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Protein, isoflavones, trypsin inhibitory and in vitro antioxidant capacities: Comparison among conventionally and organically grown soybeans

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ABSTRACT

The objectives of this work were to compare the conventional and organic crops of BRS258 soybeans obtained concurrently by the Agricultural Center of Palma (RS, Brazil) in relation to protein, total phenolics, isoflavone contents, in vitro antioxidant capacity, and trypsin inhibitory activity. The results showed that organic soybeans had a higher protein content and lower trypsin inhibitory activity than soybean cultivated by the conventional system. Levels of isoflavones and phenolic compounds from organic soybeans were also significantly lower. Isoflavone distribution was also different: the highest percentage of glycitein (glycitein plus glycitein derivatives) was found in organic soybeans and the highest percentage of daidzein (daidzein plus daidzein derivatives) in conventionally grown soybeans. Conventional soybeans presented a higher DPPH scavenging capacity but there was no difference in the ferric reducing antioxidant power (FRAP), compared to organic cultivation, despite the higher phenolics and isoflavone contents, compounds known for their antioxidant capacity.

1. Introduction

Foods of plant origin have non-nutrient compounds (phytochemicals) such as polyphenols which have health-promoting biological activities such as antioxidant, anti-inflammatory and hypocholesterolemic. Growing environmental awareness associated to increased public interest in safer foods has led to question modern farming practices. This was reflected in an increasing demand for organic products, which is perceived as less harmful to the environment and healthier than conventionally grown foods.

The consumption of soybeans in grain is traditionally part of the diet of the Orientals, but has spread in the West in the last decade mainly due to the appeal of healthy food. The Food and Drug Administration (FDA, 2008) approved in 1999 a health claim on food labels, for products containing soy protein, about the association between soy protein (25 g/d) and a reduced risk of coronary heart disease.

Soybean is a legume characterized by high contents of polyunsaturated lipids and protein (30–45%) and represents the most important source of the phytoestrogens called isoflavones, which have been associated to beneficial effects in humans, such as prevention of cancer, cardiovascular diseases, osteoporosis and menopausal symptoms (Adlercreutz & Mazur, 1997). The antioxidant activity of isoflavones is related to the number of hydroxyl groups present in their chemical structure and decreases with glycosylation or replacement of the hydroxyl group by methoxy group. Isoflavones may inhibit lipid peroxidation in vitro by acting as metal chelators or free radical scavengers (Cook & Samman, 1996; Naim, Gestertner, Bondi, & Birk, 1976).

Protease inhibitors in legume seeds can have an important impact on their nutritional value as they inhibit pancreatic serine proteases, impairing protein digestion. They belong to two different families, referred to as Kunitz (act specifically against trypsin) and Bowman-Birk (inhibit trypsin and chymotrypsin simultaneously) type inhibitors, both kinds found in soybeans. Bowman-Birk inhibitor (BBI) has been shown to be effective in preventing or suppressing carcinogenesis (Lajolo, Genovese, Pryme, & Dale, 2004).

Nutritionally relevant plant constituents are affected by growth conditions. The fundamental difference between the system of organic and conventional production is related to soil fertility, which can affect the nutritional composition of the plant, including secondary metabolites. Studies comparing the nutritional quality of organic and conventional vegetables showed inconsistent differences except for the high level of ascorbic acid (vitamin C) and low nitrate in organic products. Organically grown tomatoes seem to contain more polyphenols, carotenoids and vitamin C than conventionally grown ones (Brandt & Mølgaard, 2001; Caris-Veyrat et al., 2004; Mitchell et al., 2007).

In a study conducted by Wang, Chen, Sciarappa, Wang, and Camp (2008) which analyzed the content of total phenolic and antioxidant flavonoids in organic and conventional crops of blueberries, it was observed that the organically grown fruits had higher total phenolic and flavonoid contents and antioxidant capacity than the conventionally

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grown ones. However no data is available in relation to the effect of organic farming on bioactive compounds and antioxidant capacity of soybeans, mainly as a result of the numerous factors that have to be controlled to allow the comparison. In this way, the objectives of this work were to compare organically and conventionally grown soybeans from the same cultivar (BRS 258), crop season, and geographic region, in relation to protein, total phenolics, isoflavone contents and antioxidant capacity.

2. Materials and methods

2.1. Organic and conventionally grown soy samples

Samples of soybeans grown in organic and conventional systems were obtained from experimental crops in areas adjacent to the Agricultural Center of Palma (CAP), University of Pelotas (UFPel), Pelotas, Rio Grande do Sul, Brazil, in the 2008/2009 season. Organic soybeans were grown in an isolated area 5 km far from the conventional soybean and other conventional crops plantations, with a slope of 5% area of environmental protection, borders and windbreak. The source of water for the area had no communication with surrounding areas, and there was no flood risk and transfer of land or water in these areas. The beginning of the transition to organic production in this crop occurred in 1995 when it was held the last conventional cultivation with soil amendment, plowing, harrowing and cultivation of soybeans, followed by the cultivation of barley. Since then, the area is being handled in accordance with the recommendations of the organic system, including winter crops, summer and ground cover, trying to change species and varieties. In the case of soybeans, the crop is held every two years, alternating cultivars BR 36, BRS 154, BRS 155, BRS 213, BRS 216 and BRS 258.

Three different samples of the 2008/2009 season of the BRS 258 cultivar were analyzed, two of which were grown in an organic system (one at UFPel and one certified by another producer in the same region, here denominated COP), and BRS 258 grown in a conventional system also at the UFPel. Chemical and physical characteristics of CAP and COP soils are presented in Table 1. Each soy sample was cultivated in three different plots (A, B, and C, for COP; D, E, and F, for organic BRS 258; and G, H, and I, for conventional BRS 258). The experimental plots consisted of individual areas with 4×5 m, randomly distributed in a completely randomized design within the experimental field, with 03 replicates per treatment. Organic soybeans were grown using weeding for invading herbs control, and conventional soybeans had two applications of the herbicide Imazetapyr®. All samples were homogenized in a mill Janke & Kunkel A-10 (Ika Works, Inc., Wilmington, NC, USA) prior to analysis.

Table 1
Chemical and physical characteristics of soil from conventional and organic plantations of the Agricultural Center of Palma (CAP) and a certified organic producer (COP) from Rio Grande do Sul, Brazil.

	CAP/ conventional	CAP/organic	COP/organic
Physical parameters			
Clay (%)	28	27	30
Silt (%)	32	32	34
Sand (%)	40	41	36
Chemical parameters			
Organic matter (%)	3.9	4.1	3.8
P ($mg.dm^3$)	6.6	6.4	6.5
K ($mg.dm^3$)	85.2	86.3	87.2
S ($mg.dm^3$)	3.6	4.0	3.8
Ca($cmol_c.dm^3$)	3.2	3.3	3.5
Mg($cmol_c.dm^3$)	1.5	1.7	1.7
Al ($cmol_c.dm^3$)	0.0	0.0	0.0
Cation exchange capacity ($cmol_c.dm^3$)	7.5	7.6	7.2
pH	5.9	5.8	5.8

Soils analysis made by Official Soil Analysis of FAEM-UFPEL (ufpel.edu.br/solos/prestacao-de-servicos/).

2.2. Moisture content

The moisture content of samples was determined in triplicate after drying at 105 °C in an air oven until constant weight (AOAC, 1995).

2.3. Protein content

The protein content (N×6.25) of the samples was determined in triplicate by the micro-Kjeldahl method (AOAC, 1995).

2.4. Isoflavone analysis

The samples were extracted in triplicate with 80% aqueous methanol (20:1 v/w) under agitation for 2 h at 4 °C, according to Genovese and Lajolo (2001). The homogenates were filtered through filter paper Whatman no. 06 and concentrated until methanol elimination on a rotatory evaporator (Rotavapor RE 120, Büchi, Flawil, Sweden) at ≤40 °C. The volume of the extracts was adjusted to 5 mL with HPLC grade methanol, and aliquots were filtered through a 0.22 μm PTFE filter unit (polytetrafluoroethylene, Millipore Ltd., Bedford, MA, USA) for HPLC injection. Isoflavone separation and quantitation was performed with a C18 Synergy 4 μ Fusion RP (25 cm×4.6 mm id) column (Phenomenex, Torrance, CA, USA) and a Hewlett Packard 1100 system equipped with autosampler, diode array detector, and the ChemStation software (Agilent Technologies, Palo Alto, CA, USA). Elution solvents were: A, water:acetonitrile:acetic acid (95:5:0.1) and B, acetonitrile: acetic acid (99.9:0.1). The solvent gradient was the same used by Genovese and Lajolo (2001), at flow rate 1 mL/min. Eluates were monitored at 255 and 320 nm and samples were injected in duplicate. Identification was made based on the spectra and retention time in comparison to known standards, and quantitation was based on external calibration. The 12 isoflavone standards were from LC Laboratories (Woburn, MA, USA). Calibration was performed by injecting the standards three times at five different concentrations ($R^2 \geq 0.999$). Total isoflavones contents were expressed as mg of aglycone/100 g of sample dry weight (DW).

2.5. Total phenolic content

Total phenolic contents in the extracts above were determined by means of the Folin-Ciocalteu assay (Singleton, Orthofer, & Lamuela-Raventos, 1999) using (+)-catechin (Sigma Chemical Co., St. Louis, MO, USA) as the standard. The absorbance readings were performed at 750 nm in a spectrophotometer Ultrospec 2000 UV-visible spectrophotometer (Amersham Biosciences, Cambridge, UK) and results expressed as mg equivalents of catechin per 100 g of sample dry weight (DW).

2.6. Total antioxidant capacity

2.6.1. DPPH scavenging activity

DPPH (2,2′-diphenyl-1-picrylhydrazyl radical, Sigma Chemical Co., St. Louis, MO, USA) scavenging activity of soybeans was assessed in the above extracts according to Brand-Williams, Cuvelier, and Berset (1995), with some modifications (Duarte-Almeida, Santos, Genovese, & Lajolo, 2006). Briefly, a 50 μL aliquot of the extract previously diluted and 250 μL of a methanol solution of DPPH (0.5 mM) were shaken, and, after 20 min, the absorbance was measured at 517 nm using the Benchmark Plus microplate spectrophotometer (BioRad, Hercules, CA, USA). Results were expressed as μmol Trolox equivalents/100 g of sample (DW).

2.6.2. Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed as described by Benzie and Strain (1996). FRAP reagent was freshly prepared daily by mixing together 300 mM acetate buffer pH 3.6, 10 mM 2,4,6′-tripyridyl-s-triazine (TPTZ, Sigma Chemical Co., St. Louis, MO, USA) in 40 mM HCl, and 20 mM $FeCl_3$.6 H_2O in 0.25 M acetate buffer, pH 3.6 (10:1:1). The absorbance was read at 593 nm after 4 min-incubation at room temperature, against a blank of FRAP reagent and distilled water. Results were expressed as mmol Trolox equivalents/100 g of sample (DW).

2.7. Trypsin inhibitory activity

Trypsin inhibitory activity was measured according to Kakade, Simons, and Liener (1970) using BAPNA (benzoyl-DL-arginine-*p*-nitroanilide) as substrate. Defatted soybean flours (1 g) were extracted with 50 mL of 0.01 N NaOH for 3 h at room temperature, and after centrifugation (30.000 *g*/30 min, 4 °C), supernatants had pH adjusted to 8.2. Aliquots of 0.2 to 1.0 mL of the soybean extracts were pipetted, in triplicate, and the volume made up to 1.0 mL with Tris buffer, 50 mM, pH 8.2, containing 20 mM $CaCl_2$. One microliter of trypsin (EC 3.4.21.4, T-8253, 12.700 units/mg protein from Sigma Chemical Co., St. Louis, MO, USA) solution (0.05 mg/mL 0.001 N HCl) was added to each tube and, after 5 min in a water bath at 37 °C, 7.0 mL of BAPNA (N-α-benzoyl-DL-arginine-*p*-nitroanilide, B-4875, Sigma Chemical Co., St. Louis, MO, USA) solution (0.3 mg/mL Tris buffer, 50 mM, pH 8.2, containing 20 mM $CaCl_{2)}$ previously warmed to 37 °C was added. Exactly 10 min later, the reaction was stopped by adding 1 mL of 30% acetic acid, and the absorbance measured at 410 nm, against the blanks to which the acetic acid was added before BAPNA. One trypsin unit (TU) was arbitrarily defined as an increase of 0.01 absorbance units at 410 nm. Results were expressed as the number of trypsin units inhibited (TUI) per mg of protein.

2.8. Statistical analysis

All the analyses were performed in triplicate samples and results expressed as mean ± standard deviation. For the statistical analysis the program STATISTIC version 6.0 Stat Soft (Tulsa, OK, USA) was used. The comparison of means was performed by ANOVA ($p<0.05$) and the LSD test (Least significant difference).

3. Results and discussion

3.1. Protein content

Nutrients and bioactive compounds present in soybeans vary greatly with the cultivar, environmental conditions and geographical sowing region. To study the effect of organic versus conventional cultivation these influencing factors must be controlled. Soil quality and environmental conditions in particular modulate the rate at which primary and secondary metabolisms occur. Table 1 presents soil properties from CAP and COP areas, and no differences were observed in physical and chemical parameters among them. Soybean cultivar BRS 258 was developed as part of the breeding program of EMBRAPA Soybean (Brazil) and is characterized by a high level of protein (42.3 g/100 g DW) (Paucar-Menacho et al., 2010). For this study, BRS 258 cultivar was grown under both conventional and organic conditions for comparison, and a significant difference was observed in the protein content for the same year crop (Table 2). The moisture ranged from 8.3 (COP C) to 12.2% (BRS 258 C I) and on average organic soybeans presented lower moisture, of around 8.6%, compared to 12.1% of conventionally grown. This increase in dry matter in organically grown plants has already been reported for tomatoes (Caris-Veyrat et al., 2004). In this way, to allow a more proper evaluation of mode of cultivation, protein contents were compared in dry weight basis; with an average value for organic 11–14% higher than for conventional BRS 258 soybeans. Also, only organic BRS 258 presented protein contents similar to those previously reported for this cultivar by Paucar-Menacho et al. (2010) for seeds grown in the Paraná Sate (Brazil).

It was previously reported that there were almost no differences between organic and conventional products in contents of minerals and vitamins, but the contents of protein were consistently lowest in organic products. Brandt and Mølgaard (2001) cited that this behavior could be a consequence of being grown with lower nutrient supply. But, probably, this is not the case of soybean here studied. The environment where the organic soybeans were grown has been monitored since 1995, keeping good nutrient levels, lower soil temperature and water changes. It is possible that, despite these conditions, the photosynthetic rate has been reduced, due to higher competition with weeds, affecting the primary metabolism.

3.2. Total phenolic content

Lower levels of total phenolics were detected in BRS 258 soybeans cultivated under organic practice, and the decrease was on an average of 18.5 (organic UFPel) to 34% (Certified organic producer) in relation to conventional BRS 258 (Table 2). These results contrasted with the initial hypothesis based on work by Mittler (2002) and Oh, Trick, and Rajashekar (2009) who proposed accumulation of these compounds upon abiotic stresses. In addition, some works detected higher levels of secondary metabolites in vegetables produced under organic system (Caris-Veyrat et al., 2004; Wang et al., 2008). Caris-Veyrat et al. (2004) attributed some of the differences to the increased dry matter content of organically grown plants: when results were expressed as fresh matter, organic tomatoes had higher vitamin C, carotenoids, and polyphenol contents (except for chlorogenic acid) than conventional tomatoes. When results were expressed as dry matter, no significant difference was found for lycopene and naringenin. Asami, Hong, Barrett, and Mitchell (2003) found significantly higher content of total phenolics in corn and marionberries grown using organic agricultural methods as compared with conventionally grown ones (Asami et al., 2003).

In this work, higher phenolic compounds and isoflavones levels were detected in soybean cultivated in the conventional production system. When organic agricultural practices are conducted within well-functioning and stable organic systems, the abiotic and biotic stress could be reduced and secondary metabolism attenuated, reducing the

Table 2
Moisture (%), protein (%, N×6.25), total phenolics and total isoflavone contents (mg/100 g DW) of BRS 258 soybeans grown in organic and conventional systems (2009 crop).

Soybean sample	Moisture	Protein	Total phenolics	Isoflavone
	%	DW	DW	DW
BRS 258 COP				
A	8.4 ± 0.2 [a]	41.1 ± 0.4 [a]	181.0 ± 17.7 [d]	58.1 ± 10.3 [c]
B	9.5 ± 0.2 [b]	41.3 ± 0.2 [a]	190.4 ± 2.59 [d]	62.4 ± 0.7 [c]
C	8.3 ± 0.2 [a]	40.0 ± 0.9 [a]	186.8 ± 8.19 [d]	62.1 ± 13.7 [c]
Mean	8.7 ± 0.6	40.8 ± 0.7	186.1 ± 4.7	60.9 ± 2.4
BRS 258 O				
D	8.4 ± 0.1 [a]	41.3 ± 0.1 [a]	218.7 ± 8.5 [b]	75.0 ± 2.6 [c]
E	8.9 ± 0.4 [a,b]	42.3 ± 0.4 [a]	227.7 ± 6.8 [a,b]	76.6 ± 7.8 [c]
F	8.5 ± 0.3 [a]	41.8 ± 0.6 [a]	241.1 ± 1.4 [a]	71.5 ± 12.8 [c]
Mean	8.6 ± 0.3	41.8 ± 0.5	229.2 ± 11.3	74.4 ± 2.6
BRS 258 C				
G	12.1 ± 0.2 [c]	37.5 ± 1.3 [b]	278.3 ± 5.7 [c]	187.7 ± 22.3 [a]
H	12.0 ± 0.2 [c]	36.4 ± 1.0 [b]	278.2 ± 8.5 [c]	160.7 ± 22.2 [b]
I	12.2 ± 0.4 [c]	36.6 ± 0.9 [b]	286.9 ± 4.3 [c]	193.1 ± 4.8 [a]
Mean	12.1 ± 0.1	36.8 ± 0.6	281.1 ± 5.0	180.5 ± 17.3

BRS 258 COP, soybeans from certified organic producer. BRS 258 O, organically grown BRS 258 soybeans; BRS 258 C, conventionally grown BRS 258 soybeans from the experimental field of Agricultural Center of Palma, UFPEL, Pelotas, RS, Brazil. The results are expressed as mean ± standard deviation (n = 3). Means in the same column with different letters are significantly different ($p<0.05$). DW: dry weight.

accumulation of metabolites mainly those from the shikimate pathway (Tzin & Galili, 2010). Similar to our results, You et al. (2011) observed that not all the organic berries showed significantly higher total phenolic contents than the conventional berries.

3.3. Isoflavone contents and profile

Isoflavones are definitively the most important phenolic compounds present in soybeans, not only because of the significant concentrations of these phytoestrogens, almost not present in other legumes, but also for the important health benefits that have been attributed to them. The results obtained here showed that the total isoflavone content ranged from 58.1 to 76.6 mg/100 g DW in organically grown soybeans, meanwhile more than twice these values were found in conventionally grown soybeans, from 160.7 to 193.1 mg/100 g (Table 2). These results are in accordance with the higher total phenolic content but did not follow the same pattern, as differences were much more significant for isoflavones. The levels of the soy isoflavones, daidzein, genistein and glycitein, are affected by genetic and environmental factors, and, as presently demonstrated, are highly influenced by the mode of cultivation. The values here reported are in the same range previously found (57 to 188 mg per 100 g) for thirteen Brazilian soy varieties (Genovese, Hassimotto, & Lajolo, 2005), and conventionally grown very similar to the content previously reported for BRS 258 (172 mg/100 g DW, recalculated from Paucar-Menacho et al. (2010)).

Isoflavones seem to have a variety of roles such as act as precursors to defense compounds (phytoalexins), to inhibit the growth of various microbes, and as endogenous regulators of auxin transport in roots. They are synthesized by isoflavone synthase via the phenylpropanoid pathway, but the genetic regulation of isoflavone biosynthesis in plants is not well understood. Isoflavone synthase seems to be induced by wounding (Kim, Jung, Ahn, Kim, & Chung, 2005) and by *Bradyrhizobium japonicum* (Subramanian, Stacey, & Yu, 2006).

These bioactive substances are found in soybeans as glycosylated conjugates (malonylglycosides and underivatized β-glycosides), which, as a result of processing such as drying, defatting, and storage (Pinto, Lajolo, & Genovese, 2005) form aglycones and acetylglycosides. In this way, soy and soy products may contain three types of isoflavones, in four chemical forms: the aglycones daidzein, genistein, and glycitein; the β-glycosides daidzin, genistin, and glycitin; the acetyl-β-glycosides 6″-O-acetyl-β-daidzin, 6″-O-acetyl-β-genistin, and 6″-O-acetyl-β-glycitin; and the malonyl-β-glycosides 6″-O-malonyl-β-daidzin, 6″-O-malonyl-β-genistin, and 6″-O-malonyl-β-glycitin (Barbosa, Hassimotto, Lajolo, & Genovese, 2006). As expected, for BRS 258 isoflavones the percentage of malonylglycosides plus β-glycosides represented more than 88% of the total, with absence of acetylglycosides and low amount of aglycones (Table 3, Fig. 1). Presence of aglycones results from the action of endogenous β-glucosidase, which occurs during soaking or storage in areas of high humidity (Matsuura, Obata, & Fukushima, 1989), explaining the low percentage found in soybeans.

Regarding the distribution of isoflavones (the total percentage of daidzein and its conjugates, glycitein and its conjugates, and genistein and its conjugates) (Table 4), significant differences were observed between organic and conventional farming, the largest percentage of total glycitein being found in organic soybeans (11–18%), and the highest percentage of total daidzein (~38%) found in the conventional soybean. There was no significant difference in the amount of total genistein between samples. This result seems difficult to interpret, since this alteration in the ratio among the three main isoflavones can be the result of an up or down-regulation of their biosynthesis. Daidzein is the precursor of the phytoalexin glyceollin, and the capacity to accumulate glyceollin correlates with resistance to various fungal pathogens (Lozovaya et al., 2007). In this way, one possibility would be the increased phytoalexin synthesis in organic soybeans leading to a decrease in daidzein concentration.

Table 3
Profile of isoflavones of BRS 258 soybeans grown in organic and conventional systems (2009 crop).

Soybean sample	%			
	β-glucosides	Malonylglucosides	Acetylglucosides	Aglycones
BRS 258 COP				
A	44.6 ± 0.81 [b]	52.1 ± 0.90 [c]	n.d.	3.3 ± 0.30 [c]
B	43.5 ± 0.41 [b]	53.0 ± 0.54 [c]	n.d.	3.4 ± 0.31 [c]
C	43.3 ± 2.08 [b]	53.6 ± 2.04 [c]	n.d.	3.1 ± 0.30 [c]
BRS 258 O				
D	62.4 ± 0.77 [a]	26.4 ± 0.85 [d]	n.d.	11.1 ± 0.41 [b]
E	60.8 ± 1.38 [a]	27.6 ± 1.78 [d]	n.d.	11.6 ± 0.69 [a,b]
F	62.2 ± 0.51 [a]	26.0 ± 0.73 [d]	n.d.	12.0 ± 0.43 [a]
BRS 258 C				
G	34.0 ± 2.08 [d]	64.1 ± 2.39 [a]	n.d.	2.0 ± 0.64 [d]
H	37.0 ± 1.27 [c]	61.0 ± 1.30 [b]	n.d.	2.3 ± 0.17 [d]
I	35.6 ± 0.91 [c,d]	62.1 ± 1.01 [a,b]	n.d.	2.2 ± 0.11 [d]

BRS 258 COP, soybeans from certified organic producer. BRS 258 O, organically grown BRS 258 soybeans; BRS 258 C, conventionally grown BRS 258 soybeans from the experimental field of Agricultural Center of Palma, UFPEL, Pelotas, RS, Brazil. n.d. not detected. The results are expressed as mean ± standard deviation (n = 3). Averages in the same column with different letters are significantly different ($p<0.05$).

3.4. Trypsin inhibitory activity

Protease inhibitors are found in a wide variety of plants, including most legumes, especially in seeds, and are associated with plant defense mechanisms against herbivores and pathogens. The quantity depends on the variety, physiological state, insect infestation, harvest conditions and storage. The protease inhibitors found in legumes can be divided into two main classes: the Kunitz and Bowman-Birk types. Usually bear the name of the first protease inhibitors which were tested against, so they are generically known as trypsin inhibitors. Currently it is known that their specificity is not restricted to trypsin and, indeed, are able to inhibit other serine proteases such as chymotrypsin and elastase (Genovese & Lajolo, 2001).

Trypsin inhibitory activity of organically grown BRS 258 soybeans was significantly lower than that of conventionally grown ones (Table 5). Organic soybeans showed 96.9–109.3 units of trypsin inhibitor per mg protein (DW), and 45.0–49.9 unit of trypsin inhibitor per mg of grain (DW), meanwhile values 10–30% higher were obtained for soybeans grown by the conventional system. These results indicate that despite its higher protein content, organically grown soybeans did not synthesize larger amounts of trypsin inhibitors.

3.5. Antioxidant capacity

Total in vitro antioxidant capacity of organic and conventionally grown soybeans was evaluated through two different methodologies, DPPH radical scavenging capacity and Ferric reducing antioxidant power (FRAP). Results in Fig. 2 show that conventional soybeans presented a higher DPPH scavenging capacity but there was no difference in the FRAP, compared to organic cultivation, despite the higher phenolic and isoflavone contents, compounds known for their antioxidant capacity. A low correlation between the antioxidant activity and total phenolic content/total isoflavone content has been previously reported for thirteen Brazilian soybean varieties (Genovese et al., 2005). Xu and Chang (2008) found for 29 cultivars of conventional yellow soybeans a FRAP value ranging from 0.83 to 1.34 mmol equivalent of Fe^{+2}/100 g sample. Samples that showed no ability of scavenging DPPH showed, however, relatively high values of FRAP. These results indicate that, similar to our results, soybean antioxidants do not necessarily have the same behavior in different antioxidant systems.

The effect of organic cultivation on antioxidant properties is controversial. For lettuce, no difference was found in the DPPH radical

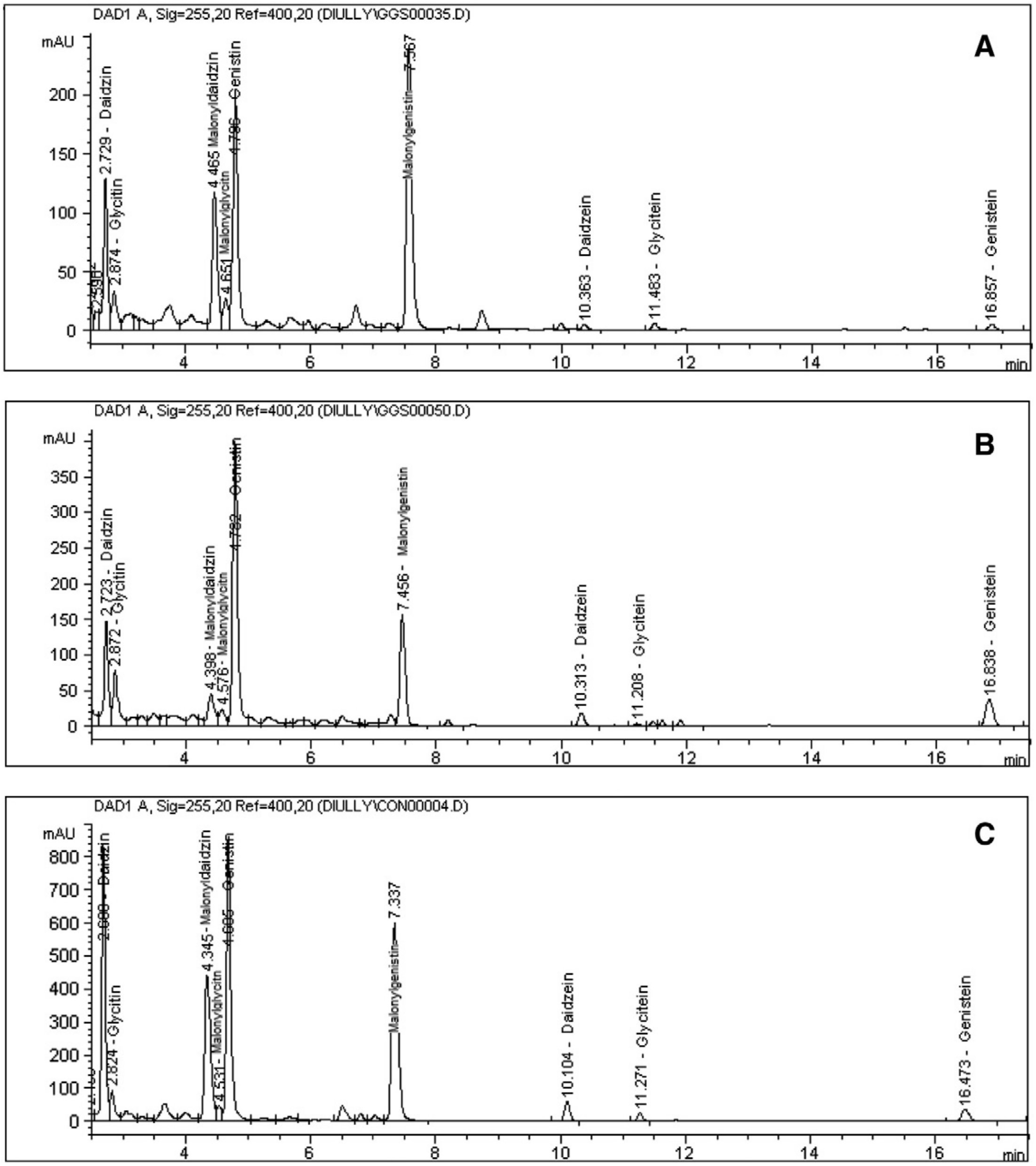

Fig. 1. Typical HPLC chromatogram (255 nm) of isoflavones from A: BRS 258 Certified organic producer B: UFPel Organic BRS 258, C: UFPel conventional BRS 258 soybeans and retention times of the reference compounds: (1) daidzin, (2) glycitin, (3) genistin, (4) malonyldaidzin, (5) malonylglycitin, (6) acetyldaidzin, (7) acetylglycitin, (8) malonylgenistin, (9) acetylgenistin, (10) daidzein, (11) glycitein, and (12) genistein.

scavenging and no relationship could be found between antiradical activity and polyphenol or anthocyanin contents (Heimler, Vignolini, Arfaioli, Isolani, & Romani, 2012). However, organic blueberries presented higher total phenolics, total anthocyanins, and antioxidant activity (ORAC) than fruit from the conventional culture, with a significant correlation between the ORAC values and total phenolics and total anthocyanins (Wang et al., 2008). Organic mangoes also presented higher DPPH scavenging activity and total phenolics (Maciel, Oliveira, Bispo, & Miranda, 2011). But Olsson, Anderson, Oredsson, Berglund, and Gustavsson (2006) reported that, for strawberries, the effect of organic cultivation on antioxidants was different depending on the cultivar, although both cultivars that had been grown organically inhibited cancer cell proliferation to a higher extent than those from conventionally grown strawberries. In the study by Stracke, Rüfer, Weibel, Bub, and Watzl (2009), comparing organic and conventional cultivation of apple for three years, it was observed that organic apples presented on average 15% higher antioxidant capacity determined by FRAP, TEAC and ORAC than conventionally produced fruits. However, the authors observed that the climatic variations from one year to another had a greater effect on the variation in the polyphenol content and antioxidant capacity of apples than the type of cultivation.

The ability of scavenging DPPH here found (207–259 μmol trolox/100 g sample, for organic, and 254 μmol trolox/100 g sample for conventional) was lower than that reported by Barbosa et al. (2006), of 370 μmol equivalents trolox/100 g of sample (DW), however was higher than those values found by Xu and Chang (2008) for 29 cultivars of yellow soybeans, which ranged from 0 to 116 μmol equivalents trolox/100 g of sample (DW).

4. Conclusions

Organic soybeans had a higher protein content and lower trypsin inhibitory activity than soybean cultivated by the conventional system. The levels of isoflavones and phenolic compounds from organic

Table 4
Distribution of total forms of isoflavones of BRS 258 soybeans grown in organic and conventional systems (2009 crop).

Soybeans samples	% TOTAL*		
	Daidzein	Glycitein	Genistein
Certified organic producer			
T1A	32.0 ± 2.10 [c]	11.0 ± 0.65 [d]	57.1 ± 3.37 [a,b]
T1B	31.1 ± 2.00 [d]	12.3 ± 0.56 [c]	56.6 ± 1.69 [a,b]
T1C	31.8 ± 2.61 [c]	15.4 ± 1.28 [b]	52.7 ± 4.33 [b]
BRS 258 Organic UFPEL			
T2D	23.0 ± 2.00 [f]	16.3 ± 0.56 [b]	60.7 ± 1.69 [a]
T2E	24.0 ± 2.00 [e]	18.0 ± 0.56 [a]	58.1 ± 1.69 [a,b]
T2F	23.7 ± 2.00 [e]	18.0 ± 0.56 [a]	58.2 ± 1.69 [a,b]
BRS 258 Conventional UFPEL			
T3G	37.3 ± 2.00 [b]	6.0 ± 0.56 [e]	56.7 ± 1.69 [b]
T3H	38.2 ± 2.00 [a]	4.1 ± 0.56 [f]	57.6 ± 1.69 [a,b]
T3I	38.2 ± 2.00 [a]	4.1 ± 0.56 [f]	57.6 ± 1.69 [a,b]

BRS 258 COP, soybeans from certified organic producer. BRS 258 O, organically grown BRS 258 soybeans; BRS 258 C, conventionally grown BRS 258 soybeans from the experimental field of Agricultural Center of Palma, UFPEL, Pelotas, RS, Brazil. The results are expressed as mean ± standard deviation (n = 3). Averages in the same column with different letters are significantly different ($p < 0.05$). * The total percentage of each aglycone represents the sum of the free and conjugated forms, expressed as aglycones, in relation to the total.

soybeans were also significantly lower than those of soybeans grown by the conventional system, and the ratio of derivatives of genistein, daidzein and glycitein was affected by cropping system. These results seem contrary to what might be expected, since both protease inhibitors and phenolic compounds are associated with plant defense mechanisms, indicating that well-functioning and stable organic systems reduce the abiotic and biotic stress caused initially by the transition. These results are all new and show the need for further studies to deepen the knowledge about the effect of the type of soybean cultivation.

Abbreviations and nomenclature

DPPH 2,2′-diphenyl-1-picrylhydrozyl radical
μL microliter
FRAP Ferric reducing antioxidant power

Table 5
Trypsin inhibitory activity of BRS 258 soybeans grown in organic and conventional systems (2009 crop).

Soybean samples	UTI/mg protein DW	UTI/mg soybean DW
BRS 258 COP		
A	102.3 ± 1.73 [d,e]	45.9 ± 0.79 [e,f]
B	96.9 ± 2.43 [e]	45.0 ± 0.15 [e,f]
C	101.6 ± 1.47 [d,e]	44.7 ± 0.42 [f]
Mean	100.3 ± 2.94	45.2 ± 0.62
BRS 258 O		
D	104.4 ± 4.89 [c,d]	47.1 ± 2.21 [d,e]
E	109.3 ± 5.68 [c]	49.9 ± 1.43 [c]
F	105.7 ± 3.68 [c,d]	48.5 ± 1.51 [c,d]
Mean	106.5 ± 2.52	48.55 ± 1.38
BRS 258 C		
G	125.0 ± 3.45 [b]	53.3 ± 1.47 [b]
H	135.0 ± 2.02 [a]	55.9 ± 0.84 [a]
I	131.1 ± 1.78 [a]	55.1 ± 1.17 [a,b]
Mean	130.4 ± 5.03	54.8 ± 1.34

BRS 258 COP, soybeans from certified organic producer. BRS 258 O, organically grown BRS 258 soybeans; BRS 258 C, conventionally grown BRS 258 soybeans from the experimental field of Agricultural Center of Palma, UFPEL, Pelotas, RS, Brazil. The results are expressed as mean ± standard deviation (n = 3). Means in the same column with different letters are significantly different ($p < 0.05$). UTI: Unit of trypsin inhibitor.

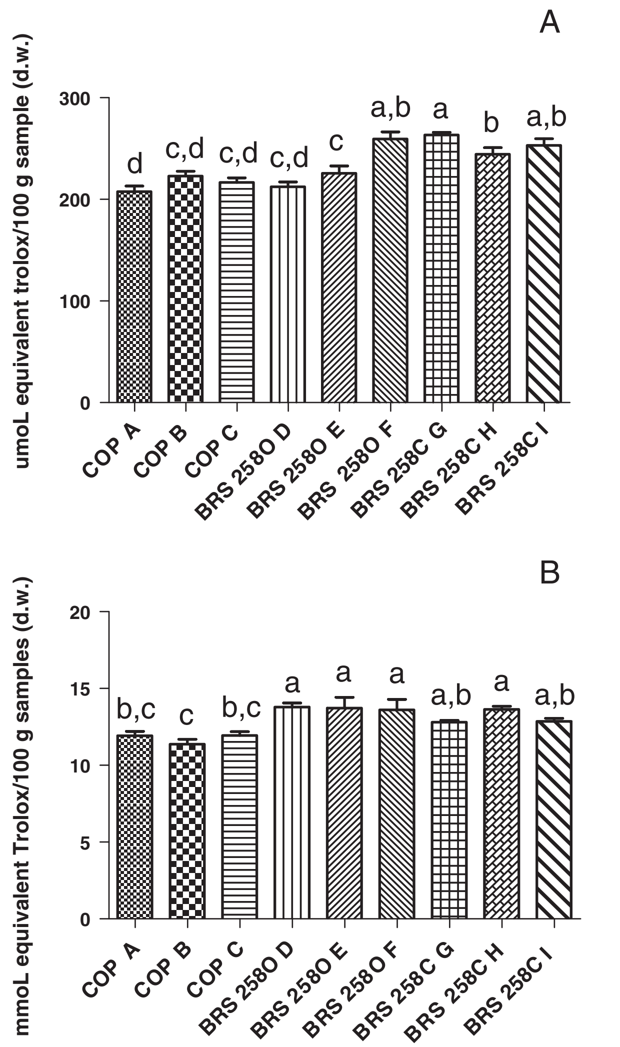

Fig. 2. Total in vitro antioxidant capacity of BRS 258 soybeans grown in organic and conventional systems. A: DPPH radical scavenging capacity; B: Ferric Reducing antioxidant power (FRAP). COP: certified organic producer; O, organically grown from UFPel; C, conventionally grown from UFPel. The results are expressed as mean ± standard deviation (n = 3). Different letters are significantly different ($p < 0.05$).

TPTZ 2,4,6′-tripyridyl-s-triazine
BAPNA 2,2′-benzoyl-DL-arginine-*p*-nitroanilide

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