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FOOD TECHNOLOGY

From a nutraceutical and anti-nutritional perspective, can a grain soybean cultivar be used as vegetable soybean?

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ABSTRACT: Vegetable soybean has high nutritional value and offers functional benefits to human body. However, its use in cooking is still limited, mainly due to the lack of knowledge about its properties. In addition, the availability of cultivars for human consumption is reduced. Therefore, this study was conducted in Botucatu, Brazil, to assess the potential of grain soybean (GS) and dual-purpose soybean (DPS) cultivars for consumption as vegetable soybean (VS). Pods were harvested at R6 stage, and the contents of bioactive compounds (total phenolic compounds, flavonoids, antioxidant activity and proteins) and antinutritional components (nitrates, oxalate, alkaloids and tannins) were evaluated. The cultivars showed the same protein content, not differing statistically from each other, as well as for nitrate and oxalate. The content of phenolic compounds and total antioxidant activity of the SG cultivar differed statistically from the content of the DPS and VS cultivars, respectively. According to the results, the GS cultivar, with pods harvested at R6 stage, showed nutraceutical quality and contents of antinutritional compounds similar to those of the two cultivars proposed for the vegetable soybean.
Key words: *Glycine max* L. Merrill, bioactive compounds, oxalate, nitrate, tannin.

Na perspectiva nutracêutica e antinutricional, cultivar de soja-grão pode ser usada como soja-hortaliça?

RESUMO: A soja-hortaliça apresenta alto valor nutritivo e oferece benefícios funcionais para o organismo humano. No entanto, seu uso na culinária ainda é limitado, principalmente devido à falta de conhecimento sobre suas propriedades. Além disso, a disponibilidade de cultivares para consumo humano é reduzida. Portanto, este estudo foi realizado em Botucatu, Brasil, com o objetivo de verificar o potencial de cultivares de soja-grão (SG) e soja de duplo propósito (SDP) para consumo como soja-hortaliça (SH). Assim, vagens foram colhidas no estádio R6, foram avaliados quanto os teores de compostos bioativos (compostos fenólicos totais, flavonoides, atividade antioxidante e proteínas) e de antinutricionais (nitratos, oxalato, alcaloides e taninos). A cultivares apresentaram mesmo teor de proteína, não diferindo estatisticamente entre si, bem como para nitrato e oxalato. O conteúdo de compostos fenólicos e atividade antioxidante total da cultivar SG, diferiu estatisticamente do conteúdo das cultivares SPD e SH, respectivamente. De acordo com os resultados, a cultivar SG, com colheita das vagens em R6, apresentou qualidade nutracêutica e teores de compostos antinutricionais compatível com as duas cultivares propostas para soja vegetal.
Palavras-chave: *Glycine max* L. Merrill, compostos bioativos, oxalato, nitrato, tanino.

INTRODUCTION

Vegetable soybean has gained prominence for being a versatile food in terms of consumption, whether as hamburgers, soy milk, or as textured soy protein and snacks, which are recognized as important food sources, especially for vegetarians and vegans (ALMEIDA et al., 2014).

In addition to processed products, vegetable soybean in its fresh form has aroused the interest of both researchers and consumers, due to its nutritional properties (ZEIPINA et al., 2022; NAIR et al., 2023). In 100 grams of raw grains, there are high contents of protein (12.95 g), iron (3.55 mg) and vitamins A (9 µg), B1 (0.435 mg), B2 (0.175 mg) and C (29 mg), and carbohydrates (up to 8.32 g in 100 g of dry mass) (SMIDERLE, 2007; SHANMUGASUNDARAM & YAN, 2010), containing all essential amino acids in its composition (AGYENIM-BOATENG et al., 2023). Vegetable soybean is also considered a functional food because it contains isoflavones, such as genistein, daidzein and glycitein, which are phytoestrogens with antioxidant, anticarcinogenic and antimicrobial action (TAIZ & ZEIGER, 2013).

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Vegetable soybean and grain soybean may show differences in visual, sensory, aromatic and nutritional characteristics (FLORES et al., 2019). Vegetable soybean has larger seeds that occupy 80-90% of the pod and 100 seeds weigh around 30 g, whereas 100 seeds of grain soybean weigh from 12 to 19 g (OLIVEIRA et al., 2013; CASAS-LEAL et al., 2022). In addition, vegetable soybean has chemical characteristics that make it more pleasant to the taste, such as the absence of bitterness and astringency (NAIR et al., 2023). When harvested at the R6 maturity stage, vegetable soybean has higher contents of sugar, starch, glycine, and other compounds compared to when harvested ripe (R8) (CASAS-LEAL et al., 2022).

Vegetable soybean is widely recognized as a significant source of antioxidants, playing an important role in reducing oxidative stress in the human body (SILVA et al., 2010; LIN & WU, 2021). Additionally, it has low levels of antinutrients, which contributes to improving digestibility and promoting human health (KUMAR et al., 2020). Among the antinutrients present in soybean, tannins stand out for positively contributing to the sensory attributes of food (DEGÁSPARI et al., 2005). However, they can precipitate proteins, confer astringency and reduce palatability (BENEVIDES et al., 2011). Another relevant antinutrient is calcium oxalate, whose consumption can cause problems in the urinary tract, especially in predisposed people (HOLMES et al., 2015; RIZZO & BARONI, 2018). Moreover, vegetable soybean contains nitrate, which, when metabolized, can lead to the formation of nitrosamines, a compound considered carcinogenic. In children, nitrate intake can promote the formation of methemoglobinemia, which affects oxygen transport and may lead to death (MANTOVANI et al., 2005; BENEVIDES et al., 2011). However, nitrate concentration depends on many factors such as species, climate and crop management (COLLA et al., 2018).

However, vegetable soybean cultivars are rare, and finding cultivars that behave as such is useful to better make this product available in supermarkets, street markets and restaurants, since there is growing market demand. Therefore, understanding the nutritional composition of soybean cultivars is essential to guide healthy food choices and promote a balanced diet. In this context, the objective of this study was to assess the potential of grain soybean and dual-purpose soybean cultivars for consumption as vegetable soybean, based on the contents of bioactive compounds and antinutrients.

MATERIALS AND METHODS

Characterization of the area and experimental material

The experiment was conducted in the field in the city of Botucatu, state of São Paulo, Brazil, located at 22º 51' S, 48º 26' W and 786 m altitude. The climate of Botucatu, according to Köppen's classification, is Cfa (warm temperate (mesothermal), with average annual rainfall and temperature of 1314 mm and 19.5 ºC, respectively (CUNHA & MARTINS, 2009). The soil was classified as a *Latossolo Vermelho Escuro* (Oxisol) (SANTOS et al., 2018).

Treatments and experimental design

Cultivars BRS 267 (vegetable soybean), BRSMG 790A (dual purpose: grain and vegetable) and 58HO124 EP RR (grain soybean) were evaluated in a randomized block design with six replicates. Each experimental unit had 30 plants.

Conduction of the experiment

Seeds of the cultivars were inoculated with *Bradyrhizobium elkanii* and *Bradyrhizobium japonicum*, sown in polystyrene trays with 128 cells, using one seed per cell. On November 24, 2020, at 10 days after sowing, the seedlings were transplanted to the field at spacing of 0.50 m between rows with 10 plants per meter.

Soil acidity correction and fertilization were performed as recommended by AMBROSANO & WUTKE (1997). For that, 2.52 t ha^{-1} of limestone (RNV = 90%) was applied at 36 days before transplantation, on October 19, 2020. Prior to planting, 1,184.48 kg ha^{-1} of P_2O_5 and 13.25 kg ha^{-1} of K_2O were applied using natural phosphate and potassium sulfate as sources, respectively.

Cultural practices adopted followed the recommendation for organic management of production, in accordance with the regulations of Law No. 10,831 (BRASIL, 2003) and the Technical Regulation of Normative Instruction 46 (BRASIL, 2011) complemented by IN 17 (BRASIL, 2014), consisting of manual weeding and disease and pest control.

Data Acquisition

Pods were harvested when they reached the R6 maturity stage, which occurred at 93, 93 and 104 days after transplantation for BRS 267, 58HO124 EP RR and BRSMG 790A, respectively.

Variables analyzed

The pods were dried in a forced air circulation oven at 60 ºC for 96 hours. Then, they were ground in a Willye TE-650 knife mill. In a sample of 0.2 g, 10 mL of 80% methanol was added and the mixture was kept in an ultrasonic bath with heating (Eco-Sonics) at 40 ºC for 15 minutes. Subsequently, it was centrifuged for 30 minutes at 4000 rpm (Clinical Centrifuge - 80-2B). The following parameters were evaluated: a) Content of total phenolic compounds - determined according to the Folin-Ciocalteau spectrophotometric method (SINGLETON et al., 1999), with results were expressed in gallic acid equivalent, based on a calibration curve; b) Flavonoid content - determined using the methodology described by PEIXOTO SOBRINHO et al. (2008), with results expressed in mg of quercetin per 100 g of dry mass; c) Total antioxidant activity - determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl – Alpha Aesar 95% purity) free radical scavenging method described by YEPEZ et al. (2002), with results expressed as percentage of free radical scavenging; d) Protein content - calculated by determining the N content according to the micro-Kjeldahl method (MALAVOLTA et al., 1989) and applying the correction factor of 6.25, with results expressed as % of dry mass; e) Oxalate content - determined using the method described by AL-WAHSH et al. (2012), with oxalate concentrations expressed in g per 100 g of dry mass; f) Nitrate content - determined using the method described by CATALDO (1975), with results converted into $N\text{-}NO_3^-$ contents in pod dry mass, with the aid of a calibration curve prepared from dilute solutions of sodium nitrate; g) Soluble tannin content - determined using the method described by TAIRA (1996), with results obtained from a gallic acid calibration curve; and h) Test for alkaloids - determined using the methodology proposed by MENEZES FILHO & CASTRO (2019).

Statistical analysis

The data were subjected to non-parametric statistical analysis, and the statistical analysis was performed using R 3.3.0 (R CORE TEAM, 2016) via the Friedman test (< 0.05).

RESULTS AND DISCUSSION

For the nutritional and antinutritional factors of the soybean cultivars, the data showed significant differences, except for protein content, nitrate, and oxalate (Table 1). According to Conover's analysis, the evaluated cultivars did not differ in terms of protein content (Table 2). Studies have indicated protein contents of vegetable soybean ranging from 34.2% to 35.4% (XU et al., 2015) and from 37.04% to 42.46% for soybean cultivars harvested at R6 stage (AGYENIM-BOATENG et al., 2023). For soybean to be considered a protein source, its content must be higher than 20% for it to be equivalent to the protein of animal origin (CABRAL & MOSDESTA, 1981). Additionally, for soybean to be classified as vegetable, it needs to have a protein content of at least 13% at the R6 stage (SHANMUGASUNDARAM et al., 2015).

It is important to emphasize that soybean is a significant source of protein, as it offers appropriate contents and is a low-cost option available in many developing countries. There are no records that the protein content of vegetable soybean is higher than that of grain soybean. Therefore, cultivars that have low content of antinutritional components and adequate protein content can be indicated for fresh consumption, additionally considering the aroma, flavor, odor and palatability.

Vegetables contain a large number of phytochemicals, such as phenolic compounds, sulfurous and nitrogenous compounds, ascorbic acid

Table 1 - Friedman test to protein content (%), total phenolic compounds (mg gallic acid/100 g DM), flavonoids (mg quercetin/100 g DM), antioxidant activity (%), nitrate (mg nitrate/100 g), tannin (mg gallic acid/100 g DM) and oxalate (mg oxalate/100 g DM) of soybean cultivars.

	Friedman	Degrees of freedom	P-value
Protein content	4.66	2	0.0969
Total phenolic compounds	6	2	0.0497*
Flavonoids	6	2	0.0497*
Antioxidant activity	6	2	0.0497*
Nitrate	4.66	2	0.0969
Tannin	6	2	0.0497*
Oxalate	4.66	2	0.0969

*Friedman chi-square (< 0.05).

Table 2 - Calculation of the Conover Test comparing the cultivars pairwise: dual-purpose soybean (DPS), vegetable soybean (VS), and grain soybean (GS).

	----------Protein content----------	
	GS	VS
VS	0.120	-
DPS	1	0.310
	----------Total phenolic compounds----------	
	GS	VS
VS	0.662	-
DPS	0.043*	0.066
	----------Antioxidant activity----------	
	GS	VS
VS	0.043*	-
DPS	0.662	0.662
	----------Flavonoids----------	
	GS	VS
VS	0.066	-
DPS	0.662	0.043*
	----------Nitrate----------	
	GS	VS
VS	0.120	-
DPS	0.310	1
	----------Tannins----------	
	GS	VS
VS	0.662	-
DPS	0.662	0.043*
	----------Oxalate----------	
	GS	VS
VS	1	-
DPS	0.310	0.120

*Significant difference by the Conover test (< 0.05).

and tocopherols, which have a strong antioxidant capacity and are associated with reducing the risk of various diseases (SILVA et al., 2010). A significant difference exists in the performance among the DPS and GS cultivars for phenolic compounds and the VS and GS antioxidant activity (Table 2). These results are correlated since phenolic compounds confer antioxidant capacity in plants; although, there are other compounds with the same function (LIN & WU, 2021). The importance of antioxidant activity lies in the fact that it blocks the oxidation of cells, reducing the concentration of reactive oxygen species (VIANA et al., 2015). Although, the human body has enzymes with this function, consuming foods with greater antioxidant properties, such as soybean, can help combat oxidative stress (GOMES et al., 2021). Cultivars with low oxidative activity exhibit antioxidant potential values lower than 60% (MELO et al., 2006).

Phenolic compounds are derived from the secondary metabolism of plants, coming from the phenylpropane metabolic pathway (MA et al., 2022). The higher proportion reported in the DPS and GS cultivars can be attributed to the interaction between environmental characteristics, such as the quality and quantity of light, and the genetic characteristics of the cultivar, since the content of phenolic compounds in plants depends on these two factors (NAKABAYASHI & SAITO, 2015). Phenolic compounds are also important for giving color and aroma to vegetables (HE & GIUSTI, 2010).

The secondary metabolism of plants includes the class of terpenes, nitrogenous compounds and phenolic compounds. Flavonoids are a group of phenolic compounds and play an important role in the defense of plants against microorganisms and insects (SILVA et al., 2018).

These compounds are widely found in fruits and vegetables and exhibit anti-inflammatory function and the ability to block the formation of reactive oxygen species (SILVA et al., 2010).

In this context, there is a significant difference in the performance of flavonoid content between the DPS and VS cultivar (Table 2). This finding supports the purpose for which these cultivars were selected, namely, as a nutritionally rich food for human consumption.

The cultural practices employed without the use of pesticides may have led to the production of more compounds with biological functions, such as flavonoids, which are also important for the metabolic regulation of nutrients in plants (AOI et al., 2021). Soybean is a food rich in isoflavones, which belong to the class of flavonoids and are effective antioxidants in fighting cancer (PEREIRA & CARDOSO, 2012). The lower expression of the antioxidant capacity of the flavonoids present in one cultivar may be a consequence of the position of the hydroxyl group and its proximity to the CO2H group, since these factors determine the antioxidant capacity of flavonoids (SILVA et al., 2010).

Regarding antinutritional factors, no significant differences were found for nitrate content between cultivars (Table 2). The amount ingested and the susceptibility of the body are determinant factors for it to cause damage, and the non-harmful consumption of nitrate is up to 3.7 mg kg^{-1} day^{-1} (BENEVIDES et al., 2011).

Tannins are phenolic compounds that are also present in the soybean cultivars analyzed, which can confer undesirable color and odor to soybean (BENEVIDES et al., 2011). It was observed that the DPS and VS cultivars have the lowest tannin content (Table 2), while the GS cultivar exhibited higher means for this antinutrient. However, the result is interesting and demonstrates that the tannin content in DPS did not differ from that obtained in VS, which is recommended for consumption as a vegetable.

Regarding the oxalate content, there was no difference between the cultivars (Table 2). Soybeans are known to have significant amounts of oxalate, which can range from 43 to 148 mg/100 g in grain soybean (AVILA-NAVA et al., 2021). However, MASSEY et al. (2001), in a study evaluating commercial grain soybean cultivars for calcium oxalate contents, reported values of 2.62 to 3.60 mg of oxalate/100 g of mass. Cultivars with lower oxalate content are more suitable for fresh consumption because the lower intake of this antinutrient reduces the risk of kidney stone formation; for a person with such predisposition, a daily intake of approximately 40 to 50 mg is recommended (TAYLOR & CURHAN, 2006).

Although, the presence of alkaloids in vegetable soybean with immunosuppressive and inhibitory function has been reported by WANG et al. (2016), no alkaloids were detected in the soybean cultivars studied here (Table 3). Alkaloids are found in a restricted group of vegetables and have a defense function due to their inhibitory capacity.

According to the results, the grain soybean cultivar when harvested early, at the R6 stage, did not show antinutrient contents that could classify it as inadequate for consumption as vegetable; additionally, its value of bioactive compounds was compatible with those of the two cultivars proposed for the vegetable soybean market, and it showed higher contents of phenolic compounds and total antioxidants.

CONCLUSION

The grain soybean cultivar, with pods harvested at R6 stage, showed bioactive compounds and antinutrients contents compatible with those of the two cultivars proposed for the vegetable soybean

Table 3 - Contents of alkaloids (presence or absence) of double-purpose soybean (DPS), vegetable soybean (VS) and grain soybean (GS) cultivars.

Cultivars	----------------------------------Alkaloids-----------------------------------
DPS	Absent
VS	Absent
GS	Absent

market, giving it the potential to be consumed as vegetable soybean.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Authors critically revised the manuscript and approved of the final version.

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