A high-protein soybean cultivar contains lower isoflavones and saponins but higher minerals and bioactive peptides than a low-protein cultivar

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ABSTRACT

Soybean is a major source of protein and other nutrients and non-nutrient bioactives for human health. The objective was to compare the bioactive compounds of a low-protein (BRS 133) soybean in comparison to a high-protein (BRS 258) soybean cultivar. The high-protein soybean contained 17% lower carbohydrates and a lower chemical score (63) in relation to the low-protein soybean, which had a higher chemical score (76), associated with the higher methionine content (1.2%). Cultivar BRS 258 had more calcium (15.5%), phosphorus (30.1%), iron (18.7%), copper (9.0%) and zinc (11.5%), and a higher concentrations of lunasin, BBI and lectin (20.3%, 19.0% and 27.1%, respectively) than the low-protein cultivar. BRS 133 had 75.4% higher concentration of total isoflavones (5.1% of total aglycones) and 31.0% total saponins, as compared to BRS 258. It was concluded that the low-protein soybean cultivar contained higher isoflavones and saponins, but lower levels of minerals and bioactive peptides, such as lunasin.

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1. Introduction

Soybean (*Glycine max* L. Merrill) is a legume that is consumed worldwide. Recently, soybean foods have generated interest because of their reported beneficial effects on nutrition and health. Studies have shown that Asian populations habitually consuming soybean products have a lower risk of osteoporosis and some chronic diseases, most notably heart disease and cancer (McCue & Shetty, 2004).

Bioactive compounds present in legumes vary greatly with the plant species, cultivar, weather and geographical sowing location. Soybean is a complex food matrix containing low or no starch, about 20% oil and 40% high-quality protein (Liu, 1997), in addition to several important bioactive compounds, including lunasin, trypsin inhibitors, isoflavones, and saponins. Lunasin is a unique 43 amino acid novel cancer preventive peptide originally isolated from soy (de Lumen, 2005; Gonzalez de Mejia, Vasconez, DeLumen, & Nelson, 2004). Bowman–Birk inhibitor (BBI) is a 71 amino acid peptide with seven disulphide bonds, which stabilize an active configuration, and has a double head with the chymotrypsin inhibitor domain and the trypsin reactive site (Birk, 1985). Soybean lectin has both anti-nutritional and anti-carcinogenic properties (Gonzalez de Mejia & Prisecaru, 2005; Vasconez-Costa, 2004). Lectins accumulate in protein storage vacuoles of the cotyledons and are degraded during seed germination and maturation (Pusztai, 1991). Lipids are an important source of the compounds responsible for flavour in soybean products. Soybean seeds are a major source of isoflavones including genistein, daidzein and glycitein and their corresponding glycosides genistin, daidzin and glycitin, as well as their malonyl and acetyl conjugates (Anderson & Wolf, 1995). The isoflavone glycosides are present primarily as β-D-glucosides and a portion of the glucosides are substituted on the C-6 hydroxyl of the glucose by a malonyl group, especially in the seed hypocotyls (Anderson & Wolf, 1995). Research concerning soybean isoflavones has revealed their protective effect in health associated

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with menopause, cancer and cardiovascular disease. Some other health benefits are under investigation (McCue & Shetty, 2004). During processing, some loss and/or shifting of the distribution profile of isoflavones may occur (Mandarino, Carrão-Panizzi, & Oliveira, 1992). The main isoflavones found in unprocessed soy flour, malonylgenistin, genistin, malonyldaidzin and daidzin, are converted into their aglycones and acetylglycoside forms. Saponins are plant terpenoids which are conjugated to two or more sugar molecules in soy. On the basis of their aglycone structures, the saponins present in the mature bean have been divided into group B and group A soy saponins (Berhow, Kong, Vermillion, & Duval, 2006). Group B soy saponins appear to exist in the intact seed tissue as conjugates of 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), at the 22-hydroxyl position (Kuduo et al., 1993). The DDMP conjugates are relatively labile and are easily degraded, most likely resulting in the formation of the non-DDMP group B soy saponins. The other forms of the group B soy saponins arise from alternate sugars attached to the 3-hydroxyl position of the aglycone. The group A soy saponins are didesmosidic with alternate sugar composition in both sets of oligosaccharides attached to the aglycone at the 3- and 21-hydroxyl positions (Shiraiwa, Kudo, Shimoyamada, Harada, & Okubo, 1991).

Based on the available scientific evidence, the Food and Drug Administration (FDA) has allowed a health claim (FDA, 2002). The beneficial effect is attributed to the protein fraction and a minimum daily intake of 25 g of soy protein should be necessary for the health effect to be significant (Messina & Erdman, 2005).

The objective of this study was to compare the relative content of a low-protein and a high-protein soybean from Brazilian cultivars on their nutrients, proteinaceous bioactive compounds (lunasin, Bowman–Birk inhibitor and lectin) and non-proteinaceous bioactive compounds (isoflavones and saponins).

2. Materials and methods

2.1. Materials

The breeding program of Embrapa Soybean, Brazil has developed the conventional low-protein cultivar (BRS 133) and the high-protein (BRS 258) cultivars aimed to meet consumer needs (EMBRAPA, 2008). BRS 133 was produced in the region of Ponta Grossa, while cultivar BRS 258 was produced in the region of Guarapuava. Both regions are in Paraná State, Brazil, and were provided by Embrapa Transferência de Tecnologia, Brazil.

Immunoaffinity purified lunasin (98%) from soy and mouse monoclonal antibody against the lunasin epitope–EKHIME-KIQGRGDDDDD were provided by Dr. Ben O. de Lumen, of the University of California at Berkeley, USA. Purified groups A and B soy saponins were prepared in the Peoria laboratory (USDA) (Berhow et al., 2006).

The primary polyclonal antibody specific for lectin from soybean was provided by Dr. Theodore Hymowitz from the Department of Crop Sciences, University of Illinois at Urbana-Champaign, IL, USA. The lectin anti-serum was obtained at his laboratory by immunizing young male New Zealand white rabbits with 5 mL subcutaneous injections of an emulsion containing 5 mg of pure lectin, 1 mL of distilled water and 1 mL of Freund's complete adjuvant. Six weeks after the first immunization, rabbits showing response to the antibodies (measured 20 days after the first injection) were injected again with a similar dose of lectin and bled two weeks later.

The beans were sanitized for 10 min with a sodium hypochlorite solution (100 mg/L) and immediately washed three times with distilled water. The sanitized grains were frozen at $-30\,°C$ for 4 h, freeze-dried and milled to produce soybean flour. Whole soy flours were obtained in a refrigerated hammer mill, model 680 from Mar-

coni (Piracicaba, Brazil), and the powders stored at $7\,°C$, conditioned in air-tight glass.

2.2. Determination of the weight of 1000 soybean seeds

The weight of 1000 seeds was determined by weighting eight replicates of 100 seeds each (BRASIL, 1992).

2.3. Proximate composition

Moisture, total proteins and ash of the whole flour (WSF) were determined by the AACC (1995) procedures 44–15, 46–13 and 08–12, respectively. The conversion factor 5.71 for protein was used. Lipids, total sugars and starch were determined according to the methods of the Adolfo Lutz Institute (Instituto Adolfo Lutz, 1985). Total carbohydrates, including total fibre, were determined by difference. Dietary, soluble and insoluble fibres were determined following the AOAC (1997) procedure 991.43. Metabolizable energy of the flours was estimated by multiplying the protein and carbohydrate contents by 4 kcal per gram and fat by the factor of 9 kcal per gram.

2.4. Physical and physicochemical characteristics

Colour was determined by means of a Color Quest II Hunterlab instrument (Reston, VA), determining the components L^* (lightness), a^* (green $-$/red $+$) and b^* (blue $-$/yellow $+$), according to the CIE-$L^*a^*b^*$ system. The chrome (C^*) and hue angle (h^*) values were calculated as described by Minolta. The chrome value was calculated as shown in Eq. (1), and the saturation angle as shown in Eq. (2).

$$\text{Chrome } (C^*) = [(a^*)^2 + (b^*)^2]^{1/2} \tag{1}$$
$$h^* = \tan^{-1}(b^*/a^*) \tag{2}$$

Particle size was determined in a Granutest, Model 295, instrument, according to procedure 965.22 of the AOAC (1997).

For pH, procedure 943.02 of the AOAC (1997) was followed and the water activity was determined in triplicate using an AquaLab, series 3, Model TE equipment.

2.5. Fatty acid composition

The procedure of Hartman and Lago (1973) was followed for the esterification step and gas chromatography (Agilent 6850 series GC System, Santa Clara, CA, USA) for the analysis of the fatty acid methyl esters, using a capillary column (Agilent DB-23; 50% cyanopropyl-methylpoly-siloxane; 60 m × 0.25 mm). Instrument operating conditions were: detector temperature (280 °C), injector temperature (250 °C), oven temperature (110 °C) for the first 5 min, followed by increases of 5 °C/min to reach 215 °C and holding the temperature at 215 °C for 24 min. Helium was used as carrier gas, and the injection volume was 1.0 μL, split 1:50.

The iodine index was determined according to procedure Cd 1d-92 of the AOCS (2002), and the saponification index according to procedure Cd 1c-85, of the AOCS (2002) and calculated on the basis of the fatty acid composition.

2.6. Minerals

Duplicate samples were calcinated at 500 °C to determine the dry ash content. Calcium and microelements were determined by atomic absorption spectrometry, according to procedure 968.08 of the AOAC (AOAC, 1997) in Metrolab equipment, Model 250. Phosphorus was determinate by spectrophotometry in UV Hitachi U-2000 equipment.

2.7. Total and free amino acid composition

After a 24 h hydrolysis in 6 M HCl at 100 °C, the amino acids were reacted with phenylisothiocyanate (White, Hart, & Fry, 1986) and the derivatives separated using a Luna C-18, 100 Å; 5 μ, 250 mm × 4.6 mm (00G-4252-E0, Torrance, CA, USA) column, at 50 °C. Quantification was carried out by comparison with a standard mixture (Thermo Scientific, Rockford IL, USA) and DL-2-aminobutyric acid was used as an internal standard from Sigma–Aldrich Corp., St. Louis, MO; (Hagen, Frost, & Augustin, 1989). Running time was 24 min. The free amino acids were determined by extracting 1.25 g flour samples in 80% ethanol solution containing 0.1 M HCl, and 500 μL of α-aminobutyric acid, added as internal standard, in a 5 mL volumetric flask. The mixture was sonicated for 10 min and further homogenised for 1 h, followed by centrifugation at 8500g for 15 min. The supernatant was filtered through a 0.22 mm membrane and a 40 μL aliquot derivatized as described above, for the injection of 20 μL into the HPLC.

2.8. Protein extraction

The protein extraction procedure consisted in placing 50 mg of soybean flour and 1 mL of the extracting buffer (0.05 M Tris–HCl pH 8.2) in an Eppendorf tube. After mixing, the samples were placed in an ultrasonic bath (Branson Ultrasonic Corporation, Danbury, CT) for 70 min, mixing them at every 10 min to avoid settlement. The water temperature was adjusted to 40 °C using a recirculation bath (Endocal model RTE-9, Neslab Instruments, Portsmouth, NH). Following extraction, the samples were centrifuged at 20,000 g for 30 min at 8 °C in an Eppendorf centrifuge (Brinkmann Instruments, model 5417R, Westbury, NY), and the obtained supernatant was transferred to a new Eppendorf tube. All samples were kept at −80 °C until analysis.

2.9. Determination of soluble protein concentration by DC assay

The protein concentration was determined using the Bio-Rad DC Microplate Assay Protocol (Bio-Rad Laboratories, Hercules, CA). Briefly, 5 μL of samples (1:20 dilution) were placed in a 96-well plate and treated with 25 μL of reagent A (alkaline copper tartrate solution) and 200 μL of reagent B (dilute Folin reagent) (Bio-Rad Laboratories, Hercules, CA). The plate was gently agitated and incubated for 15 min at room temperature. After incubation, the absorbance was measured at 630 nm. The protein concentration was calculated using pure bovine serum albumin standard curve ($y = 0.0002x − 0.0021$, $R^2 = 0.997$).

2.10. Enzyme-linked immunosorbent assay (ELISA) for lunasin and BBI

Lunasin concentration in soy flour was determined by ELISA (Gonzalez de Mejia et al., 2004) with the following modifications: 100 μL of protein extracts (1:5000 dilution) were placed in a 96-well plate and stored overnight (14 h). Lunasin mouse monoclonal antibody (1:4000 dilution) was used as first antibody and anti-mouse IgG alkaline phosphatase conjugate (1:7000) from Sigma–Aldrich Corp., St. Louis, MO as the secondary antibody. The reaction was stopped adding 25 μL NaOH (3 M) at 30 min and the absorbance read at 405 nm after 35 min. Similar procedure was used for BBI analysis, samples of 100 μL of protein extracts (1/10,000 dilution) were placed in a 96-well plate, except that BBI mouse monoclonal antibody (1:1000 dilution), Agdia Inc., Elkhart, IN, was used as the first antibody and anti-mouse alkaline phosphatase (AP) conjugated IgG (1:2000) Sigma–Aldrich Corp., St. Louis, MO as the secondary antibody.

Standard curves were determined using purified lunasin ($y = 0.0054x + 0.001$, $R^2 = 0.993$) and purified BBI ($y = 0.0108x + 0.0465$, $R^2 = 0.998$).

2.11. Western blot procedures

Identity of lunasin was established by Western blot analysis in protein extracts of soybean flour. Samples were centrifuged (20,000 g) at 8 °C to eliminate any precipitate. Unstained gels were soaked in 20 mL of blotting buffer (20% methanol, 80% Tris-glycine SDS) for 15 min. A Western blot sandwich was assembled by the following order: a sponge, filter, gel, polyvinylidene difluoride (PVDF) membrane Inmobilon™-FL (Millipore Corporation, Billerica, MA, USA), and another filter and sponge, being careful to avoid formation of bubbles, and then developed for 1 h at 110 V at 4 °C. After the complete transfer membrane was then saturated by incubation in 5% nonfat dry milk (NFDM) in 0.01% TBST (0.1% Tween 20 in Tris-Buffered saline) buffer for 1 h at 4 °C, and washed three times for 5 min with fresh changes of 0.01% TBST. The washed gel was incubated with lunasin mouse monoclonal antibody (1/1000 dilution) prepared in 1% NFDM and TBST buffer for 16 h at 4 °C. After washing the incubated membrane, the membrane Inmobilon TM-FL (Millipore Corporation, Billerica, MA, USA) was incubated with anti-mouse IgG alkaline phosphatase conjugate (1/10,000 dilution) prepared in 1% NFDM in TBST buffer for 3 h at room temperature. The membrane was prepared for detection using chemiluminescent reagent, 500 μL of solution A and 500 μL of solution B (Lumigen TM, GE Healthcare, Buckinghamshire, UK).

2.12. Enzyme-linked immunosorbent assay (ELISA) for lectin

Lectin concentration in soy flour was determined by ELISA (Vasconez-Costa, 2004) with the following modifications. One hundred microlitres of protein extracts (1:10,000 dilution) were placed in a 96-well plate. Lectin mouse polyclonal antibody (1:500 dilution) was used as the first antibody, and anti-rabbit IgG alkaline phosphatase conjugate (1:1000) from Sigma–Aldrich Corp., St. Louis, MO, as the secondary antibody. The reaction was stopped adding 25 μL of NaOH (3 M) at 30 min and the absorbance read at 405 nm after 35 min. Standard curves were determined using purified lectin ($y = 0.0101x + 0.0025$, $R^2 = 0.998$).

2.13. Isoflavone determination by HPLC

Quantitative analysis of isoflavones was carried out according to Berhow (2002). Approximately 250 mg defatted soybean flour were extracted in test tubes with 3.0 mL of dimethyl sulphoxide/methanol (1:4, v/v) placed in sealed containers and heated at 50 °C for 18 h. The extracts were centrifuged and the supernatants were filtered using 0.45 μm filters. For isoflavone quantification 20 μL aliquots of the extracts were injected into a Shimadzu (Columbia, MD, USA) HPLC system (LC-10AT VP) equipped with a SPDM10A VP photodiode array detector (CTO-10AS VP, providers, Columbia, MD, USA) an oven column to maintain temperature at 40 °C, all operating under the Class VP software. Isoflavone separation was carried out in a C18 reverse-phase column YMC – Pack ODS-AM 303, 250 mm × 4.6 mm and 5 μm particle size (YMC Inc., Wilmington, NC, USA). The initial gradient conditions consisted of 100% H_2O containing 0.025% trifluoroacetic acid (TFA), and 0% acetonitrile, to 45% H_2O and 55% acetonitrile, over 25 min with a flow rate of 1 mL/min. Isoflavones were detected at 260 nm and quantified by comparison with standard curves of genistin, daidzin and glycitin. The concentrations of the malonyl-glucosides and aglycones were calculated from standard curves of their corresponding β-glucosides, using the similarity of the molar extinction coefficients of malonyl-isoflavones and β-glucosides.

Isoflavone concentrations were expressed in mg/100 g of defatted samples.

2.14. Saponin determination by HPLC

Saponins from the soybean flour were extracted with dimethyl sulphoxide/methanol (1:1, v/v) solution at room temperature for 4 h, followed by a 15 min sonication at 50 °C and another 2 h extraction at room temperature. The extracts were then filtered through a 0.45 μm nylon filter. HPLC analysis was carried out on a Hewlett–Packard Series 1100 HPLC system (G1311A pump, G1322A degasser, G1313A auto injector, G1314A variable wavelength detector, and G1316A column oven) equipped with an Inertsil ODS-3 reverse-phase C-18 column (250 mm × 4.6 mm ID) and particle size of 5 μm, with a metaguard column (Varian, Torrance, CA, USA) and a G1316A column oven. The system was controlled by HPChem Station version A.06.01. For saponin analysis, a linear water–acetonitrile gradient from 30% to 50% in 45 min was used, with 0.025% TFA added to both solvents. The flow rate was 1 mL/min and the effluent was monitored at 210 nm. Saponin concentrations were calculated by using standard curves prepared from a standardised mix of B group saponins prepared in the Peoria laboratory. The nanomolar extinction coefficient for soy saponin I, was used to quantitate the remaining B group saponins, the A group saponins, and the DMPP (1,l-dimethyl-4-phenylpiperazinium) conjugated B group saponins. Identification of saponin peaks was confirmed by comparison of standards and/or LC–MS analysis (Berhow et al., 2006).

2.15. Statistical analysis

The data were submitted to analysis of variance by the SAS program, and the means of three replicates (unless otherwise stated) compared by the Tukey test, adopting the standard criterion of significance $p \leqslant 0.05$.

3. Results and discussion

3.1. Proximate composition

Cultivars BRS 133 and BRS 258 differed significantly in terms of size and weight. The weights of 1000 seeds were 129 and 227 g, respectively.

Proximate composition and the physicochemical characteristics of both cultivars are shown in Table 1. As expected, the remarkable difference in protein concentration was reflected in their carbohydrate concentration in agreement with previous publications (Mandarino et al., 1992; Viera, Cabral, & Oliveira de Paula, 1999). As pointed out by Morais and Silva (2000), although all oligosaccharides and disaccharides from soybean are fermentable, the 1–2% of raffinose and 3.5–4.5% of stachyose have an important bifidogenic role in the human intestine. In spite of the exact significance of soybean fibre in reducing the risk of colon cancer, and cardiovascular diseases not being completely elucidated, the potential health benefits of this fraction should not be neglected. Soybean hulls contain about 87% total fibre, made up mainly of cellulose, hemicellulose, lignin and uronic acids. The values found in this study were similar to those reported by Toledo, Brazaga, Arthur, and Piedade (2007) for various soybean cultivars.

3.2. Instrumental colour of the flours

The values found for the L^* parameter of the whole flours were 84.58 for cultivar BRS 133 and 81.37 for cultivar 258. Such high values indicated that both cultivars can produce light-coloured

Table 1
Chemical composition of the soybean cultivars BRS 133 and BRS 258 and physical characteristics of their whole flours.[A]

Analysis	Cultivar	
	BRS 133	BRS 258
Composition (g/100 g dry basis)		
Total protein	37.36 ± 0.23[b]	42.29 ± 0.16[a]
Lipids	21.42 ± 0.22[b]	22.74 ± 0.07[a]
Ash	4.92 ± 0.08[a]	4.83 ± 0.02[a]
Carbohydrates (by difference)	36.30	30.14
Total dietary fibre	25.98 ± 0.02[a]	25.80 ± 0.02[a]
– Soluble fibre	2.78 ± 0.12[a]	2.87 ± 0.12[a]
– Insoluble fibre	23.20 ± 0.02[b]	22.95 ± 0.02[b]
Total sugars	10.02 ± 0.03[a]	4.03 ± 0.03[b]
Starch	0.29 ± 0.01[a]	0.31 ± 0.02[a]
Metabolizable energy (kcal 100 g)	487.42	494.38
Colour		
L^* lightness	84.58 ± 0.48[a]	81.37 ± 0.07[b]
C^* chrome	20.37 ± 0.11[b]	22.89 ± 0.39[a]
h^* hue angle	89.41 ± 0.10[a]	87.49 ± 0.13[b]
Particle size (% retained in each screen)		
20 mesh (840 μm)	3.25 ± 0.01[a]	3.57 ± 0.01[a]
28 mesh (600 μm)	17.27 ± 0.01[a]	17.72 ± 0.02[a]
35 mesh (500 μm)	35.75 ± 0.01[a]	34.85 ± 0.03[a]
60 mesh (250 μm)	38.48 ± 0.02[a]	40.27 ± 0.01[a]
80 mesh (180 μm)	3.13 ± 0.04[a]	3.33 ± 0.04[a]
100 mesh (150 μm)	1.82 ± 0.02[a]	0.23 ± 0.02[b]
Bottom (<150 μm)	0.31 ± 0.01[a]	0.02 ± 0.00[b]
pH at 25 °C	6.60 ± 0.01[a]	6.57 ± 0.03[a]
Water activity at 25 °C	0.66 ± 0.02[a]	0.55 ± 0.00[b]

[A] Means (three replicates ± SE) with different superscript letters in the same row are significantly different ($p < 0.05$).

flours. The chrome parameters C^*, in turn, indicated that cultivar BRS 258 had a higher degree of saturation (22.89), than BRS 133. Considering the h^* parameter, the values found were 89.41 and 87.49 for BRS 133 and BRS 258, respectively. It could be stated therefore that the flours of both cultivars are of a rather intense and light yellow colour.

3.3. Particle size

The particle size analysis revealed that, despite the differing composition of both cultivars, grains in both cultivars have similar matrix structures. Approximately 90% of the mass of the meals produced was retained within screens 28, 35 and 60 mesh. The remaining 10% present particle size was lower than 80 mesh.

3.4. Fatty acid composition and minerals

Fatty acid composition is shown in Table 2. A high content, about 80%, of unsaturated fatty acids, with linoleic acid (*cis* 18:2) being the predominant fatty acid (~56%), followed by oleic acid (~17%) was observed. The most abundant saturated fatty acid in both cultivars was palmitic acid (~11%). Between 1% and 3% of the oil was unsaponifiable material, such as steroids (stigmasterol, kaempesterol and sitosterol), tocopherols and provitamin-A carotenoids (Pereira & Oliveira, 2004). The elevated iodine value, between 134 and 136 g/100 g, indicates the high degree of unsaturation. These results show that there was a statistical difference in fatty acid distribution, mainly linolenic and oleic acids, between low and high-protein cultivars.

Minerals composition of cultivars BRS 133 and BRS 258, is shown in Table 2. Cultivar BRS 258 had a higher concentration of both macro- and microminerals as compared to BRS 133. The values reported in our study are comparable to those observed by Mandarino et al. (1992) for soybean produced in different locations

Table 2
Fatty acid composition and minerals of soybean cultivars BRS 133 and BRS 258.[A]

	Cultivar	
	BRS 133	BRS 258
Fatty acid (%)		
(C14:0) myristic	0.09 ± 0.00[a]	0.08 ± 0.00[a]
(C16:0) palmitic	11.78 ± 0.02[a]	11.24 ± 0.01[a]
(C16:1) palmitoleic	0.09 ± 0.00[a]	0.09 ± 0.00[a]
(C18:0) stearic	4.21 ± 0.13[a]	3.56 ± 0.00[b]
(C18:1 cis) oleic	17.17 ± 0.05[b]	20.25 ± 0.00[a]
(C18:2 cis) linoleic	56.01 ± 0.10[a]	56.37 ± 0.01[a]
(C18:3 cis) linolenic	9.40 ± 0.05[a]	7.37 ± 0.03[b]
(C20:0) araquidic	0.41 ± 0.00[a]	0.35 ± 0.00[a]
(C20:1) gadoleic	0.16 ± 0.00[a]	0.16 ± 0.00[a]
(C22:0) behenic	0.51 ± 0.01[a]	0.38 ± 0.00[b]
(C24:0) lignoceric	0.17 ± 0.02[a]	0.15 ± 0.00[a]
Saturated FA	17.16 ± 0.18	15.76 ± 0.01
Monounsaturated FA	17.42 ± 0.05	20.50 ± 0.00
Polyunsaturated FA	65.42 ± 0.15	63.74 ± 0.04
Calculated iodine index (g/100 g)	136.54	134.52
Calculated saponification index	191	191
Minerals (mg/100 g, dry basis)		
Macroelements		
Calcium	290.41 ± 1.47[b]	335.37 ± 1.27[a]
Phosphorus	524.40 ± 1.24[b]	682.17 ± 0.23[a]
Microelements		
Iron	22.30 ± 0.07[b]	26.46 ± 0.25[a]
Copper	2.88 ± 0.04[b]	3.14 ± 0.06[a]
Zinc	7.42 ± 0.16[b]	8.27 ± 0.10[a]

[A] Means (three replicates ± SE) with different superscript letters in the same row are significantly different ($p < 0.05$).

of Brazil. Calcium and phosphorus have high relevance in human nutrition. Calcium bioavailability from soy milk (22.2%) has been estimated to be 90% of that from cow's milk. As far as phosphorus,

main forms in soybean are phytic acid, inorganic phosphates, phospholipids and nucleic acids. Phytic acid may account for 50–70% and phospholipids for about 15% of the total amount (Mandarino et al., 1992).

3.5. Amino acid composition

The total amino acid composition of a food protein, particularly of the essential amino acids, has classically been considered a measure of its biological adequacy. The total amino acid composition of both cultivars is presented in Table 3. These values are compared to the official amino acid profile of an ideal protein as established by WHO-FAO-UNU (2007). There was no significant difference between the two cultivars; however, the chemical score for the two soybean cultivars was different ($p < 0.05$). This is explained by the higher methionine concentration of the low-protein cultivar.

Although the total free amino acids percentage (Table 3) was very similar, cultivar BRS 133 showed a significantly higher concentration of free amino acids than cultivar BRS 258 except for histidine, aspartic and glutamic acids, and hydroxyproline.

3.6. Bioactive compounds

The results for the functional compounds lunasin, BBI and lectin are reported in Table 4. The quantified lunasin was further identified and confirmed in a band (5.45 kDa) by Western blot analysis. Lunasin concentration was within the ranges for different soybean genotypes reported by Gonzalez de Mejia et al. (2004). Although there was no statistical difference in soluble protein concentration between the two cultivars, the concentrations of lunasin, BBI and lectin in cultivar BRS 258 were statistically higher ($p < 0.05$) than in the low-protein BRS 133 cultivar.

Table 3
Total and free amino acid composition of the whole flours of the soybean cultivars BRS133 and BRS 258, compared with the WHO/FAO/UNU standard (2007).[A]

Amino acid (g/100 g of protein, dry basis)	Requirements WHO/FAO/UNU			Cultivars	
	Children (years)		Adults	BRS 133	BRS 258
	1–2	3–10			
Histidine (Hys)	–	–	1.5	2.63 ± 0.01[a]	2.75 ± 0.01[a]
Isoleucine (Ile)	–	–	3.0	4.44 ± 0.01[a]	4.49 ± 0.03[a]
Leucine (Leu)	–	–	5.9	7.57 ± 0.04[a]	7.50 ± 0.01[a]
Lysine (Lys)	5.2	4.8	4.5	6.16 ± 0.04[a]	6.10 ± 0.01[a]
Methionine (Met)	–	–	1.6	1.22 ± 0.01[a]	1.01 ± 0.02[b]
Cysteine (Cys)	–	–	0.6	1.93 ± 0.02[a]	1.67 ± 0.01[a]
Phenylalanine (Phe)	–	–	–	4.82 ± 0.03[a]	4.88 ± 0.03[a]
Tyrosine (Tyr)	–	–	–	3.55 ± 0.04[a]	3.37 ± 0.00[a]
Threonine (Thr)	2.7	2.5	2.3	3.93 ± 0.01[a]	3.68 ± 0.04[b]
Tryptophan (Trp)	7.4	6.6	0.6	nd	nd
Valine (Val)	–	–	3.9	4.49 ± 0.03[a]	4.31 ± 0.02[a]
Total amino acid essential				40.74	39.76
	15.3	13.9	23.9		
Arginine (Arg)	–	–	–	8.68 ± 0.01[b]	9.42 ± 0.03[a]
Alanine (Ala)	–	–	–	4.30 ± 0.00[a]	4.27 ± 0.01[a]
Aspartic acid (Asp)	–	–	–	11.61 ± 0.01[a]	11.59 ± 0.04[a]
Glutamic acid (Glu)	–	–	–	18.54 ± 0.01[a]	18.97 ± 0.02[a]
Glycine (Gly)	–	–	–	5.11 ± 0.01[a]	4.95 ± 0.07[a]
Proline (Pro)	–	–	–	4.98 ± 0.00[a]	4.99 ± 0.01[a]
Serine (Ser)	–	–	–	6.06 ± 0.02[a]	6.04 ± 0.01[a]
Total amino acid				100%	100%
Sulfur amino acids (Met + Cys)	2.6	2.4	2.4	3.15	2.68
Aromatic (Phe + Tyr)	–	–	3.8	8.37	8.25
Total free amino acid				2.36%	2.01%
Sulfur amino acids (Met + Cys)				0.31	0.26
Aromatic (Phe + Tyr)				0.27	0.23
Chemical score				76	63

[A] Means (two duplicates ± SE) with different superscript letters in the same row are significantly different ($p < 0.05$).

Table 4
Bioactive compounds of soybean cultivars BRS 133 and BRS 258.[A]

Analyse	Cultivar	
	BRS 133	BRS 258
Soluble protein (mg/g flour)	248.13 ± 2.21[a]	244.19 ± 2.02[a]
Bioactive compounds (mg/g soluble protein)		
Lunasin	12.29 ± 0.54[b]	14.78 ± 0.13[a]
BBI	23.62 ± 0.36[b]	28.11 ± 0.74[a]
Lectin	16.96 ± 0.72[b]	23.28 ± 0.14[a]
Bioactive compounds (mg/g flour)		
Lunasin	3.05 ± 0.14[b]	3.61 ± 0.28[a]
BBI	5.86 ± 0.33[b]	6.86 ± 0.42[a]
Lectin	4.21 ± 0.17[b]	5.68 ± 0.03[a]

[A] Means (three replicates ± SE) with different superscript letters in the same row are significantly different ($p < 0.05$).

Table 5
Mean isoflavone concentrations of soybean cultivars BRS 133 and BRS 258.[A]

Isoflavones (mg/100 g defatted flour, dry basis)	Cultivar	
	BRS 133	BRS 258
Aglycones		
Daidzein	10.98 ± 0.10[a]	7.69 ± 0.04[b]
Genistein	14.40 ± 0.02[b]	15.41 ± 0.05[a]
Glycitein	1.31 ± 0.05[b]	2.30 ± 0.06[a]
β-glucosides		
Daidzin	42.46 ± 0.02[a]	14.71 ± 0.04[b]
Genistin	36.12 ± 0.10[a]	23.09 ± 0.06[b]
Glycitin	10.40 ± 0.04[a]	6.02 ± 0.02[b]
Malonylglucosides		
Malonyldaidzin	131.62 ± 0.06[a]	57.56 ± 0.03[b]
Malonylgenistin	100.75 ± 0.10[a]	72.96 ± 0.02[b]
Malonylglycitin	41.96 ± 0.04[a]	22.64 ± 0.04[b]
Total aglycones	26.69 ± 0.07[a]	25.40 ± 0.05[b]
Total isoflavones	390.00 ± 0.10[a]	222.37 ± 0.09[b]

[A] Means (two duplicates ± SE) with different superscript letters in the same row are significantly different ($p < 0.05$). Acetylglucosides (acetyldaidzin, acetylgenistin and acetylglycitin) were not found.

3.7. Isoflavone concentration

The outstanding difference between the two cultivars was that cultivar BRS 133 contained a greater concentration of total isoflavones (390.00 mg/100 g of defatted flour), in comparison to 222.37 mg for BRS 258 (Table 5).

The concentrations of aglycones, β-glucosides and malonyl-glucosides, as referred to total isoflavones, for cultivar BRS 133 were 6.8%, 22.8% and 70.3%, respectively, and 11.4%, 19.7% and 68.8%, respectively, for cultivar BRS 258.

3.8. Saponin concentration

As with the isoflavones, cultivar BRS 133 contained a higher concentration of total saponins, 9.7 mg/g of defatted soy flour, as opposed to 7.4 found in the cultivar BRS 258 (Table 6).

In summary, the comprehensive chemical characterisation gathered for these two Brazilian soybean cultivars permits to conclude that a distinctive pattern emerges for some nutrients and bioactive compounds as a result of a different protein concentration. The higher protein concentration of cultivar BRS 258 seemed to have occurred mostly at the expense of the carbohydrate fraction of the grain.

Both cultivars exhibited expected total and free amino acid composition in spite of the fact that the low-protein soybean cultivar (BRS 133) had a higher amino acid score (76) than the high-protein soybean cultivar (BRS 258). This was probably due to an

Table 6
Saponin concentration in the soybean cultivars BRS 133 and BRS 258.[A]

Saponins (mg/g defatted flour, dry basis)	Cultivar	
	BRS 133	BRS 258
Group B and DMPP group B		
Soy saponins I	1.67 ± 0.04[a]	1.26 ± 0.00[b]
Soy saponins II	0.23 ± 0.01[a]	0.22 ± 0.00[b]
Soy saponins III	0.74 ± 0.01[a]	0.27 ± 0.01[b]
Soy saponins IV	0.13 ± 0.01[a]	0.10 ± 0.01[b]
Soy saponins V	0.29 ± 0.01[a]	0.21 ± 0.00[b]
Soy saponins βg	2.25 ± 0.07[a]	1.69 ± 0.07[b]
Soy saponins βa	0.24 ± 0.02[a]	0.12 ± 0.01[b]
Soy saponins γg	0.94 ± 0.03[a]	0.57 ± 0.03[b]
Soy saponins γa	0.14 ± 0.01[a]	0.09 ± 0.00[b]
Soy saponins αg	0.55 ± 0.02[a]	0.42 ± 0.01[b]
Group A acetyl-saponins		
Soy saponins aA$_1$	2.30 ± 0.03[b]	0.29 ± 0.10[a]
Soy saponins aA$_2$	0.23 ± 0.01[a]	0.14 ± 0.01[b]
Soy saponins aA$_7$	0.04 ± 0.01[b]	0.06 ± 0.00[a]
Total soy saponins	9.75 ± 0.18[a]	7.44 ± 0.05[b]

[A] Means (two duplicates ± SE) with different superscript letters in the same row are significantly different ($p < 0.05$).

unidentified storage protein fraction low in methionine, which may be responsible for the extra protein of the protein richer grain. Since both cultivars were produced in Paraná State – Brazil and under similar cultivation techniques, the concentration of minerals, ranging from 9% to 30% higher in the cultivar BRS 258 in relation to cultivar BRS 133, could be directly related to the protein deposition in the seed. A similar physiological mechanism could explain the higher concentrations of the proteinaceous components lunasin, Bowman–Birk inhibitor and lectin in cultivar BRS 258.

On the other hand, the nearly 75.4% higher total isoflavone concentration of the low-protein cultivar BRS 133, with 5.1% more aglycones, was rather unexpected. Analogously, the non-proteinaceous total soy saponins were about 31.0% higher in this cultivar as compared to the protein-rich cultivar (BRS 258).

Thus, protein concentration affected the final distribution of nutrients and bioactive components in soybean.

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