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Antioxidant, bioactive, and nutritional potential of soybeans with brown and yellow seed coats[1]

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ABSTRACT - The use of soybeans for human consumption is primarily attributed to their high protein content and diverse phytochemical composition, which are key for improving human nutrition. Given the growing interest in biofortification to enhance the nutritional value of crops, this study aimed to assess the agronomic performance, nutritional, functional and physicochemical composition of soybean lines with both brown and yellow seed coats, with a particular focus on their biofortification potential. To achieve this, we evaluated ten F6 generation soybean genotypes (G1M, G2M, G3A, G4A, G5A, G6A, G7A, G8A, G9A, and G10A), along with a commercial control (G11T), using a randomized block design. Sowing occurred in the second half of November 2020, and after harvesting, grain samples were analyzed for agronomic and biochemical traits, including fatty acids, neutral and acid detergent fibers, minerals (Na and K), proteins, lipids, total flavonoids, total phenolic content, and antioxidant activity (ABTS and FRAP). A previous experiment with extractive conditions related to temperature and extraction method was carried out to evaluate the antioxidant properties. Significant variation in agronomic and proximate traits was observed among the genotypes, and the interaction between genotypes and extraction methods notably influenced antioxidant properties. Genotypes with brown seed coats, particularly G2M, showed high potential for protein and oleic acid content, making them suitable for biofortification and food product applications. The Ultrasonic Bath extraction method proved more effective in extracting ABTS and total flavonoids. These findings suggest that both the seed coat color and extraction method play crucial roles in determining the bioactive and nutritional potential of soybeans, supporting their use in biofortification strategies for enhanced human nutrition.

Key words: *Glycine max* L. (Merr.). Phytochemical grain composition. Extraction methods. compounds. Mineral content.

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INTRODUCTION

Soybean is one of the main agricultural commodities consumed in the world, produced in several regions of Brazil (CONAB, 2023). The growing interest in soybean grain production is associated with its use in the chemical agroindustry, food manufacturing, oil production, flours, bran, animal feed, and biofuels (Silva *et al*., 2019). The use of soybeans for human consumption occurs mainly due to the high protein content and diverse phytochemical composition in these grains (Loro *et al*., 2021).

Soybean is a complete food, consisting of 42% protein, 33% carbohydrates and 20% lipids (Oliveira *et al*., 2016). Its grains contain fatty acids including 55% linoleic acid, 21% oleic acid, 12% palmitic acid, 9% linolenic acid and 4% stearic acid (Padalkar *et al*., 2023). Furthermore, they are rich in phenolic compounds, anthocyanins and isoflavones, which are important for human nutrition (Desta *et al*., 2022).

The expression and quantification of phytochemical compounds in soybeans are influenced by both genetic and environmental factors (Thomas, 2018). Genotypic characteristics, such as seed coat color, seed weight, hilum color, and cycle length, significantly contribute to determining the nutraceutical composition and quality of the soybean grains (Desta *et al*., 2022). Soybeans exhibit various coat colors, including yellow, black and brown, and these variations affect their phytochemical content (Yang *et al*., 2021). Desta *et al*. (2022), revealed average levels of palmitic, stearic, oleic, linoleic and linolenic fatty acids, respectively, of 10.89%, 3.53%, 19.59%, 57.22% and 8.76% for brown seed coats soybeans, and 10.75%, 3.45%, 26.75%, 52.46% and 6.59% for yellow seed coats soybeans. When comparing soybeans with yellow seed coats to those with brown seed coats according to Ciabotti *et al*. (2016), there was a difference in protein levels, with levels of 39.80% for yellow-seeded soybeans and 38.85% for brown-seeded soybeans.

Some studies (Agyenim-Boateng *et al*., 2023; Kim *et al*., 2014; Lee *et al*., 2011; Pierce *et al*., 2015) demonstrate examples of biofortification in soybeans aimed at improving nutritional quality and reducing nutrient deficiencies. Kim *et al*. (2014), reported the biofortification of β-carotene content in grains using recombinant genes, while Lee *et al*. (2011) achieved a 77% increase in a α-tocopherol content In the study by Pierce *et al*. (2015), the crtB transgene effectively enhanced carotenoid biosynthesis in soybean, resulting in β-carotene accumulation ranging from 60 to 741 μg g^{-1}. Additionally, the transgenic seeds accumulated phytoene and α-carotene, compounds not detected in the non-transgenic control seeds, highlighting the successful biofortification potential of this genetic modification. Understanding the factors that influence soybean composition is crucial for the development of biofortification strategies, which are essential in addressing global nutritional deficiencies associated with mineral and vitamin-poor diets. Biofortification can be achieved either through genetic enhancement (genetic biofortification) or agronomic practices (agronomic fortification), aiming to improve the nutritional quality of crops (Silva *et al*., 2019). Given this context, the present study aimed to assess the agronomic performance and nutritional composition of soybean lines with both brown and yellow seed coats, with a particular focus on biofortification potential. In addition, two extraction methods (Water Bath and Ultrasonic Bath) were performed in parallel to extract total phenolic compounds and total flavonoids.

MATERIAL AND METHODS

The study was carried out in the municipality of Augusto Pestana - Rio Grande do Sul state (latitude 28º23'16"S, longitude 53º54'53"W and altitude of 390 m), Brazil. The experimental design used was randomized blocks, with three replications, evaluating ten F_6 generation genotypes and one commercial control (BMX ZEUS 55I57 RSF IPRO - Table 1). Sowing took place in the second half of November in 2020. In experimental units of 10 square meters, rows spaced 0.45 meters apart, density of 12 plants per linear meter, base fertilizer of 05-20-20 at a dose of 250 kilos per hectare, sowing took place on November 25th, 2020 and harvested on April 2021.

After harvesting, a sample of grains from each genotype was taken to evaluate the following qualitative variables: *purple spot* (PS): presence or absence; *coat color* (CC): yellow, green, brown and black; *green seeds* (GS): presence or absence; *hypocotyl color* (HYC): green or purple; *halo color* (HAC, yellow, gray or brown); *hilum color* (HC): yellow, gray or brown; *whole seeds* (WHO, unit); *seed size* (SSI): small, medium and large; *presence of fungus* (PF): presence or absence; *physical damage* (PD): presence or absence; *seed shape* (SSH): spherical, oval or round; and one *hundred seed weight* (HSW, g).

The following biochemical variables were determined: *oleic fatty acid* (OL_AC_C, %); *neutral detergent fiber* (NDF, %); *acid detergent fiber* (ADF, %); *peroxidase reaction* (PR): positive or negative; *palmitic fatty acid* (PAL_AC_C, %); *stearic fatty acid* (STE_AC_C, %); and *linolenic fatty acid* (LINOLEN_AC_C, %), total phenolic compound content (TPC, mg GAE g^{-1}); *antioxidant activity by 2,2-azino-bis* (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, mM TEAC g^{-1}) and *ferric reducing antioxidant power* (FRAP, mM Fe^{+2} g^{-1}). The sodium (Na) and potassium (K) content was performed according to Shelke *et al*. (2017), and the results were expressed in mg g^{-1}.

Table 1 - Genealogy and morphological characteristics of the genotypes used in the experiment

Genotypes	Genealogy	MP	PP	Hilo	CC	HAC	SB	SSH	SSI
G1M	5 (19F3)	FPS JÚPITER	URNS	Medium brown	Brown	Brown	Opaque	Round	Medium
G2M	23 (17F3)	FPS JÚPITER	URNS	Light brown	Brown	Brown	Opaque	Round	Medium
G3A	50 (74F6)	DM 5.8 BMX APOLO RR	MAR.M4	Light brown	Light yellow	Brown	Medium	Oval	Big
G4A	46 (85F6)	FPS NETUNO RR	DM 5.8 BMX APOLO RR	Medium brown	Light yellow	Yellow	Medium	Round	Big
G5A	60 (99F6)	BMX POTÊNCIA RR	MAR.M4	Very light brown	Light yellow	Brown	Medium	Oval	Medium
G6A	58 (62F6)	BMX TURBO RR	9471 IPRO	Dark brown	Light yellow	Brown	Medium	Round	Big
G7A	45 (91F6)	FPS NETUNO RR	DM 5.8 BMX APOLO RR	White	Light yellow	Yellow	Medium	Oval	Big
G8A	59 (103F6)	BMX POTÊNCIA RR	NS 5909 RR	Medium brown	Light yellow	Brown	High	Round	Big
G9A	47 (90F6)	MAR.M4	NS 5909 RR	Black	Light yellow	Black	Medium	Oval	Big
G10A	54 (10F6)	FPS NETUNO RR	DM 5.8 BMX APOLO RR	Black	Light yellow	Black	Medium	Oval	Big
G11T	55i57RSF IPRO	-	-	Brown	Light yellow	Brown	Medium	Spherical	Big

PP: paternal parent; MP: maternal parent; CC: coat color; HAC: halo color; SB: seed brightness; SSH: seed shape; SSI: seed size

To quantify *total phenolic compounds* (TPC), *total flavonoids* (FLA) and *antioxidant activity* (ABTS), a previous experiment was carried out with extractive conditions related to temperature and extraction method. As there is no consensus or even complete optimization of these compounds for soybeans, two methods were tested, adapting the methodologies of previous studies (Chung *et al*., 2010; Malenčić *et al*., 2008, 2012). For this, 0.5 g of ground and dried grains were placed in tubes, with the addition of 15 mL and 10 mL of 70% ethanolic solution in each Falcon tube. Subsequently, each sample was homogenized by vortexing for one minute. This step was performed equally in both methods. In one test, extraction was tested in a water bath (method 1) and in the other at room temperature (25 ^{0}C) in an ultrasonic bath (method 2). The same amounts and type of solvent were used for both methods. From the homogenized sample, in method 1, the samples were left in a water bath for 20 min at a temperature of 60 °C. For method 2, the samples were subjected to an ultrasonic bath for 20 min at room temperature. Every 10 min, samples from both methods were homogenized again using vortexing. Following homogenization, the samples were centrifuged at 10,000 rpm for 10 minutes to obtain the supernatant, which represented the extract. This extract was then used for the subsequent analyses described below. All analyzes were conducted in triplicate to ensure accuracy and reproducibility.

Total phenolic compounds (TPC) of soybean extracts were determined by the Folin-Ciocalteu method, as described by Singleton *et al*. (1999). The absorbance of the extract was measured at 740 nm in a spectrophotometer (UV–Vis Bel Photonics, 2000 Piracicaba, Brazil) and expressed in mg GAE g^{-1} (GAE: gallic acid equivalent). *Total flavonoids* (TF) were obtained by the method described by Park *et al*. (1995). The absorbance of the extract was measured at 415 nm in a spectrophotometer (UV–Vis Bel Photonics, 2000 Piracicaba, Brazil), expressed in µg of quercetin g^{-1}. The *antioxidant activity* called *ABTS* (ABTS+ scavenging activity) was measured according to the methodology described by Re *et al*. (1999). Absorbance was measured at 734 nm and results were expressed in mM TEAC g^{-1} of sample (TEAC: antioxidant capacity equivalent to Trolox). The ferric reducing antioxidant power (FRAP) of the extracts was determined by the procedure described by Pulido *et al*. (2000), based on the antioxidant's ability to reduce Fe^{+3} to Fe^{+2} in the presence of 2,4,6-tri(2-pyridyl)1,3,5-triazine (TPTZ). Absorbance readings were performed on a spectrophotometer at 595 nm. The results were expressed in mM Fe^{+2} g^{-1}.

The data were subjected to the Shapiro-Wilk, Bartlett and Durbin-Watson tests to verify compliance with the assumptions of normality and homogeneity of residual variances, respectively. After the assumptions were met, analysis of variance was performed to identify the effect of genotypes and extraction methods, as well as their interaction between genotypes x extraction methods, using the F test ($p < 0.05$). If a significant effect was found, the means were grouped by Tukey at 5% significance. The linear correlation coefficients between pairs of characters were calculated, and the significance of the coefficients was verified by Student's t test at 5% probability. The grouping of genotypes regarding antioxidants, bioactive and nutritional characters was carried out using the k-means method, to partition the points into k groups using the Hartigan and Wong algorithm (1979). Mean values were analyzed through eigenvalue and eigenvector estimation by compiling pedigree *x* variable effects using a Biplot principal components analysis. All analyzes were performed using the *emmeans*, *k-means*, metan, and *ExpDes.pt* packages in the R software environment (R Core Team, 2023).

RESULTS AND DISCUSSION

The analysis of variance revealed that all agronomic and proximate traits varied significantly ($p < 0.05$) depending on the soybean genotypes (Table 2). This indicates the existence of wide genetic variability among genotypes for components presented in Table 2, corroborating Sales *et al*. (2016) who point out that the chemical composition of grains is strongly influenced by the genetics of the cultivars.

In Table 3, it is observed that linoleic acid is the predominant component, ranging from 55.89% to 61.44%. Following that, oleic acid appears with values between 20.96% and 29.56%. Palmitic acid ranges from 8.16% to 10.47%, while stearic acid is present in concentrations ranging from 3.43% to 3.95%. Finally, linolenic acid shows the smallest variation, with values between 0.39% and 3.42%. These data are in accordance with other reported studies (Desta *et al*., 2022; Lee *et al*., 2017). Among the evaluated genotypes, the G9A genotype showed higher performance for the proximate characters palmitic fatty acid (10.48%) and linolenic fatty acid (3.43%) (Table 3). The G8A genotype presented higher mean values of stearic fatty acid (3.96%), while the G2M genotype presented superiority in oleic fatty acid (29.57%). The G6A genotype stood out for its linoleic fatty acid content, with an average of 61.44%. In relation to agronomic characters, the superiority of the G11T genotype was demonstrated for the one hundred seed weight and lipids, with averages of 20.34 and 22.55%. When evaluating the protein content, the G2M and G10A genotypes differed from the others, with values of 33.54 and 33.67%. As assessed by Lee *et al*. (2017), the increase in the one hundred grain weight results in a reduction in protein content, even demonstrating that genotypes with yellow and brown seed coats are more stable for this character, which corroborates the findings of the present study. For potassium (K) content, genotypes G9A and G11T had the best performance, and G10A was intermediate, with values of 7.27%, 7.71% and 6.95%, respectively. Regarding sodium (Na) content, superiority was observed for the G8A group; G9A; G10A, with averages of 237.13%, 237.76%, and 236.99%, respectively. Therefore, it can be inferred that these genotypes tend to present satisfactory levels of electrolytes in the seed.

There was a significant interaction between genotypes and extraction methods for the variables ABTS (ABTS+ scavenging activity) and total flavonoids (FLA). However, for total phenolic compounds (TPC) and ferric reducing antioxidant power (FRAP), significant effects were observed for both factors independently, with no interaction (Table 4). The coefficient of variation was 12.23% for ABTS, 5.66% for phenols, 5.53% for flavonoids and 5.20% for FRAP.

For the ABTS variable, the G6A genotype was found to be superior in both methods, besides to the G10A genotype for the Ultrasonic Bath (UB) method (Table 5). In the case of the total flavonoids variable, the genotypes G10A and G11T showed superiority for the Water Bath (WB) method, while for the UB method only G10A was superior. A similar response was seen for total flavonoids,

Table 2 - Summary of analysis of variance represented by mean square for agronomic and proximate components in different soybean genotypes for palmitic fatty acid (PAL_AC_C), stearic fatty acid (STE_AC_C), oleic fatty acid (OL_AC_C), linoleic fatty acid (LIN_AC_C), linolenic fatty acid (LINOLEN_AC_C), hundred seed weight (HSW), neutral detergent fiber (NDF), acid detergent fiber (ADF), potassium (K), sodium (Na), moisture, and ash

SV	DF	PAL_AC_C	STE_AC_C	OL_AC_C	LIN_ AC_C	LINOLEN_AC_C
Genotype	10	2.33E + 00*	6.98E - 02*	1.94E + 01*	9.22E + 00*	2.98E + 00*
Block	2	3.67E + 00	3.67E + 00	3.67E + 00	3.67E + 00	3.67E + 00
Residuals	20	1.93E - 29	1.06E - 30	1.57E - 28	2.65E - 30	2.90E - 31
SV	DF	HSW	K	Na	Moisture	Ash
Genotype	10	8.21E + 00*	11.03E + 00*	1.91E + 04*	2.10 E + 00*	1.18E + 00*
Block	2	3.67E + 00	4.00E + 00	3.00E + 00	4.14E + 00	4.07E + 00
Residuals	20	8.61E - 31	0.08E + 00	1.42E - 01	0.08E + 00	4.53E - 03
SV	DF	Protein	Lipids	NDF	ADF	
Genotype	10	13.64E + 00*	24.34E + 00*	264.34E + 00*	49.18 E + 00*	
Block	2	3.44E + 00	2.82E + 00	24.14E +00	4.32E + 00	
Residuals	20	0.05E + 00	0.16 E + 00	11.36E + 00	3.90E + 00	

SV: source of variation; DF: degrees of freedom

and the G10A genotype obtained superior performance, regardless of the method, together with G11T for WB. When comparing the extraction methods, higher averages of ABTS and FLA were found for the UB method, abstracting from the genotype used. Such results can be justified considering that the optimal temperature for

Table 3 - Grouping of means (%) for eleven soybean genotypes

Genotypes	PAL_AC_C	STE_AC_C	OL_AC_C	LIN_ AC_C	LINOLEN_AC_C
G1M	8.41 i	3.43 h	27.73 b	57.14 j	0.86 h
G2M	8.75 f	3.41 i	29.56 a	55.89 k	0.39 j
G3A	8.71 g	3.62 f	22.74 g	59.84 e	2.96 b
G4A	9.51 e	3.56 g	22.52 h	61.42 b	0.63 i
G5A	9.93 d	3.76 b	20.96 k	59.96 c	2.44 c
G6A	8.49 h	3.68 d	22.40 i	61.44 a	0.86 h
G7A	8.16 k	3.68 d	24.31 d	59.90 d	1.50 e
G8A	10.44 b	3.95 a	24.12 e	58.07 g	1.23 g
G9A	10.47 a	3.69 c	22.39 j	57.93 h	3.42 a
G10A	10.02 c	3.76 b	23.69 f	58.59 f	2.21 d
G11T	8.38 j	3.67 e	25.36 c	57.86 i	1.47 f
Genotypes	HSW	K	Na	Moisture	Ash
G1M	14.29 k	3.82 c	78.56 c	11.52 ab	4.63 a
G2M	15.25 j	3.38 cd	78.61 c	12.30 ab	2.43 d
G3A	18.49 b	3.32 cd	78.56 c	11.34 bc	4.01 c
G4A	17.07 d	3.92 c	78.87 c	11.37 bc	4.21 b
G5A	16.12 g	3.87 C	79.03 c	10.65 cd	4.36 b
G6A	16.33 e	2.69 d	78.61 c	10.66 cd	4.31 b
G7A	16.32 f	2.78 d	78.82 c	9.93 d	4.30 b
G8A	17.66 c	6.34 b	237.13 ab	12.16 Ab	4.21 b
G9A	15.71 i	7.26 a	237.76 ab	10.54 cd	4.65 a
G10A	16.05 h	6.94 ab	236.99 ab	9.91 d	4.77 a
G11T	20.33 a	7.71 a	236.35 b	10.19 d	4.17 bc
Genotypes	Protein	Lipids	NDF	ADF	
G1M	29.07 e	16.89 e	45.76 abc	21.75 a	
G2M	33.53 a	14.21 fg	46.07 abc	19.71 ab	
G3A	29.57 de	16.32 e	44.59 abc	20.07 a	
G4A	30.20 de	13.88 g	42.31 abc	19.05 ab	
G5A	32.74 B	14.93 fg	45.83 abc	20.86 a	
G6A	34.19 a	15.12 fg	50.00 ab	19.94 ab	
G7A	28.94 e	17.11 de	50.60 a	17.29 ab	
G8A	31.54 c	19.76 c	37.59 c	17.55 ab	
G9A	31.91 c	18.17 de	40.54 bc	14.16 b	
G10A	33.66 a	21.06 b	16.57 d	7.97 c	
G11T	28.24 f	22.54 a	46.98 abc	21.73 a	

PAL_AC_C: palmitic fatty acid; STE_AC_C: stearic fatty acid; OL_AC_C: oleic fatty acid; LIN_ AC_C: linoleic fatty acid; LINOLEN_AC_C: linolenic fatty acid; HSW: hundred seed weight; K: potassium; Na: sodium; NDF: neutral detergent fiber; ADF: acid detergent fiber. Average in the same column with different superscripts (a-k), for the same group of samples, show statistical differences ($p \leq 0.05$) according to the Tukey test

Table 4 - Analysis of variance for the effects of genotype and extraction method

SV	DF	ABTS	TPC	FLA	FRAP
		MS	MS	MS	MS
Genotypes	10	20.32	0.18	44947.1	11.84
Methods	1	74.02	3.29	200086	116.71
Genotypes x Methods	10	1.86	0.04	5876.1	0.45
Residuals	44	0.24	0.03	133.84	0.03
Total	65	4.71	0.09	10987.8	4.02
CV (%)	-	12.23	5.66	5.53	5.20

ABTS: ABTS•+ scavenging activity; TPC: total phenolic compounds; FLA: total flavonoid compounds; FRAP: ferric reducing antioxidant power; SV: source of variation; DF: degrees of freedom; MS: mean square; CV: coefficient of variation; *: significant at 5% by F test

Table 5 - Breakdown of the simple effects of the interaction between genotypes and extraction methods on the variables ABTS (ABTS+ scavenging activity) and FLA (total flavonoid compounds) and analysis of the main effects of genotypes on the variables FRAP (ferric reducing antioxidant power) and TPC (total phenolic compounds). CV: coefficient of variation. Means followed by the same lowercase letter (a-f) in the column and capital letter (A-B) in the row are not different from each other by the Tukey test grouping of means ($p < 0.05$)

	ABTS		FLA	
	Extraction Method		Extraction Method	
Genotypes	Water Bath	Ultrasonic Bath	Water Bath	Ultrasonic Bath
G1M	0.61 fB	1.04 eA	40.27 eB	151.79 fA
G2M	1.97 dB	4.37 dA	120.87 cB	245.26 dA
G3A	1.62 eB	4.14 dA	111.10 cB	239.99 dA
G4A	1.44 eB	3.63 dA	181.89 bB	202.88 eA
G5A	3.30 cB	3.87 dA	108.55 cB	265.11 cA
G6A	6.10 aB	8.20 aA	94.69 cB	271.89 cA
G7A	2.59 dB	4.51 dA	74.81 dB	248.77 dA
G8A	3.65 cB	5.57 cA	179.64 bB	349.98 bA
G9A	4.12 cB	5.57 cA	77.98 dB	185.18 eA
G10A	4.80 bB	8.86 aA	358.13 aB	385.76 aA
G11T	2.68 dB	6.42 bA	347.73 aB	360.38 bA
CV (%)	12.23		5.53	

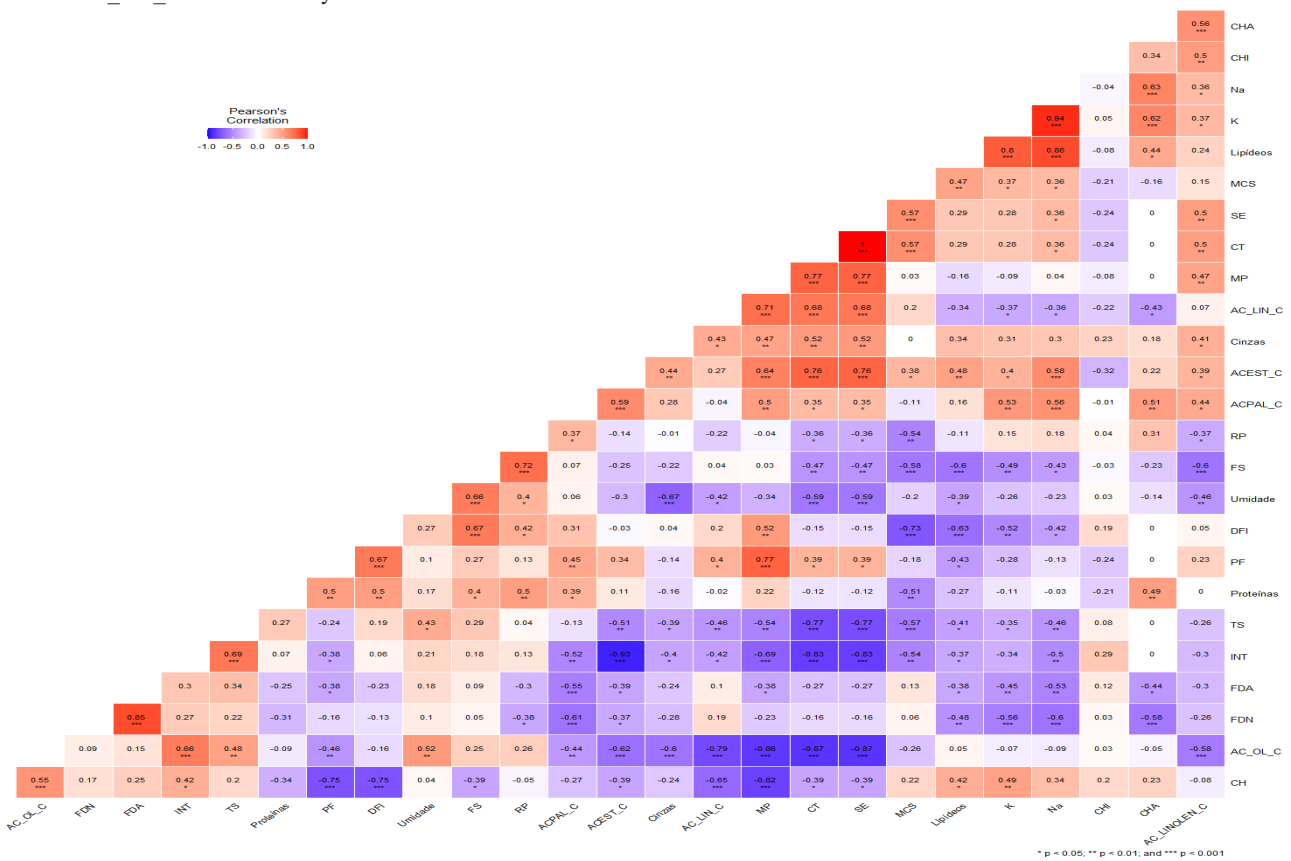
Genotypes	FRAP	TPC
G1M	9.71 d	3.23 c
G2M	9.05 d	3.27 c
G3A	11.30 b	3.84 a
G4A	10.43 c	3.38 c
G5A	10.23 c	3.38 b
G6A	11.10 b	3.54 b
G7A	10.17 c	3.29 c
G8A	12.49 a	3.58 b
G9A	12.60 a	3.49 b
G10A	13.28 a	3.27 b
G11T	12.72 a	3.59 b
CV (%)	5.20	5.66

extracting antioxidant compounds is 40 ºC (Khonchaisri *et al*., 2022), which may explain the gains in the extraction of bioactive compounds obtained by the UB method. According to Yang *et al*. (2021), the solubility of the compounds increases up to the extraction range of 40 ºC, with a reduction of 17.2% when raising the temperature to 50 ºC. The contribution of temperature to the solubility of soluble proteins is related to intermolecular and intramolecular interactions (Zenker *et al*., 2020).

For the main effect of genotypes on the variable FRAP, the genotypes G7A, G8A, G9A, G10A and G11T were grouped, being considered superior, with averages of 12.49, 12.60, 13.28 and 12.72%, respectively. For the TPC variable, only G3A was superior, with an average of 3.84%, and superiority was again given to the UB extraction method.

Figure 1 presents the linear correlations (r value) between the agronomic and proximate attributes, and a positive correlation was inferred among linolenic fatty acid and halo color (r = 0.56), hypocotyl color (r = 0.5), sodium (r = 0.36), potassium (r = 0.37), green seeds (r = 0.5), seed coat color (r = 0.5), purple spot (r = 0.47), ash (r = 0.41), stearic acid (r = 0.39), palmitic acid (r = 0.44); negative correlation with peroxidase (r = -0.37), seed shape (r = -0.6), moisture (r = -0.46), and oleic acid (r = -0.58). There was a positive correlation among stearic fatty acid (STE_AC_C), palmitic acid (r = 0.59), ash (r = 0.44), purple spot (r = 0.64), IC (r = 0.76), green seeds (r = 0.76), one hundred seed weight (r = 0.38), lipids (r = 0.48), potassium (r = 0.4), sodium (r = 0.58), and linolenic fatty acid (r = 0.39), while a negative correlation was estimated with hilum color (r = -0.39), oleic fatty acid (r = -0.62), neutral detergent fiber (r = -0.37), acid detergent fiber (r = -0.39), whole seeds (r = -0.93), and seed size (r = -0.51). Some other correlations are also worth highlighting, such as the positive linear relationship between lipids content and sodium (r = 0.86) and potassium (r = 0.8), as well as the existence of a perfect correlation (r = 1.0) between seed coat color and green seeds. Of the negative correlations, it was assessed that the oleic fatty acid content is inversely proportional to most of the characters evaluated.

Figure 1 - Linear correlation between agronomic and proximate attributes evaluated in soybeans. OL_AC_C: oleic fatty acid; NDF: neutral detergent fiber; ADF: acid detergent fiber; WHO: brightness intensity; SSI: seed size; PF: presence of fungi; PD: physical damage; SSH: seed format; PR: peroxidase; PAL_AC_C: palmitic fatty acid; STE_AC_C: stearic fatty acid; PS: purple spot; CC: coat color; GS: green seed; HSW: hundred seed weight; K: potassium; Na: sodium; HYC: hypocotyl color; HAC: halo color; LINOLEN_AC_C: linolenic fatty acid and HC: hilum color



Based on Figure 1, linolenic acid, a polyunsaturated fatty acid (omega-3), showed positive correlations with several agronomic traits (halo color, r = 0.56; hypocotyl color, r = 0.5; green seeds, r = 0.5; seed coat color, r = 0.5; and purple spot, r = 0.47). These correlations suggest a favorable relationship between linolenic acid content and phenotypic traits such as color and hypocotyl color, which could be useful in the visual selection of genotypes with high levels of beneficial fatty acids. Furthermore, the positive correlation between linolenic acid and minerals such as sodium (r = 0.36) and potassium (r = 0.37), as well as the ash content (r = 0.41), suggests a potential interaction between these nutrients and lipid compounds, which could influence plant mineral nutrition and the nutritional value of the seeds. The positive correlation of stearic acid with important chemical and agronomic traits also suggests that this saturated fatty acid may be associated with a more balanced nutritional composition and overall seed quality. The negative correlation of stearic acid with some traits, such as oleic acid, suggests a balance between different fatty acids in the lipid composition, which may impact nutritional quality. Abdelghany *et al.* (2020) observed a positive correlation between linolenic acid and linoleic acid (r = 0.29), as both are polyunsaturated fatty acids and share biosynthetic pathways. Additionally, the negative correlation between oleic acid and linolenic acid, observed by other researchers (Abdelghany *et al.*, 2020; Lee *et al.*, 2017) and in this study, suggests a consistent antagonistic behavior between these compounds. In another study (Fallen *et al.*, 2012), linoleic acid was highlighted as a central point, with significant correlations, including a strong inverse relationship with oleic acid (r = -0.8), while the results from this study generally presented relevant correlations with linolenic acid. Overall, the reported findings are important for understanding the biochemical and physiological mechanisms involved in seed formation and may guide management and breeding strategies to balance productivity, nutritional quality, and desirable physical traits.

Positive correlations were estimated among ABTS, TPC, FRAP and flavonoids for both the WB method (Figure 2a) and the UB method (Figure 2b). This indicates that the extraction of one compound does not result in antagonism or inhibition of the extraction of another. Carvalho *et al.* (2017), characterizes the magnitudes of correlations as null (r = 0), weak (r = 0.01 and r = 0.30), medium (r = 0.31 and r = 0.60), strong (r = 0.61 and r = 0.90) and very high coefficients greater than r = 0.91. For the WB method, there was a strong correlation between ferric reducing antioxidant power and phenols (r = 0.65) and between ferric reducing antioxidant power and flavonoids (r = 0.64), as well as a medium magnitude correlation between phenols and ABTS (r = 0.43) and ferric reducing antioxidant power and ABTS (r = 0.59). Linear relationships of strong magnitude were observed between flavonoids and ABTS (r = 0.74), flavonoids and FRAP (r = 0.65) and ABTS and ferric reducing antioxidant power (r = 0.68) when using the UB method. To this end, the quantitative superiority of compounds extracted by the UB method can be attributed to the multiple extraction capacity, which is greater than the WB method.

Figure 2 - Linear correlation for the compounds extracted considering the Water Bath extraction method (a) and the Ultrasonic Bath extraction method (b). ABTS: TPC: total phenolic compounds; FRAP: ferric reducing antioxidant power and FLA: flavonoids

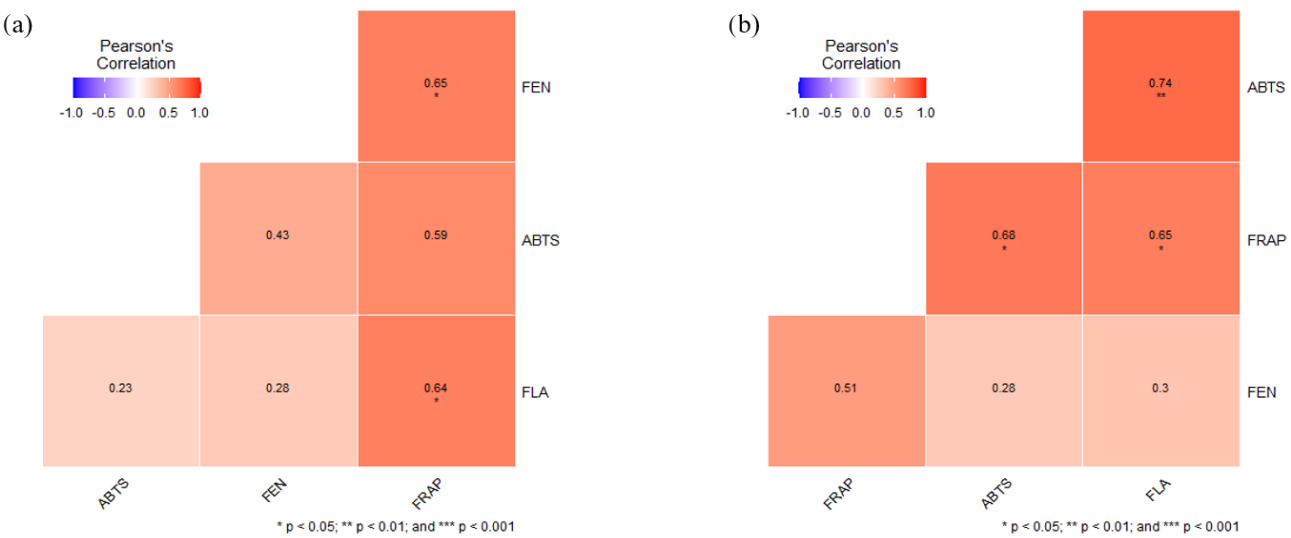


Figure 3 - K-Means clustering of principal components Biplot for agronomic and proximate attributes of 11 soybean genotypes (G1M; G2M; G3A; G4A; G5A; G6A; G7A; G8A; G9A; G10A; G11T). OL_AC_C: oleic fatty acid; NDF: neutral detergent fiber; ADF: acid detergent fiber; PAL_AC_C: palmitic fatty acid; STE_AC_C: stearic fatty acid; HSW: hundred seed weight; K: potassium; Na: sodium and LINOLEN_AC_C: linolenic fatty acid

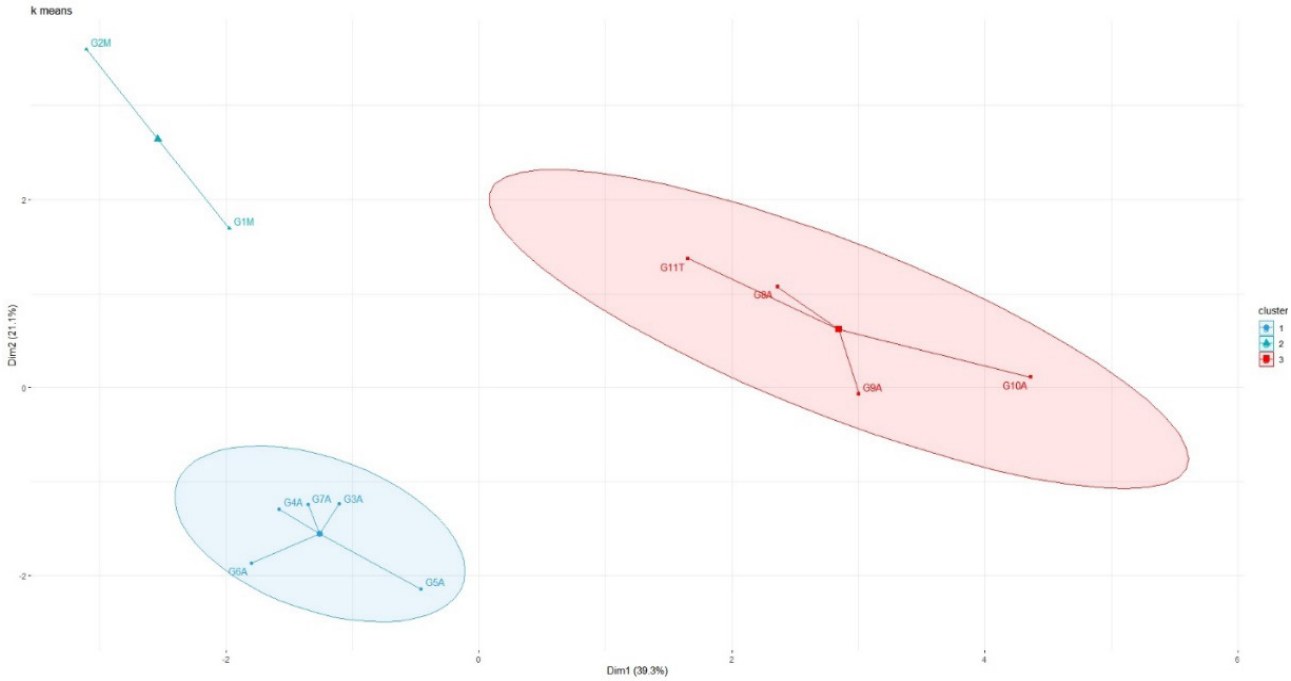
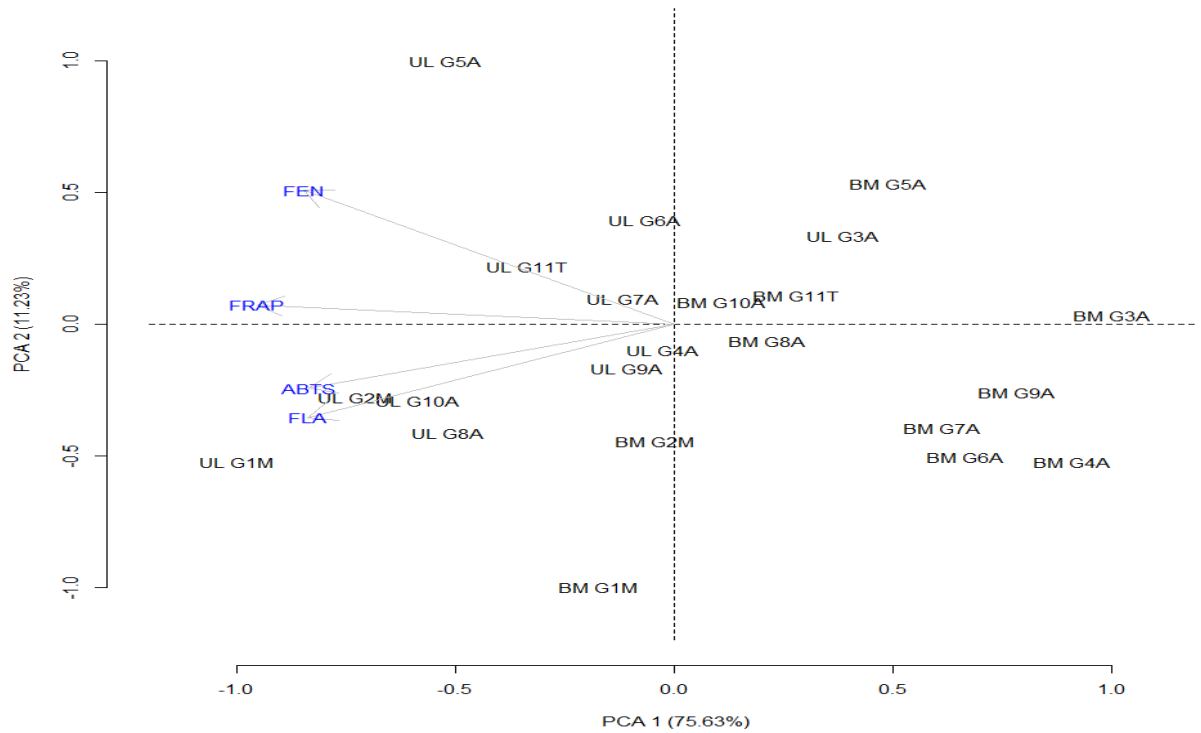


Figure 4 - Main component analysis Biplot of compounds extracted from 11 soybean genotypes (G1M; G2M; G3A; G4A; G5A; G6A; G7A; G8A; G9A; G10A; G11T) and two extraction methods (Water Bath – WB; Ultrasonic Bath - UB). ABTS (ABTS+ scavenging activity); TPC: total phenolic compounds; FLA: flavonoids; FRAP: ferric reducing antioxidant power



The first two principal components explained 60.4% of the total variation in the data (39.3% and 21.1%, respectively), which was sufficient to associate the genotypes based on their similarities. In this context, the G1M and G2M genotypes were grouped in the same cluster, as they have similar fatty acid levels, with the brown coat distinguishing them from the other genotypes that have a yellow coat. Considering the characteristics presented by the control (G11T), the genotypes G8A, G9A, and G10A were grouped due to the characteristics of proximate composition, which presented higher potassium and sodium content in the grain, in addition to a FRAP in these genotypes.

For the analysis of main components (PCA1: 75.63%; PCA2: 11.23%) regarding the interaction between genotypes and extraction methods (Figure 4), it was observed that the UB method allowed a higher intensity extraction of compounds ABTS, flavonoids, FRAP, and TPC. Due to the proximity presented between ABTS and flavonoids and UBG10A and UBG8A, it is estimated that the UB extraction method favored the extraction of these compounds in the genotypes. Furthermore, it is estimated that G1M and G2M have an innate ability to express and promote higher levels of ABTS and flavonoids. This observation is supported by the fact that, even though the WB method resulted in a lower number of extracted compounds, the eigenvalues of these genotypes were positioned close to those of the genotypes subjected to the UB method.

There was no specific proximity between the phenol and FRAP eigenvectors and the evaluated genotypes suggesting that the amount of these compounds extracted was not significantly different.

CONCLUSIONS

Genotypes with brown seed coats, particularly G2M, demonstrated high potential for protein and oleic acid content, reinforcing their suitability for biofortification and as a base for food products with enhanced nutritional value. Additionally, the Ultrasonic Bath extraction method proved to be the most effective for extracting the ABTS antioxidant radical and total flavonoids, further emphasizing the importance of optimizing extraction conditions to maximize bioactive compound recovery. Notably, both G1M and G2M exhibited higher levels of ABTS and flavonoids, underscoring their strong antioxidant capacity and bioactive potential. These findings highlight the relevance of genotype selection and extraction methods in enhancing the nutritional and functional properties of soybeans, supporting their application in biofortification strategies aimed at improving human nutrition.

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