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Original

Effect of harvest at different times of day on the physical and chemical characteristics of vegetable-type soybean

Efeito da colheita em diferentes horários do dia sobre as características químicas e físicas de soja tipo hortaliça

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Abstract

The objective of this study was to evaluate the effect of harvest at different times of day on the chemical and physical characteristics of vegetable-type soybean BRS 267 cultivar, harvested at the R6 stage (seed development) and to compare it with that on the grains harvested at the R8 stage (maturation). The pods of the BRS 267 cultivar were harvested at the R6 stage (at 8:00 AM, 12:00 AM, and 6:00 PM), the color parameters were evaluated, and the grains were analyzed for chemical composition, activity inhibitor trypsin, phytic acid content, starch, sugars, fatty acids, and isoflavones. No differences were observed among the different harvest times in terms of the chemical constituents of vegetable-type soybean BRS 267 cultivar harvested at the R6 stage. Isoflavones content did not change with different harvest times, and the aglycone forms (daidzein, glycitein, and genistein) were found in smaller quantities at the R6 stage compared to the R8 stage. The color of the pods of soybean BRS 267 cultivar, harvested at the R6 stage did not change with different harvest times. The grains harvested at the R6 stage had lower protein content, phytic acid, and sucrose and higher levels of lipids, carbohydrates, starch, glucose, fructose, stachyose, and linolenic acids than those collected at the R8 stage. The different times of harvest did not affect the quality of the vegetable-type soybean BRS 267 cultivar harvested at stage R6. Nevertheless, it is recommended to harvest in the morning, when the temperature is milder, like other vegetables, to facilitate and optimize its marketing and in natura consumption.

Keywords: *vegetable-type soybean; centesimal composition; phytic acid; trypsin inhibitor; isoflavones.*

Resumo

O trabalho teve como objetivo avaliar o efeito da colheita em diferentes horários do dia sobre as características químicas e físicas dos grãos de soja da cultivar tipo hortaliça BRS 267, colhidos no estádio R6 (desenvolvimento da semente), e comparar com os colhidos no estádio R8 (maturação). As vagens da cultivar BRS 267 foram colhidas no estádio de desenvolvimento R6 nos horários das 8, 12 e 18 horas e avaliadas a cor e os grãos analisados quanto à composição química, atividade do inibidor de tripsina, teor do ácido fítico, amido, açúcares, ácidos graxos e isoflavonas. O teor de isoflavonas não diferiu entre os diferentes horários de colheita no estádio R6. No estádio R8 foi encontrado maior teor das formas agliconas. Os diferentes horários de colheita não influenciaram na cor das vagens e nos constituintes químicos dos grãos da cultivar BRS 267 colhida no estádio R6. Os grãos colhidos no estádio R6, quando comparados com os colhidos no estádio R8, apresentaram menor teor de proteínas, ácido fítico e sacarose e maior teor de lipídios, carboidratos, amido, glicose, frutose, estaquiose e ácido linolênico. A qualidade da soja tipo hortaliça BRS 267 no estádio R6 não diferiu com os diferentes horários de colheita, contudo, como produto tipo hortaliça, recomenda-se que a colheita seja realizada no período da manhã, cuja temperatura é mais amena, para facilitar a rápida comercialização e consumo como alimento in natura.

Palavras-chave: *soja tipo hortaliça; composição centesimal; ácido fítico; inibidor de tripsina; isoflavonas.*

1 Introduction

Vegetable-type soybean, traditionally known as edamame in Japan, is harvested at the R6 stage, in which the grains are fully developed but still green and immature. The reproductive stages Rl and R2 are based on flowering, R3 and R4 on pod development, R5 and R6 on seed development, and R7 and R8 on maturation (FERH et al., 1971). In the oriental cuisine, the dish edamame is traditionally eaten with this type of soybeans. This product is little known to Brazilian people, but it is popular in Asia and the United States. In Brazil, there is a demand for this type of product although its production and chemical composition need further investigation, as proposed by this study.

The edamame has a mild and sweet flavor, and thus it is widely appreciated and consumed as an appetizer, in which the pods containing the beans are cooked in salted water (SHURTLEFF; LUPKIN, 2001).

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The quality of the vegetable-type soybean or edamame can be assessed in terms of major five characteristics, namely appearance, taste, flavor, texture, and nutritional value (MASUDA, 1991). Due to these characteristics, especially its soft and sweet flavor that can contribute to the increase of soybean consumption, the Brazilian Agricultural Research Corporation, the National Center for Soybean Research, Embrapa Soybean developed the vegetable-type soybean BRS 267 cultivar for its consumption as edamame.

As for appearance, the pods of vegetable-type soybean should have bright-green color and good shape with spotless surface to fetch a good price at the wholesale and retail market. The surface condition of the pods may indicate the amount of chemical components in the grain, for example, yellowish reflects freshness decline and degradation of sugars, amino acids, and ascorbic acid. The grains should be large, and the weight of 100 seeds should be equal or greater than 20 g (SHANMUNGASUNDARAM, 1991). The mild sweet flavor of the vegetable-type soybean cultivars can be attributed to the higher concentration of sucrose, glutamic acid, and alanine in relation to conventional cultivars (MASUDA, 1991). The BRS 267 vegetable-type soybean described by Silva, Carrão-Panizzi and Prudencio (2009) showed contents of 43.50 $g.kg^{-1}$ of sucrose, 0.39 $mol.mg^{-1}$ of glutamic acid, and 0.21 $mmol.mg^{-1}$ of alanine,while the conventional BRS 133 soybean showed levels of 40.60 $g.kg^{-1}$ of sucrose, 0.37 $mol.mg^{-1}$ of glutamic acid, and 0.20 $mmol.mg^{-1}$ of alanine.

In addition to the sweet flavor and mild flavor, edamame has tender texture, which can be influenced by several factors such as cultivar, stage of harvesting, and cooking time of beans (TSOU; HONG, 1991). When the cooking time is prolonged, degradation of the color of the pods and change in the texture of the grains may occur.

The nutritional value of vegetable-type soybean cultivars can be confirmed by the higher protein content (12.95 $g.100\ g^{-1}$), vitamins A (180 $IU.100\ g^{-1}$), C (29 $mg.100\ g^{-1}$), B1 (0.435 $mg.100\ g^{-1}$), B2 (0.175 $mg.100\ g^{-1}$), minerals such as K with 620 $mg.100\ g^{-1}$, P (194 $mg.100\ g^{-1}$) and Ca (197 $mg.100\ g^{-1}$), and dietary fiber (4.2 $g.100\ g^{-1}$) (UNITED STATES, 2005).

Anti-nutritional factors that could reduce vegetable-type soybean consumption include phytate, trypsin inhibitor, saponins, isoflavones, and certain oligossacharides (MOHAMED; RAO; MEBRAHTU, 2001). Phytic acid is the main source of phosphorus in soybean seed and is known to form complexes with phosphorus, proteins, and minerals such as Ca, Mg, Zn, and Fe. This reduces the bioavailability of these minerals. The trypsin inhibitors can bind proteins and reduce protein efficiency ratio. However, thermal treatments such as bleaching can reduce this antinutritional factor. (MEBRAHTU; MOHAMED; ELMI, 1997). According to Shamsuddin (2002), both phytic acid and trypsin inhibitors may have beneficial effects on human health: it lowers the risk of colon cancer, breast, lung and skin, prevents kidney stones (ZHOU; ERDMAN, 1995), and have antioxidant activity (EMPSON; LABUZA; GRAF, 1991).

Saponins and isoflavones are associated with bitter and astringent taste, however, they are important phytochemicals associated with benefits to human health (ELLINGTON; BERHOW; SINGLETARY, 2005). Stachyose and raffinose cause flatulence and abdominal discomfort, but they can promote the growth of beneficial bacteria to the human organism (OBATOLU; OSHO, 2006).

Environmental factors, such as location, season, and weather (light intensity and water content) can affect greatly the quality attributes of vegetable-type soybean (MASUDA, 1991). The time of harvest and the time of day are parameters considered as important and may interfere with the content of certain chemical constituents. When vegetable-type soybean is harvested under high temperature, its unique and distinctive flavor may be changed due to the reduction in sugar content and free amino acids (MBUVI; LITCHFIELD, 1995).

The objective of this study was to evaluate the effect of harvest at different times of day on the chemical and physical characteristics of the vegetable-type soybean BRS 267 cultivar harvested at the R6 stage comparing with those harvested at the R8 stage.

2 Material and methods

2.1 Materials

Grains of vegetable-type soybean BRS 267 cultivar, developed by the breeding program of Embrapa Soybean, Londrina-PR, were used. The pods (2007/2008 season) were harvested in February at the R6 growth stage (62 days after flowering) at three different times of day (8:00 AM, 12:00 AM and 6:00 PM). Pods containing beans at the R8 growth stage were also harvested.

2.2 Chemical composition

For chemical analysis, the grains at the R6 and R8 stages were manually threshed, dried in an oven, cooled (Fanem 320 SE) at 45 °C, and grounded using an analytical mill (Tecnal TE 631) to obtain a finely powder (35 mesh). Moisture, protein, lipid, and ash were determined according to a method developed by Instituto Adolfo Lutz (2005) and total carbohydrate was determined by difference.

2.3 Phytic acid content

Phytic acid content was determined according to Latta and Eskin (1980) with some modification by using Dowex-AGX-4 resin as proposed by Ellis and Morris (1986).

2.4 Trypsin inhibitor activity

Trypsin inhibitor activity was determined according to Kakade et al. (1974) and expressed as $mg.g^{-1}$ IT.

2.5 Starch content and sugars

The content of starch was determined by enzymatic hydrolysis (RICKARD; BEHN, 1987). The sugars were

quantified according to Masuda, Kaneko and Yamashita (1996) by ion exchange chromatography (Dionex BioLC mark) with a pulse amperometric detector, AgCl electrode (Dionex brand, model ED 50), cell amperometric gold, and auto-injector samples. Separation was accomplished using a CarboPac PA 10 column (250 × 4 mm internal diameter, particle size 5 mm) adopting the isocratic system using 50 mM NaOH as the mobile phase solution at a flow rate of 1.0 mL/min at 25 °C. Sugar quantification was performed using external standard (calibration curves) with the respective standards (Sigma) of known concentration.

2.6 Fatty acids content

The content of fatty acids was determined by gas chromatography (GC) according to Rayford et al. (1994) and Min et al. (2005) using a gas chromatograph (Hewlett Packard brand - model 6890) with sample auto-injector and equipped with silica capillary column (30 m long, 0.32 mm internal diameter and 0.2 mm in film thickness) (Supelco brand - Model SP 2340). The analysis was conducted by isothermal chromatography at 190 °C with a flame ionization detector with a temperature of 300 °C and injector set at 250 °C. Fatty acid quantification was performed by external standard (calibration curves) with the respective standards (Fame) of known concentration.

2.7 Isoflavones analysis

The extraction of isoflavones was performed according to Carrão-Panizzi, Góes-Favoni and Kikuchi (2002) and quantified according to Berhow (2000) by liquid chromatography (Waters brand, model 2690). Separation was performed on a C18/ODS reversed phase column (YMC-Pack ODS-AM) (S-5 mm, 120 A, 250 mm long × 4.6 mm in diameter) adopting a linear binary gradient system using methanol-trifluoroacetic acid as the mobile phase at flow rate 1 mL/min at 25 °C. A photodiode array detector (Mark Waters, model 996) with wavelength at 254 nm was used for detection of isoflavones. Quantification of isoflavones was performed by external standard (calibration curves) with the respective standards (Sigma) of known concentration.

2.8 Color parameters

The color parameters of the pods such as lightness (L*), red-green component (a*), and yellow-blue component (b*) were determined using a colorimeter (MINOLTA CO., JAPAN, model CR - 13) at observer angle 10° under D65 illuminant.

2.9 Experimental design and statistical analysis

The experimental design was a randomized block with four replications. The results were subjected to analysis of variance (ANOVA) and mean comparison test, Tukey test, at 5% significance using the scientific software package SAS-Statistical Analysis System, version 8.2 (SAS INSTITUTE, 2001).

3 Results and discussion

Moisture, protein, lipids, ash, and carbohydrates contents (Table 1) of the vegetable-type soybean BRS 267 cultivar at the R6 growth stage did not differ among themselves in different harvest times. The high temperature and low relative humidity at the harvest time of 12:00 AM, compared to that of 8:00 AM and 6:00 PM, probably was not enough to cause variations in the composition of the constituents. The average protein content at the R6 stage was 36.72 g.100 g^{-1}, and it is in accordance with the result reported by Rao, Bhagsari and Mohamed (2002) for the vegetable-type soybean with contents between 33.32 and 38.60 g.100 g^{-1}. The average protein content of BRS 267 at the R8 stage, was higher than that at the R6 indicating that the proteins were not fully synthesized at that stage of development. The vegetable-type soybean, harvested at the R6 stage, had a higher protein content compared to that of fresh peas, 28.72 g.100 g^{-1} (CANNIATTI-BRAZACA, 2006), and corn, 3.22 g.100 g^{-1} (UNITED STATES, 2008).

The average lipids content at the R6 stage, 19.98 g.100 g^{-1}, was higher than that at the R8 stage, 18 g.100 g^{-1}. No differences were found in the phytic acid content of vegetable-type soybean BRS 267 cultivar harvested at the R6 stage at different harvest times. The average content of phytic acid at the R6 stage was lower (1.86 g.100 g^{-1}) than that at the R8 (2.61 g.100 g^{-1}). Investigating vegetable-type soybean cultivars Rao, Bhagsari and Mohamed (2002) found that the phytic acid content ranged from 1.08 to 1.39 g.100 g^{-11}, and these values were lower than those observed in this study. The activity of trypsin inhibitor (IT) did not vary with the time of harvest and among the R6 and R8 growth stages. Obatolu and Osho (2006) found, in different cultivars of the vegetable-type soybean, trypsin inhibitor activities values between 19.70 and 23.80 mg.g^{-1} IT, which are close to those observed in this study.

Starch and sugars contents (Table 2) in vegetable-type soybean BRS 267 cultivar did not differ in the different times of harvest. The average content of starch at the R6 stage was 4.85 g.100 g^{-1}, which is higher than that at the R8 stage, 0.65 g.100 g^{-1}. Masuda (2004) also observed a higher average content of starch, 8.32 g.100 g^{-1}, for soybean harvested at the R6

Table 1. Chemical composition, phytic acid content, and trypsin inhibitor activity of vegetable -type soybean BRS 267 cultivar harvested at the R6 stage at different times of day and average values at the R6 and R8 the stages[1].

Chemical composition	Harvest at R6			Average value	
	8:00 AM	12:00 AM	6:00 PM	R6	R8
Mositure	8.50^{a}	8.33^{a}	8.48^{a}	8.44^{A}	6.70^{B}
Proteins	36.62^{a}	36.46^{a}	37.09^{a}	36.72^{B}	40.27^{A}
Lipids	19.21^{a}	20.42^{a}	20.33^{a}	19.98^{A}	18.00^{B}
Ash	4.67^{a}	4.58^{a}	4.56^{a}	4.60^{B}	6.08^{A}
Carboydrates	31.00^{a}	30.22^{a}	30.36^{a}	30.26^{A}	28.95^{B}
Phytic acid	1.80^{a}	1.88^{a}	1.85^{a}	1.86^{B}	2.61^{A}
Trysin inhibitor	21.75^{a}	21.22^{a}	20.88^{a}	21.28^{A}	20.16^{A}

[1]Results expressed as g.100 g^{-1} (dry weight) for chemical composition and phytic acid and trypsin inhibitor activity by mg IT g^{-1} (dry weight); means followed by equal letters in the lines do not differ by Tukey's test, at 5% probability for each component.

Table 2. Starch and sugars contents of vegetable-type soybean BRS 267 cultivar harvested at the R6 stage at different times of day and average values at the R6 and R8 stages[1].

Constituents	Harvest at R6			Average value	
	8:00 AM	12:00 AM	6:00 PM	R6	R8
Starch	4.84[a]	4.84[a]	4.87[a]	4.85[A]	0.65[B]
Glucose	0.05[a]	0.04[a]	0.05[a]	0.05[A]	**
Frutose	0.05[a]	0.05[a]	0.06[a]	0.05[A]	**
Sucrose	4.44[a]	4.62[a]	4.44[a]	4.50[B]	6.50[A]
Raffinose	1.01[a]	1.04[a]	1.04[a]	1.03[A]	1.61[A]
Stachyose	4.52[a]	4.14[a]	4.47[a]	4.38[A]	1.79[B]
Carbohydrates total	10.07[a]	9.89[a]	10.06[a]	10.01[A]	9.90[A]

[1]Results expressed as g.100 g^{-1} (dry weight); means followed by equal letters in the lines do not differ by Tukey's test, at 5% probability for each component. **Not detected constituents.

stage than that at the R8 stage, 0.07 g.100 g^{-1}. The sucrose content (4.50 g.100 g^{-1}) was higher than that described by Kumar, Rani and Chauran (2007), who also analyzed soybean cultivars at the R6 stage and obtained a maximum of 2.02 g.100 g^{-1} of sucrose. The average content of sucrose of the BRS 267 cultivar at the R8 stage was higher than that at the R6 stage. However, glucose and fructose content in the R6 stage was 0.05 g.100 g^{-1}, whereas these sugars were not detected at the R8 stage. Silva, Carrão-Panizzi and Prudencio (2009) also found higher levels of fructose in vegetable-type soybean BRS 267 cultivar, 0.80 g.kg^{-1}, and suggested that this sugar with sucrose may be associated with the unique and distinct flavor of edamame. With the ripening of the grains (R8) (Table 2), a reduction in the starch content and an increase in stachyose and sucrose were observed.

The content of fatty acids in vegetable-type soybean did not differ in the different harvest times (Table 3). The total content of fatty acids in soybean BRS 267 cultivar harvested at the R8 stage was higher than at the R6 stage indicating that these components were probably not yet be fully developed at the R6 growth stage. The content of fatty acids was close to that described by the United States (2008) (14.83 g.100 g^{-1}) for vegetable-type soybean. According to United States (2008), the peas and corn have total fatty acid content of 0.52 and 4.03 g.100 g^{-1} on a dry basis, respectively. Therefore, the vegetable-type soybean becomes a differentiated product in terms of fatty acids when compared with fresh peas or corn.

According by Góes-Favoni, Carrão-Panizzi and Beléia (2010), the values for the isoflavones may be presented in aglycone equivalents or as the sum of the different forms (YUE; MOURSY; XU, 2010), and the isoflavones content may vary with environmental conditions, processing conditions, and analytical techniques. The content of isoflavones did not change with harvest at different times of day (Table 4). Aglycone forms (daidzein, glycitein and genistein) that have biological activity were found in smaller quantities at R6 stage compared to those at the R8 stage. The total isoflavone content of vegetable-type soybean harvested at the R6 stage was 186.94 mg.100 g^{-1}, and it was less than that of the soybean harvested at the R8 stage, 270.27 mg.100 g^{-1}. With the maturation of the grains, was there was an increase in the concentration of glucosydes, malonyl-genistin, and aglycones, while the forms malonyl-daidzin and malonyl-glycitin did not differ. These results confirm the findings of Mandarino, Carrão-Panizzi and Crancianinov (1999), who stated that the malonyl-daidzin form is present in significant quantities throughout the period of grain development.

Table 3. Fatty acid content of vegetable-type soybean cultivar BRS 267 cultivar harvested at the R6 stage at different times of day and average values at the R6 and R8 stages[1].

Fattic acids	Harvest at R6			Average value	
	8:00 AM	12:00 PM	6:00 PM	R6	R8
Palmitic	1.80[a]	1.69[a]	1.76[a]	1.75[A]	1.58[A]
Estearic	0.52[a]	0.51[a]	0.53[a]	0.52[B]	0.58[A]
Oleic	3.07[a]	2.95[a]	3.03[a]	3.02[A]	3.75[A]
Linoleic	8.44[a]	8.02[a]	8.18[a]	8.21[A]	8.75[A]
Linolenic	0.05[a]	0.05[a]	0.05[a]	1.30[A]	1.15[B]
Araquidic	1.38[a]	1.20[a]	1.31[a]	0.05[A]	0.05[A]
Fatty acids total	15.27[a]	14.48[a]	14.86[a]	14.85[B]	16.13[A]

[1]Results expressed as g.100 g^{-1} (dry weight); means followed by equal letters in the lines do not differ by Tukey's test, at 5% probability for each component.

Table 4. Isoflavones content of vegetable-type soybean cultivar BRS 267 cultivar harvested at the R6 stage at different times of day and average values at the R6 and R8 stages[1].

Isoflavones	Harvest at R6			Average value	
	8:00 AM	12:00 AM	6:00 PM	R6	R8
Daidzin	13.91[a]	14.00[a]	13.15[a]	13.65[B]	39.33[A]
Glycitin	4.50[a]	4.36[a]	4.84[a]	4.57[B]	10.53[A]
Genistin	13.71[a]	14.40[a]	14.52[a]	14.21[B]	47.63[A]
Malonyl-Daidzin	66.00[a]	65.16[a]	64.22[a]	65.13[A]	66.03[A]
Malonyl-Glycitin	29.01[a]	28.21[a]	28.81[a]	28.68[A]	29.26[A]
Malonyl-Genistin	60.00[a]	59.77[a]	59.64[a]	59.80[B]	71.72[A]
Daidzein	0.32[a]	0.31[a]	0.31[a]	0.31[B]	1.78[A]
Glycitein	0.28[a]	0.27[a]	0.28[a]	0.28[B]	1.52[A]
Genistein	0.28[a]	0.29[a]	0.28[a]	0.28[B]	2.49[A]
Isoflavones total	188.01[a]	186.77[a]	186.05[a]	186.94[B]	270.27[A]

[1]Results expressed as g.100 g^{-1} (dry weight); means followed by equal letters in the lines do not differ by Tukey's test, at 5% probability for each component.

Table 5. Color parameters of the pods of vegetable-type soybean BRS 267 cultivar harvested at the R6 stage at different times of day and average values at the R6 and R8 stages[1].

Color parameters	Harvest at R6			Average value
	8:00 AM	12:00 AM	6:00 PM	
L*	18.77[a]	19.97[a]	19.17[a]	19.30
a*	–7.93[a]	–7.23[a]	–7.38[a]	–7.51
b*	19.90[a]	20.23[a]	20.38[a]	20.17

[1]Average of five replicates being the means followed by the same letter do not differ by Tukey test at 5% probability for each component. L* value indicates white (100) or black (0), a* value indicates red (+) or green (–) and b* value indicates yellow (+) or blue (–). Maximum values for a* and b* are between 100 and –100, respectively.

The color parameters (L*, a*, b*) of the pods of the vegetable-type soybean BRS 267 cultivar harvested at the R6 stage did not change with different harvest times (Table 5). The pods of soybean BRS 267 cultivar showed a tendency of light to dark tone with an average L* value of 19.30. The average value

of the negative a* may indicate greenish pods since the more negative this value is, the more intense the green color. The average value of b* (20.17) may suggest a tendency to yellow, which is probably due to the premature ripening of the pods.

While the length of harvest and time of day are important parameters for the quality of the edamame and may also interfere with the content of certain constituents (MBUVI; LITCHFIELD, 1995; MASUDA, 1991; OBATOLU; OSHO, 2006), in the present study, it was confirmed that the different harvest times of vegetable-type soybean BRS 267 cultivar in the R6 stage did not influence the composition, the activity of trypsin inhibitors, the content of phytic acid, starch, soluble sugars, fatty acids, and isoflavones, and the color parameters L*, a*, b* of the pods, even with variations of temperature and relative humidity. At the R6 stage, the presence of the glucose and fructose may be associated with the mild and sweet flavor of the vegetable-type soybean. With the ripening of the grains, there was an increase in protein, phytic acid, total fatty acids, and isoflavones contents.

4 Conclusion

The quality of the vegetable-type soybean BRS 267 cultivar harvested at the R6 stage did not change with different harvest times, yet, it is recommended to harvest in the morning when the temperature is more pleasant, like other vegetables, to facilitate its rapid commercialization and consumption as in natura food. When compared with grains harvested at the R8 stage, the grains harvested at the R6 stage had a lower protein, phytic acid and sucrose content and higher levels of lipids, carbohydrates, starch, glucose, fructose, stachyose, and linolenic acids,

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