilian commercial cultivars, under field conditions. In greenhouse conditions, Bellaloui & Gillen (2010) obtained protein contents from 39.61% to 48.60% in North American cultivars, at physiological maturity stage (R8), in experiments carried out considering the position of the pods in different plant segments for seed sampling. Soybean lines with higher protein levels can be negatively correlated with isoflavone levels. Thus, the selection for higher protein content could imply a relative decline in the isoflavone content (Chiari et al., 2004).

Table 2. Protein and oil contents (dry matter basis) in the seeds of eight (8) triple-null soybean lines, compared to their parents.

Genotypes	Protein content (%)	Oil content (%)
IAC 10-0003-1	39.27b	20.05e
IAC 10-0010-1	39.83b	20.00e
IAC 10-0010-2	40.95a	22.88a
IAC 10-0012	38.80b	18.86f
IAC 10-0043	40.38a	22.02b
IAC 10-0046	41.89a	20.93d
IAC 10-0107-1	39.82b	20.72d
IAC 10-0115-2	38.84b	21.41c
BRS 213 (TN)	40.93a	22.71a
IAC PL-2 (TP)	39.60b	22.24b
F	5.44**	53.68**
CV (%)	1.86	1.46

Values followed by different letters in the columns indicate significant differences by Scott-Knott's test at 5% probability. TN: triple-null standard, lipoxygenase-free; TP: triple-positive standard, with the three isoenzymes; CV (%) Coefficient of Variation of experimental data. **Significant at 1% probability by the F test.

The oil contents (Table 2) indicated a range of variation from 18.86% (IAC 10-0012) to 22.88% (IAC 10-0010-2), similar to that reported by Sales et al. (2016), from 15.27% to 24.80% in field cultivation and also by Bellaloui & Gillen (2010), from 17.31% to 24.69% in greenhouse, in different soybean cultivars. The results of the oil content showed significant differences among triple-null lines. The lines IAC 10-0010-2 and IAC 10-0043 were similar to the BRS 213 and IAC PL-2 standards, respectively.

Table 3 shows the results of the contents of phytic acid, tannins, total phenols and total flavonoids. Significant differences were evident among the eight (8) triple-null soybean lines for all characteristics. The variation range of phytic acid from 1.03 to 2.11 g 100 g^{-1} DM was similar to that reported by Moreira et al. (2012) from 1.13 to 2.44 g 100 g^{-1} DM for different cultivars in different locations and under field conditions. The lines IAC 10-0010-2 and IAC 10-0012 showed phytic acid levels higher than those of standard cultivars. The lines IAC 10-0003-1, IAC 10-0010-1, IAC 10-0046, IAC 10-0107-1 and IAC 10-0115-2 showed low values of phytic acid and did not differ from the standard cultivars IAC PL-2 and BRS 213. Only the IAC 10-0043 line stood out from the others with a significantly lower amount. Nevertheless, all soybean lines exhibited adequate phytic acid concentration for human consumption. It is worth mentioning that a daily intake of phytic acid above 10% is considered very high, acting as an anti-nutritional substance. However, in normal diets, the concentration of phytic acid is low (around 0.035%), prevailing the functional action (Onomi et al., 2004).

Table 3. Mean values (dry matter basis) of phytic acid, tannins, total phenols, flavonoids in two standard cultivars and eight (8) triple-null soybean lines.

Genotypes	Phytic acid (g 100 g^{-1})	Tannins (mg LE 100 g^{-1})	Total Phenols (mg GAE 100 g^{-1})	Total Flavonoids (mg CE 100 g^{-1})
IAC 10-0003-1	1.62c	49.93d	278.78b	31.15e
IAC 10-0010-1	1.64c	77.84b	245.63c	85.08b
IAC 10-0010-2	2.11a	38.41e	223.57c	97.95a
IAC 10-0012	1.83b	60.02c	155.73e	37.75d
IAC 10-0043	1.03d	45.19e	202.07d	104.66a
IAC 10-0046	1.52c	39.36e	262.33c	98.44a
IAC 10-0107-1	1.44c	41.94e	294.57b	102.87a
IAC 10-0115-2	1.37c	50.26d	323.67a	43.51d
BRS 213 (TN)	1.55c	95.41a	282.43b	70.50c
IAC PL-2 (TP)	1.47c	57.30c	110.27f	80.20b
F	15.48**	68.46**	36.16**	171.94**
CV (%)	8.02	6.90	8.01	4.96

Values followed by different letters in the columns indicate significant differences by Scott-Knott's test at 5% probability. LE: leucoathocyanidin equivalent; GAE: gallic acid equivalent; CE: catechin equivalent; TN: triple-null standard, lipoxygenase-free; TP: triple-positive standard, with the three isoenzymes; CV (%), coefficient of variation of experimental data. **Significant at 1% probability by the F test.

The results of the analysis of soluble condensed tannins revealed significant differences among lines (Table 3), ranging from 38.41 to 95.41 mg LE 100 g^{-1} DM, using the DMACA method. The values obtained were lower than the levels of tannins reported by Malencić et al. (2008), from 72.7 to 158.5 mg CE 100 g^{-1} DM, using the Folin-Ciocalteu method. The diverse results can be attributed to differences in environment, genotypes and methods of determination. In the present study, the seeds were obtained in a greenhouse, in Campinas (SP), and in Malencić et al. (2008) genotypes of different origins were used, grown in an experimental field, in Novi Sad, Servia. Low levels of tannins are recommended for human consumption (Silva & Silva, 1999). All triple-null lines had significantly lower tannin levels than the standard cultivar BRS 213.

The contents of total phenols ranged from 155.73 to 323.67 mg GAE 100 g^{-1} DM, higher than the value of the standard cultivar IAC PL-2 of 110.27 mg GAE 100 g^{-1} DM. The line IAC 10-0115-2 was significantly higher in its content of total phenols in relation to the other lines and to the standard cultivar BRS 213. The lines IAC 10-0003-1 and IAC 10-0107-1 were similar to the BRS 213, whereas the other lines were inferior. The range of variation was greater than that reported by Xu & Chang (2008), from 207 to 290 mg GAE 100 g^{-1} DM, in North American yellow soybean cultivars, extracted with 50% acetone. In Croatian soybean cultivars, extracted with methanol, the total phenol content ranged from 87.2 to 216.3 mg GAE 100 g^{-1} DM (Mujić et al., 2011), and in advanced Croatian breeding lines, Josipović et al. (2016) reported values from 212.1 to 322.7 mg GAE 100 g^{-1} DM, closer to those obtained in this study.

Total flavonoid contents (Table 3) ranged from 31.15 to 104.66 mg CE 100 g^{-1} DM, more broadly, reaching slightly higher levels than the values reported by Josipović et al. (2016) from 42.8 to 65.9 mg CE 100 g^{-1} DM for advanced Croatian lines and by Xu & Chang (2008), from 18 to 59 CE 100 g^{-1} DM, for American yellow grain cultivars. The lines IAC 10-0043, IAC 10-0107-1, IAC 10-0046 and IAC 10-0010-2 were superior to the other lines and to the standard cultivars BRS 213 and IAC PL-2.

Quantitative characteristics, such as oil, protein and bioactive components (phenols, flavonoids, tannins and phytic acid) are polygenic and respond more to environmental fluctuations than qualitative (morphological) characteristics. In this context, the use of a multiline in soybean, that is, a mixture of genotypes with greater population homeostasis, would provide greater phenotypic stability than the inbred lines (Carneiro et al., 2019). A selective mixture of lines would be a recommended strategy to stabilize and maximize favorable components (oil, protein and antioxidants) and minimize antinutritional components (such as phytic acid and tannins), preserving the best agronomic performance and the homogeneity of qualitative characteristics (flower and pod color, etc.).

4 Conclusions

The lipoxygenase-free IAC soybean lines showed average contents of protein and oil within the range of variation of commercial soybean cultivars, thus presenting good nutritional characteristics as a cheaper source of protein and suitable for plant-based meat diets. The line IAC 10-0010-2 stood out from the others, with high levels of protein and oil, similar to BRS 213. The new lipoxygenase-free lines, compared to the BRS 213 standard, had lower levels of soluble condensed tannins, lower presence of phytic acid in line IAC 10-0043 and higher presence of total phenols in line IAC 10-0115-2; whereas lines IAC 10-0010-1, IAC 10-0010-2, IAC 10-0043, IAC 10-0046 and IAC 10-0107-1 stood out with higher values of total flavonoids. Eventually, these lipoxygenase-free lines could be used in a multiline strategy to ensure greater stability of their quantitative characteristics.

Triple-null lines (lacking L-1, L-2 and L-3 lipoxygenase isoenzymes), IAC 10-0003-1, IAC 10-0010-1, IAC 10-0010-2, IAC10-0012, IAC10-0043, IAC10-0046, IAC 10-0107-1 and IAC 10-0115-2 can be indicated as promising parents, following the genetic improvement of soybeans. Other eleven lines without the presence of L-3 isoenzyme would be favorable to obtaining new lipoxygenase-free lines by genetic easier removal of L-1 and L-2 isoenzymes.

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Funding: None.

Received: Feb. 19, 2021; Accepted: Oct. 15, 2021

Associate Editor: Giuseppina Pace Pereira Lima.