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Food Technology/ Original Article

# Germination conditions influence the physical characteristics, isoflavones, and vitamin C of soybean sprouts

**Abstract** – The objective of this work was to evaluate the effects of the germination conditions of 'BRS 216' soybean (*Glycine max*) on the length, yield, and isoflavone contents of sprouts. A 2<sup>3</sup> factorial arrangement was used to evaluate the variables soaking time, irrigation frequency, and germination time. Sprouts that showed better length and yield and higher isoflavone contents were evaluated for their chemical composition and vitamin C content. Soaking and germination time of soybean showed a positive and significant linear effect on sprout length. However, only germination time showed a significant positive linear effect on yield, and a significant negative linear effect on the content of malonylglycosides, aglycones, and total isoflavones. Soybean germination conditions were established at 6 hours of soaking, three days of germination, and 8 hours of irrigation frequency. Under these conditions, the obtained sprouts show a more preserved chemical composition, besides higher contents of β-glycosides, malonylglycosides, aglycones, total isoflavones, and vitamin C than the nongerminated soybean. Under suitable germination conditions, it is possible to produce soybean sprouts with better physical characteristics, higher yield, and higher contents of isoflavones, aglycones, and vitamin C.

**Index terms:** *Glycine max*, aglycones, ascorbic acid, proximate composition, soaking.

## Condições de germinação influenciam as características físicas, isoflavonas e vitamina C de brotos de soja

**Resumo** – O objetivo deste trabalho foi avaliar os efeitos das condições de germinação da soja (*Glycine max*) 'BRS 216' sobre o comprimento, o rendimento e o teor de isoflavonas dos brotos. Um delineamento fatorial 2<sup>3</sup> foi utilizado para avaliar as variáveis tempo de maceração, frequência de irrigação e tempo de germinação. Os brotos que apresentaram melhor comprimento e rendimento e maior teor de isoflavonas foram avaliados quanto à composição química e ao teor de vitamina C. Os tempos de maceração e germinação da soja apresentaram efeito linear positivo e significativo sobre o comprimento dos brotos. No entanto, apenas o tempo de germinação apresentou efeito linear significativo e positivo sobre o rendimento, e efeito linear significativo e negativo sobre o teor de malonilglicosídeos, agliconas e isoflavonas totais. As condições de germinação da soja foram estabelecidas em 6 horas de maceração, três dias de germinação e 8 horas de frequência de irrigação. Nessas condições, os brotos obtidos apresentam composição química mais preservada, além de maior teor de β-glicosídeos, malonilglicosídeos, agliconas, isoflavonas totais e vitamina C do que a soja não germinada. Em condições adequadas de germinação, é possível produzir brotos de soja com melhores características físicas, maior rendimento, e maiores teores de isoflavonas, agliconas e vitamina C.

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**Termos para indexação:** *Glycine max*, agliconas, ácido ascórbico, composição aproximada, maceração.

## Introduction

Soybean – *Glycine max* (L.) Merr. – is widely cultivated worldwide due to its nutritive value with high content of lipids, proteins, and carbohydrates. It also contains isoflavones that have beneficial effects on human health. The production and consumption of soybean and its by-products have increased significantly. According to the Food and Agriculture Organization (FAO), it is the most important legume crop cultivated in the world (Fao, 2019). In a dry weight basis, soybean grains are composed of 21% lipids, 40% proteins, 34% carbohydrates, and 5% ash. Soybean can be used to prepare various types of food that are versatile, tasty, and digestible, such as tofu, sauce, paste, extract, and sprouts (Ali, 2010).

Soybean isoflavones are bioactive compounds that benefit human health and are divided into four groups:  $\beta$ -glycosides (daidzin, genistin, and glycitin); 6''-*O*-acetylglycosides (acetyl daidzin, acetyl genistin, and acetyl glycitin); 6''-*O*-malonylglycosides (malonyl daidzin, malonyl genistin, and malonyl glycitin); and aglycones (daidzein, genistein, and glycitein) (Falcão et al., 2018). Soybean isoflavones have also been extensively investigated because of their positive effects, including antioxidant and hypocholesterolemic activity, prevention of menopausal symptoms, and cardiovascular diseases; besides, such isoflavones protect against bone loss in postmenopausal symptoms, in women, and against the development of breast and prostate cancers (Haron et al., 2011; Dong et al., 2013; Wada et al., 2013; Liu et al., 2017; Ramdath et al., 2017).

Germination is a biological process by which seed emerge from a latent stage, after they are supplied with the appropriate moisture, temperature, and nutrient conditions that are necessary for growth and development (Nogueira & Sedyama, 2013). During the germination, a sequence of metabolic changes is triggered, improving the nutritional value of the soybean, while reducing the amount of undesirable substances, such as flatulence-inducing components (raffinose and stachyose) and antinutrients (trypsin inhibitors, lectins, phytates) (Martín-Cabrejas et al., 2008; Paucar-Menacho et al., 2010; Kim et al., 2013). Bioactive compounds such as ascorbic acid,

riboflavin, and thiamine, according to Raghuvanshi & Bisht (2010), phytosterols, tocopherols, and flavonoids, according to Shi et al. (2010), and total phenolics, glycosides saponins, and isoflavone aglycones, according to Quinhone Júnior & Ida (2015), have been found to be positively affected by germination. Furthermore, seed germination may also improve the antioxidant potential of soybean (Yoshiara et al., 2018).

The influence of germination conditions on the nutrient content and composition depends on variables such as soaking time, humidity, temperature, time of germination, and cultivar type (Paucar-Menacho et al., 2010).  $\beta$ -glucosidases are activated during soybean germination, thus changing the isoflavone profile of this feedstock (Yoshiara et al., 2018). Furthermore, soybean sprouts containing bioactive isoflavones have already been researched as potential feedstocks in the development of soymilk and tofu (Murugkar, 2015), and their consumption is highly recommended due to the presence of these compounds.

Although several studies have supported the use of germination to improve soybean quality, the production of soybean sprouts in Brazil is very restricted, or almost nonexistent. However, the lack, or scarcity of information on the conditions of germination of such a product may compromise their popularization, especially when it comes to the effects of such a process on the potential bioactivity of soybean sprouts. In turn, the knowledge on the effects of this process may enhance the knowledge of the soybean industry for the development of soybean sprouts as functional foods.

The objective of this work was to evaluate the effects of the germination conditions of 'BRS 216' soybean on the length, yield, and isoflavone contents of sprouts.

## Materials and Methods

The soybean cultivar 'BRS 216' was provided by Embrapa Soja, located in the municipality of Londrina, in the state of Paraná, Brazil. This conventional cultivar is appropriate for sprout production due its seed size, average weight of 10.4 g per 100 seed, and high-protein content (43.6%), according to Cultivares... (2005). All experiments and physical and chemical analysis (oil, protein, ash, isoflavones, and vitamin C) were performed at Embrapa Soja.

The different forms of isoflavone were determined using high-performance liquid chromatography (HPLC) with a photodiode array detector (Model 996), and an automatic sample injector (Model 717 Plus), both manufactured by Waters (Milford, USA), using a reverse phase column (YMC-Pack ODS-AM, C18, S-5  $\mu\text{m}$ , diameter of  $250 \times 4.6$  mm) according to the method described by Benedetti et al. (2013). Each form of isoflavone has a distinct molar mass, and the different forms of isoflavones and the sum of all forms of isoflavones – that is,  $\beta\text{gly}$ s ( $\beta$ -glycosides),  $\text{Mgly}$ s (malonylglycosides),  $\text{Agly}$ s (aglycones), and  $\text{IsoTs}$  (total isoflavones, which means the sum of  $\beta$ -glycosides, malonylglycosides, and aglycones) – were expressed on a molar basis as  $\mu\text{mol}$  of isoflavones  $\text{g}^{-1}$  of defatted sample (mean  $\pm$  standard deviation), on a dry basis. The 6''-*O*-acetylglycosides and the 6''-*O*-malonylglycosides (Wako Pure Chemical Industries, Ltd., Osaka, Japan), the  $\beta$ -glycosides and the aglycones (Sigma-Aldrich Co., St. Louis, MO, USA) were used as standards for the isoflavones. All chemicals were of analytical grade, or liquid-chromatography grade.

In order to evaluate the effects of germination conditions on 'BRS 216' soybean sprouts length, yield, and isoflavones content, the factorial design  $2^3$  of the response surface methodology was applied. The independent variables investigated were soaking time (hours), irrigation frequency (hours) and germination time (days). The design was composed of 8 factorial points, and 3 additional replicates at the central point, resulting in a total of 11 randomized assays. The response functions were determinate as: LSPR, length of soybean sprouts in cm; % YSPR, yield of soybean sprouts in percentage;  $\beta\text{gly}$ s,  $\mu\text{mol}$  of  $\beta$ -glycosides  $\text{g}^{-1}$  of soybean sprouts;  $\text{Mgly}$ s,  $\mu\text{mol}$  of malonylglycosides  $\text{g}^{-1}$  of soybean sprouts;  $\text{Agly}$ s,  $\mu\text{mol}$  of aglycones  $\text{g}^{-1}$  of soybean sprouts; and  $\text{IsoTs}$ ,  $\mu\text{mol}$  of total isoflavones  $\text{g}^{-1}$  of soybean sprouts. The contents of malonylglycosides, aglycones, and total isoflavones were expressed on a dry basis.

For each assay, germination was performed as described by Oliveira et al. (2013), with modifications, with 250 g of soybean seed at room temperature ( $26 \pm 2^\circ\text{C}$ ). Soybean seed, previously sanitized with 1% sodium hypochlorite for 4 min, were placed in a container of 100 L capacity with a cap to prevent light exposure. This container included a stainless-steel screen 2 cm from the bottom, and an outlet at the bottom to drain excess water. The soybean sprouts

were placed in resealable plastic zipper storage bags, frozen at  $-86^\circ\text{C}$  in an ultrafreezer (Indrel IULT 355D, Londrina, PR, Brazil), freeze-dried at  $-55^\circ\text{C}$  (Liotop L101, São Carlos, SP, Brazil), milled in a grinder model DCG-12BC (Cuisinart Grind Central Coffee, Stamford, CT, USA), and stored at  $4^\circ\text{C}$  until the determination of response functions. The model for each response function was expressed as  $Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 + \beta_{123}x_1x_2x_3 + \varepsilon$ , in which:  $Y$  is the response function;  $x_1$ ,  $x_2$ , and  $x_3$  are the levels of the coded variables;  $\beta$  is the estimated coefficient on the response surface; and  $\varepsilon$  is the error. The models were obtained by the analysis of variance (Anova at 5% probability) followed by regression analysis, using the software Statistic 8.0 (StatSoft, Tulsa, OK, USA). Response surface plots were constructed from the adjusted models.

After performing each assay, the response functions LSPR and %YSPR were measured and calculated. To measure the LSPR of sprouts, the container was divided into eight parts, from which seventeen sprouts were collected as follows: one sample from the center of the container, eight samples from the intermediate region, and eight samples from the ends of each part of the container. The LSPR of sprouts was measured with a millimeter ruler and expressed as an average of these seventeen sprouts. The %YSPR was calculated as the ratio of the mass of soybean sprouts to the mass of seed multiplied by 100.

Soybean sprouts showing better length and yield, and higher-total isoflavone content were evaluated for chemical composition and vitamin C content. Nongerminated soybean was used as a control. The chemical composition and vitamin C content were determined according to the method described by the Latimer Jr. (2012).

## Results and discussion

The soybean germination conditions had different effects on the length (LSPR) and yield (%YSPR) of the sprouts (Table 1). The parameters of analyses of variance and regression for LSPR showed that soaking ( $X_1$ ) and germination time ( $X_3$ ) had positive and significant linear effects, whereas irrigation frequency ( $X_2$ ) did not show a significant effect on germination. The lack of fit of the model was not significant, and the proposed model ( $\text{LSPR} = 13.545 + 1.599x_1 + 6.055x_3$ ) explained 98% ( $R^2$ ) of the experimental data. The

response surface (Figure 1 A) indicated that the LSPR was maximal (21.1 cm) when 5-hour soaking  $\leq X_1 \leq$  6-hour soaking and  $x_3 = 7$  days of germination were used, which corresponded to assays 6 and 8. However, soybean sprouts which had grown to 21.2 cm in length were not suitable due to the marked appearance of the roots and transformation of cotyledons into leaves. Nevertheless, the soybean sprouts length was minimal (5.89 cm) when 2-hour soaking  $\leq X_1 \leq$  3-hour-soaking and  $x_3 = 3$  days of germination, which corresponded to assays 1 and 3. Under these germination conditions, the cotyledons were still preserved, and no root had emerged. The soybean sprouts that were approximately 8.75 cm long still exhibited proper characteristics of sprouts, and they were observed in assays 2 and 4 ( $X_1 = 6$  hour-soaking and  $X_3 = 3$  days of germination). According to Huang et al. (2014), the elongation percentage of soybean radicles at 2, 3, 4, and 5 days of germination were 177, 80, 55, and 21%, respectively. The elongation efficacy decreased with germination time and, in general, elongation occurred most effectively at the early stage of germination, that is, from 1 to 3 days. Therefore, in order to obtain soybean sprouts of adequate length (about 8.75 cm) and specific characteristics, germination should be carried out with 6-hour-soaking ( $X_1$ ) and 3 days of germination ( $X_3$ ), irrespective of irrigation frequency ( $X_2$ ).

The regression and analysis of variation of %YSPR indicated that only the germination time  $X_3$  showed a significant and positive linear effect. The lack of fit of the model was not significant ( $p > 0.05$ ), and the proposed model ( $\%YSPR = 195.454 + 46.250x_3$ ) explained 89% ( $R^2$ ) of the experimental data. The response surface (Figure 1 B) indicated that %YSPR reached a maximum value (241.7%) when  $x_3 = 7$  days of germination, which corresponded to assays 5 and 7. However, the integrity of the cotyledon in the sprouts was no longer preserved, and there was a marked appearance of roots, whose sprouts were 18.5 and 16.9 cm length. The soybean sprouts that were germinated for 3 days with 2-hour-soaking resulted in a %YSPR of 128% (assay 1) and 144% (assay 3), whereas those that were germinated for 3 days with 6-hour-soaking resulted in a higher % YSPR of 152% (assay 2) and 166% (assay 4). The higher yield of sprouts can be attributed to the absorption of water during irrigation in germination, and the fact that it decreased gradually as germination progressed indicated that some components were metabolized and used in the form of energy during germination (Nogueira & Sediya, 2013). Therefore, assay 4 corresponded to the best germination conditions that produced soybean sprouts of 8.75 cm length and intact cotyledons, without the marked appearance of roots,

**Table 1.** Independent variables of the germination conditions, and the response functions evaluated in ‘BRS 216’ soybean (*Glycine max*) sprouts.

Assay	Independent variable and coded level <sup>(1)</sup>			Response function <sup>(2)</sup>					
	$x_1(X_1, h)$	$x_2(X_2, h)$	$x_3(X_3, d)$	LSPR (cm)	YSPR (%)	$\beta$ glys ( $\mu\text{mol g}^{-1}$ )	Mglys ( $\mu\text{mol g}^{-1}$ )	Aglys ( $\mu\text{mol g}^{-1}$ )	IsoTs ( $\mu\text{mol g}^{-1}$ )
1	-1 (2)	-1 (4)	-1 (3)	5.9±1.0	128	0.88±0.29	10.42±2.54	0.42±0.11	11.72±2.07
2	1 (6)	-1 (4)	-1 (3)	9.1±1.1	152	1.09±0.32	10.27±2.37	0.39±0.08	11.75±1.99
3	-1 (2)	1 (8)	-1 (3)	5.6±1.4	144	0.86±0.26	9.81±2.33	0.38±0.08	11.05±1.93
4	1 (6)	1 (8)	-1 (3)	8.4±1.1	166	1.51±0.47	10.43±2.48	0.44±0.11	12.38±2.02
5	-1 (2)	-1 (4)	1 (7)	18.5±3.1	254	1.41±0.45	8.31±2.61	0.11±0.05	9.83±1.84
6	1 (6)	-1 (4)	1 (7)	22.1±2.3	216	1.32±0.39	8.17±2.55	0.23±0.06	9.72±1.79
7	-1 (2)	1 (8)	1 (7)	16.9±3.1	258	1.12±0.35	8.51±2.66	0.29±0.06	9.92±1.87
8	1 (6)	1 (8)	1 (7)	19.9±2.0	232	1.45±0.46	8.19±2.56	0.22±0.03	9.86±1.79
9	0 (4)	0 (6)	0 (5)	14.5±1.9	196	1.28±0.39	8.89±2.57	0.29±0.05	10.46±1.88
10	0 (4)	0 (6)	0 (5)	13.4±3.1	204	1.42±0.42	10.02±2.79	0.36±0.06	11.8±2.08
11	0 (4)	0 (6)	0 (5)	14.6±2.4	200	1.57±0.50	8.17±2.34	0.53±0.18	10.27±1.69

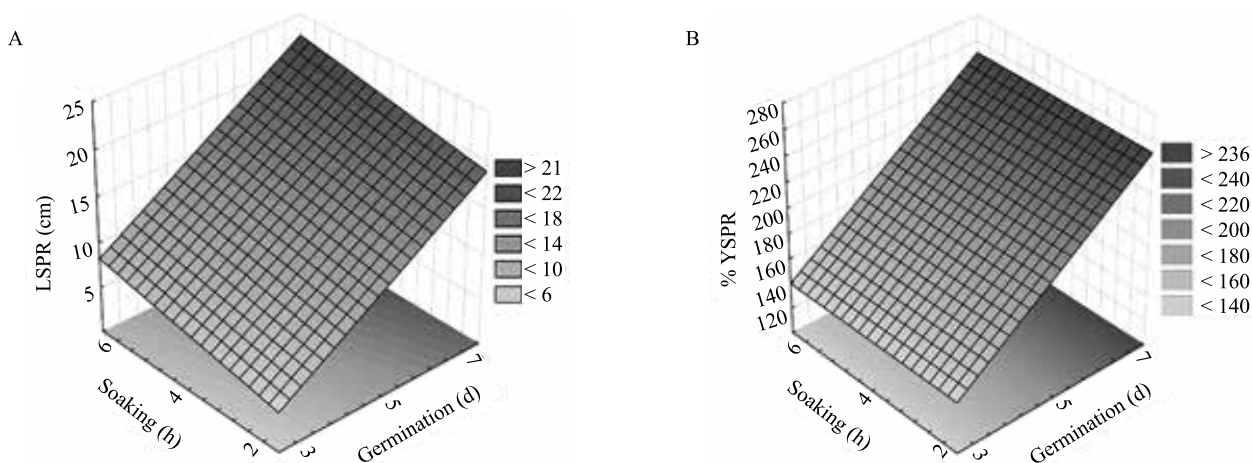
<sup>(1)</sup> $x_1$ , soaking time in hours;  $x_2$ , irrigation frequency in hours; and  $x_3$ , germination time in days. <sup>(2)</sup>LSPR, length of soybean sprouts; YSPR, yield of soybean sprouts;  $\beta$ glys, content of  $\beta$ -glycosides; Mglys, content of malonylglycosides; Aglys, content of aglycones; and IsoTs, content of total isoflavones. The  $2^3$  factorial design was composed of eight factorial points and three additional replicates at the central point, resulting in a total of 11 assays that were performed in a randomized design.

and showed the highest %YSPR. There was no need to validate the model, since assay 4 was performed according to the experimental design. Thus, assay 4, whose germination conditions were 6-hour-soaking ( $X_1$ ), 8 hours of irrigation frequency ( $X_2$ ), and 3 days of germination ( $X_3$ ), was found to produce soybean sprouts with adequate length and higher yield.

Regression analysis and the analysis of variance of  $\beta$ glys indicated that the independent variables  $X_1$ ,  $X_2$ , and  $X_3$  did not show significant effects and, therefore, the model equation was not estimated. In nongerminated soybean, daidzin, glycitin and genistin contents corresponded to 9.94, 1.68, and 5.88%, respectively, of the total isoflavones content (Table 2). To produce soybean sprouts of adequate length and higher yield, germination should be carried out according to assay 4. In this assay, the daidzin content was 45.07% higher and the genistin content was 14.29% lower than in nongerminated soybean. A similar predominance of daidzin content was found in 233 soybean cultivars grown in Brazil (Carrão-Panizzi et al., 2009). However, Ribeiro et al. (2007) observed 18 Brazilian soybean cultivars which had higher genistin content than daidzin.

For the Mglys, only the variable  $X_3$  (germination time) caused a significant and negative linear effect. The lack of fit of the model was not significant, and 77% ( $R^2$ ) of the experimental data was explained by the model ( $Mglys = 9.199 - 0.968x_3$ ). The response surface (Figure 2 A) indicated that the Mglys was maximal ( $10.168 \mu\text{mol g}^{-1}$  sample), when 3 days of

germination  $\leq X_3 \leq 4$  days of germination. These results indicated that within this range of germination time, the malonylglycoside content in soybean sprouts was 78.38% greater than that in nongerminated soybean ( $5.70 \pm 1.39 \mu\text{mol g}^{-1}$  sample) (Table 2). An average increase of 110- and 33-fold malonylglycoside content was observed in seven cultivars of soybean germinated with or without light, respectively, for 120 hours (5 days) at  $25^\circ\text{C}$  by Kim et al. (2006). However, Shi et al. (2010) observed that the malonyldaidzin, malonylgenistin, and malonylglycitin content in germinated soybean decreased by 7.7-, 1.4-, and 2.0-fold, respectively, after 168 hours (7 days) of germination at  $25^\circ\text{C}$  and in the presence of light, indicating that a longer germination time decreases the malonylglycoside content. In nongerminated soybean (Table 2), the malonylglycoside content was observed as predominant and represented 78.83% of total isoflavones, whereas malonyldaidzin, malonylglycitin, and malonylgenistin accounted for 30.25, 56.0, and 43.98% of total isoflavones, respectively. In soybean sprouts (assay 4), malonylglycoside content was predominant (82.98%), whereas malonyldaidzin, malonylglycitin, and malonylgenistin contents were 125.00, 55.00, and 57.64% higher, respectively, than in the nongerminated soybean. Our results were similar to those described by Tsukamoto et al. (1995), who observed in seven soybean cultivars that the content of malonylglycosides ranged from 67.00 to 93.00% of the total isoflavone content, whereas it was different in case of malonyldaidzin and malonylgenistin contents which



**Figure 1.** Response surface plots as a function of soaking and germination time of 'BRS 216' soybean (*Glycine max*). A, LSPR, length of soybean sprouts; and B, %YSPR, yield of soybean sprouts.

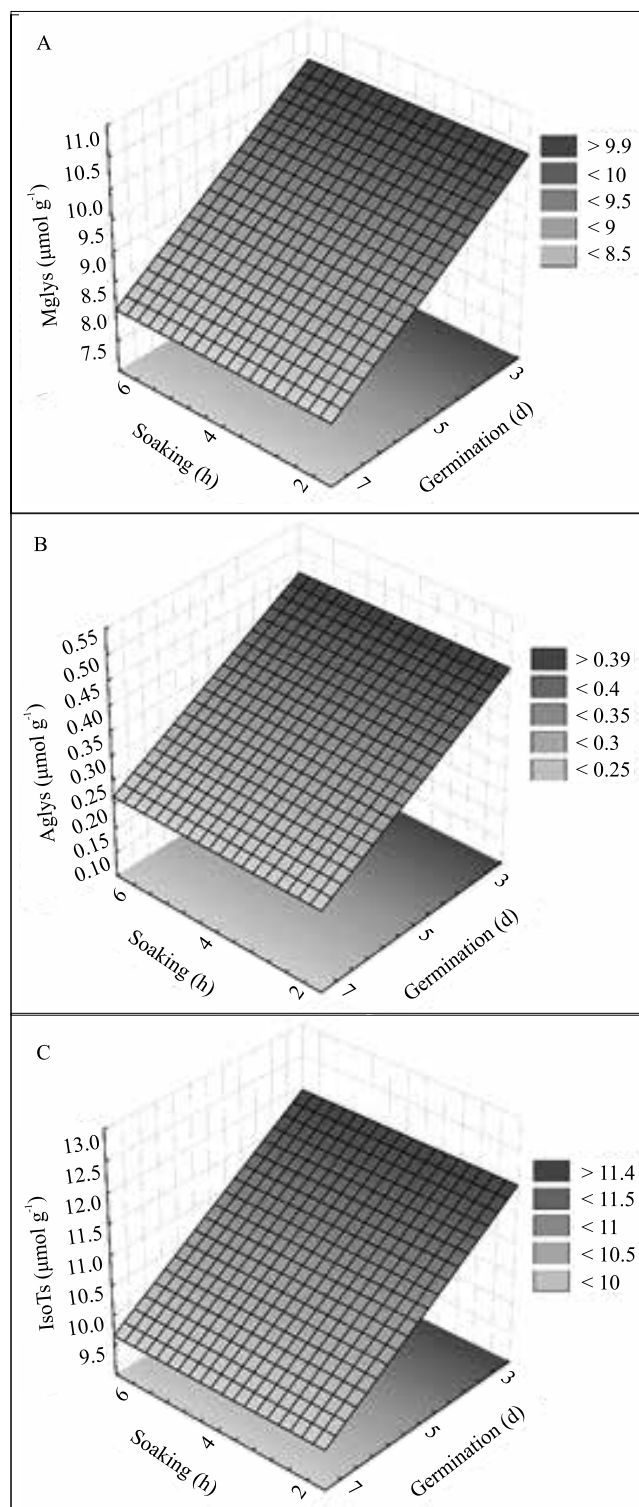
represented 44.00 and 41.00% of the total isoflavone content, respectively. Quinhone Júnior & Ida (2015) observed that soybean 'BRS 284' germinated for 168 hours at 35°C and showed 2.5- and 1.6-fold higher malonyldaidzin and malonylgenistin contents than nongerminated soybean, respectively.

Regression analysis and analysis of variance of Aglys indicated that only the variable  $X_3$  (germination time) showed a significant and negative linear effect. The lack of fit of the model was not significant ( $p > 0.05$ ), and the model proposed ( $\text{Aglys} = 0.332 - 0.097x_3$ ) explained only 54% ( $R^2$ ) of the experimental data. The response surface (Figure 2 B) indicated that Aglys was maximal ( $0.43 \mu\text{mol g}^{-1}$  sample) when 3 days of germination  $\leq X_3 \leq 4$  days of germination. These results indicated that for this germination time interval, the aglycone content was 126.31% greater than that of the nongerminated soybean ( $0.19 \pm 0.05 \mu\text{mol g}^{-1}$  sample) (Table 2). A reason for these results may be attributed to the activity of  $\beta$ -glucosidases that plays an important role, during germination, in the conversion of glycoside isoflavones into aglycones. According to Huang et al. (2014), the soybean germination may increase the aglycone content. These authors observed that in soybean germinated for 1 day, the content of aglycones increased 84%, and with 3 days of germination the increase was 147% higher than the

**Table 2.** Isoflavone contents in 'BRS216' soybean (*Glycine max*) seed and sprouts.

Isoflavone ( $\mu\text{mol g}^{-1}$ )	Soybean	
	Seed	Sprout <sup>(1)</sup>
$\beta$ -Glycosides	1.25 $\pm$ 0.29	1.51 $\pm$ 0.47
Daidzin	0.71 $\pm$ 0.13	1.03 $\pm$ 0.02
Glycitin	0.12 $\pm$ 0.02	0.12 $\pm$ 0.01
Genistin	0.42 $\pm$ 0.08	0.36 $\pm$ 0.01
Malonylglycosides	5.70 $\pm$ 1.39	10.43 $\pm$ 2.48
Malonyldaidzin	2.16 $\pm$ 0.27	4.86 $\pm$ 0.09
Malonylglycitin	0.40 $\pm$ 0.07	0.62 $\pm$ 0.01
Malonylgenistin	3.14 $\pm$ 0.58	4.95 $\pm$ 0.11
Aglycones	0.19 $\pm$ 0.05	0.44 $\pm$ 0.11
Daidzein	0.05 $\pm$ 0.01	0.26 $\pm$ 0.00
Glycitein	0.12 $\pm$ 0.02	0.13 $\pm$ 0.01
Genistein	0.03 $\pm$ 0.01	0.05 $\pm$ 0.00
Total isoflavones	7.14 $\pm$ 1.10	12.38 $\pm$ 2.02

<sup>(1)</sup>The values were obtained from soybean sprouts germinated for three days, using six hours of soaking and eight hours of irrigation frequency. Values represent mean  $\pm$  standard deviation ( $n = 3$ ) in dry basis.



**Figure 2.** Response surface plots as a function of soaking and germination time of 'BRS 216' soybean (*Glycine max*). A, Malonylglycosides (Mglys); B, aglycones (Aglys); and C, total isoflavones (IsoTs).

nongerminated soybean plants; this increase was similar to the trend found in the present study. A similar trend was observed by Paucar-Menacho et al. (2010), who evaluated the effect of time and temperature of 'BRS 258' soybean germination on the aglycone isoflavone content. They observed that soybean germinated for 63 hours (6 days) at 30°C had a maximum increase in the content of these isoflavones, which is about 153.93% higher than the nongerminated soybean (25.4 mg per 100 g of sample). In nongerminated soybean, the aglycone content (Table 2) was small, in comparison to other forms of isoflavones, and daidzein, glycitein, and genistein contents corresponded to 0.70, 1.68, and 0.42% of total isoflavones, respectively. The aglycone content of soybean sprouts (assay 4) increased significantly – 420.00, 8.33, and 66.67% for daidzein, glycitein, and genistein respectively – in comparison to the corresponding contents in the nongerminated soybean. The same trend was observed by Shi et al. (2010) in soybean germinated for 48 hours and 168 hours, whose daidzein and genistein contents increased 6.6- and 4.5-fold, respectively, at 25°C in the presence of light. Quinhone Júnior & Ida (2015) detected daidzein after 72 hours of soybean germination, and the maximum contents occurred between 120 hours and 168 hours, while genistein content was minimal between 48 hours and 120 hours and, then, increased 1.5-fold between 120 hours and 168 hours of germination. According to Zhu et al. (2005), the germination conditions had little effect on glycitein content, in contrast to effects on daidzein and genistein. Kim et al. (2006) did not detect any aglycone in seven soybean cultivars; however, after germination for 120 hours at 25°C, the average content of aglycone was 0.558 and 0.383 mmol g<sup>-1</sup> of sample with light and without light, respectively, and the total aglycone content represented 18.0 and 29.0% of the total isoflavone content of soybean sprouts. The glycitein content did not change in soybean sprouts (assay 4). It was observed by Shi et al. (2010) that the glycitein content gradually decreased during germination; however, after 144 hours (6 days), this decrease was not detected. However, Huang et al. (2014) reported that the glycitein content of germinated soybean was 59.0% lower than that of nongerminated ones.

Acetylglycosides were not detected in the present study. This was as expected because soybean was not treated by heat and, according to Jung et al. (2008), these isoflavones are formed only when soybean and

soy-based products are subjected to drying at high temperatures.

The analyses of regression and variance of IsoTs indicated that X<sub>3</sub> (germination time) showed a significant and negative linear effect. The lack of fit of the model was not significant ( $p > 0.05$ ), and the proposed model (IsoTs = 10.796 – 0.946X<sub>3</sub>) explained 75% (R<sup>2</sup>) of the experimental data. The response surface (Figure 2 C) indicated that the IsoT content was maximal (11.74 μmol g<sup>-1</sup> of sample) when 3 days of germination  $\leq X_3 \leq 4$  days of germination. In assay 4, the content of IsoTs was 12.38 ± 2.02, and it did not differ from the maximum observed by the model. These results indicated that for these germination conditions, the IsoT content of soybean sprouts was 64.46% higher than nongerminated soybean (7.14 ± 1.1 μmol g<sup>-1</sup> of sample). The IsoT content increased until 3 to 4 days of germination, and after 5 and 7 days it decreased. This decline may be associated with the exudation of isoflavones to the external environment, or to the metabolism of isoflavones to other constituents. Huang et al. (2014) observed that after 1 day of germination at 25°C, the IsoTs content was 22.0% (4.19 mg g<sup>-1</sup>) higher than that of nongerminated soybean (3.42 mg g<sup>-1</sup>); after 3 days of germination, the content reached a maximum of 37.0% (4.68 mg g<sup>-1</sup>) and, then, decreased to 27.0% (3.73 mg g<sup>-1</sup>) after 4 days.

Therefore, in order to obtain soybean sprouts of 8.75 cm long, proper characteristics, higher yield and higher content of Mglys, Aglys, and IsoTs, germination conditions should include 6 hours of soaking, 8 hours of irrigation frequency, and 3 days of germination. The protein, lipid, ash, and total carbohydrate content of soybean sprouts of assay 4 did not differ from those of the nongerminated soybean (Table 3). These results are consistent with that described by Ramadan (2012), who observed that the chemical composition of two soybean cultivars ('Giza 21' and 'Giza 35') germinated at 30°C for 40 hours and 60 hours, respectively, did not change in comparison to that of nongerminated soybean. In the present study, although the chemical composition did not change during germination, the values are within the average range for soybean, corroborating those observed by Camargo et al. (2019) in whole seed: 4.42-6.29% ash, 14.9-23.3% lipid, and 36.3-47.0% protein.

It is worth noting that the vitamin C content of soybean sprouts of assay 4 showed 61.0% increase

**Table 3.** Chemical composition and vitamin C content in 'BRS 216' soybean (*Glycine max*) seed and sprouts.

Chemical composition	Soybean	
	Seed	Sprouts <sup>(1)</sup>
Proteins (%)	44.77±2.89	47.25±0.63
Lipids (%)	17.66±0.89	14.57±0.58
Ash (%)	6.59±0.03	5.45±0.14
Carbohydrates (%)	30.97±1.46	32.73±0.29
Vitamin C (mg g <sup>-1</sup> )	1.47±0.06	2.38±0.10

<sup>(1)</sup>The values were obtained from soybean sprouts germinated for three days, using six hours of soaking and eight hours of irrigation frequency. Values represent mean ± standard deviation (n = 3) in dry basis.

in comparison with the nongerminated soybean (1.47 mg of vitamin C g<sup>-1</sup> of sample in dry basis) (Table 3). Therefore, these germination conditions help to obtain soybean sprouts with high-vitamin C content. In nongerminated soybean, vitamin C was not detected by Fernandez-Orozco et al. (2008). However, Huang et al. (2014), observed that during the germination of soybean for 1 day at 25°C, the vitamin C content was 5.27 mg 100 g<sup>-1</sup> of sample and, after 3 and 5 days of germination, this content increased by 13.3% and decreased by 22.9%, respectively.

## Conclusions

1. Both soaking and germination times show positive and significant linear effects on the length of 'BRS 216' soybean (*Glycine max*) sprouts; however, for sprout yield, only the germination time show a positive and significant linear effect.

2. To obtain soybean sprouts with adequate length, higher yield, and higher malonylglycoside, aglycone, and total isoflavone content, the germination should be carried out for 3 days after 6 hours of soaking, and 8 hours of irrigation frequency; these soybean sprouts can exhibit a preserved chemical composition, and vitamin C content 61% higher than nongerminated soybean.

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