IMPROVEMENT IN SOYBEAN SPROUTS PRODUCTION
WITH ULTRASOUND POWER

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The present work aimed the application of different ultrasound powers (10, 50 and 90W) to evaluate the germination and characteristics of soybean sprouts from soybeans of cultivar BRS 216 and 3 lines (A, B and C). In the germination test, the line A showed the highest percentage of germination (96% for 90W). The cultivar BRS 216 and line A present lower values of weight and higher yield of sprouts. The viable sprouts that presented the highest weight (for each 50g of soybean) were: line A followed by BRS 216, line C and B, with statistical differences (p<0.05) between them. For each 50g of soybean of cultivar BRS 216 and Lines A, B and C the production of viable sprouts was 231.47; 237.87; 170.74 and 199.94g, respectively, for 90W. In relation to the length of the sprouts using the power of 10W the line C presented a longer length. The line A using 90W showed better color characteristics. The highest protein value was found in cultivar BRS 216, for all applied potencies. With this work it can be concluded that the line A would be the most suitable for sprout production regardless of the ultrasound power applied.

KEY WORDS: CULTIVAR, GERMINATION, PROTEIN, POWER.

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1 INTRODUCTION

Brazil is the second largest producer of soybeans in the world, behind only of the United States. Soybeans are one of the most important Brazilian commodities. In the 2013/2014 crop season 86.120.8 million tons of soybeans were harvested, while in the 2014/2015 crop season the production was 96.222.1 million tons. For the 2015/2016 harvest, the estimate is for growth in production and productivity, reaching 102.1 million tons and for the next years it is expected an even higher production (CONAB, 2015).

Soybeans contains several bioactive constituents, such as isoflavones, protease inhibitors, saponins and phytic acids. Its health benefits have been investigated, either because of its high protein content of nutritional quality or because of its significant content in minerals and fibers, or because of its content of phenolic compounds or polyphenols, which are secondary metabolites of plants, with different chemical structures, presenting an extensive antioxidant action and consequently beneficial health effects (MARTINEZ *et al.* 2011).

Among the soybean derivatives, sprouts stand out because they are rich in dietary fiber, nutrients and bioactive components (YANG *et al,* 2015). Sprouts are obtained from germinated beans by contact with water, air and heat. According to Vieira and Lopes (2001), the germination process does not require direct sunlight, is produced in a short time, and with up to six days is possible harvest sprouts with size suitable for consumption. With germination, some significant changes occur in the centesimal composition in soybean grains. Germination is the simplest and most economical way of improving the nutritional value of grains, besides reducing antinutritional factors and increasing digestibility (LOURES *et al.* 2009).

To guarantee the sprouts quality, grains with high germinative vigor are required. It is interesting to make use of technologies that aid in the stimulation of germination, where the ultrasound is presented as an alternative, accelerating the absorption of water in the grains (MIANO *et al.* 2016), besides being considered a safe process, non-toxic and environmentally correct.

The most common interaction mechanism involved in ultrasonic technology is cavitation that causes thermal and chemical effects. In addition, mechanical effects, causing shock waves, can also accelerate the water absorption into grains by ultrasound. Although ultrasound treatment has been applied to stimulate germination in many different types of grains, little information is available on the effects of ultrasound on germinated grains in the food science view (YANG *et al.* 2015).

In Brazil the production of soybean sprouts is still very restricted or almost non-existent. Therefore, studies are needed to improve the production and germination of the sprouts, as for example the application of ultrasound in grains before germination. Embrapa Soja has developed new soybean cultivars and lines genetically improved with small grains to be used in the production of sprouts. In this sense, this work aimed the use of these lines with application of ultrasound power to verify the germination and obtaining soybean sprouts, in addition characterize physical and chemical.

2 MATERIAL AND METHODS

2.1 SOYBEANS

Soybeans (*Glycine max* (L.) Merrill) of cultivar BRS 216 and of 3 lines (A, B and C) of National Soybean Research Center - Embrapa, Londrina, PR in 2014/2015 crop seasons were used for sprout production, were the grains where produced in Passo Fundo/RS.

2.2 SOYBEAN QUALITY ANALYSIS

For soybean sprout production, it is important to evaluate the grains quality. For this, it was evaluated the weight of 100 grains and the germination test was carried out according to Brazil

(1992). For the evaluation of the weight of 100 grains, were separated randomly three samples with 100 grains of all the soybeans, were weighed in a precision scale (0.001 g) (before and after germination), and the analyzes were performed in triplicate.

In the germination analysis, four replicates of 100 grains were used. The grains were counted manually, inserted in vials with 500 mL of distilled water, for the treatment in ultrasonic bath (Unique, Model USC-1800A, 40 kHz). Subsequently, different ultrasonic powers (10, 50, and 90 W) were applied for 30 min at 25 °C (YANG *et al.* 2015). Next, the grains were seeded in humid paper towel and placed in a chamber at 25 °C and 99 % of relative humidity. After 4 days, the sprouts were counted and the results expressed in percentage.

2.3 SPROUTING

The soybeans were visually selected, removing toothpicks, stones, wilted and cracked grains. 50 g of soybeans (cultivar BRS 216 and lines A, B and C) were sanitized with sodium hypochlorite (1 %) for 4 min. For the ultrasound treatment, these grains were placed in a glass Becker containing 500 mL of distilled water and exposed to different power (10, 50 and 90 W), for 30 min, at 25 °C. Next, the soybeans were immersed in water for 4 h at 25 °C (OLIVEIRA 2013, adapted of EMATER, 2007).

The soybeans were placed into plastic trays (35 cm of diameter) with holes in the bottom to drain the water after irrigation and kept in the dark at 25 °C and 99 % of relative humidity to sprout. The irrigation was conducted every 12 h, spraying 25 mL of water at 25 °C. Sprouting was stopped by removing the samples after 4 days, time needed to reach approximately 7 cm (CANTELLI *et al*. 2016).

The sprouts were physically analyzed in relation to length, weight and color. The length of the sprouts was measured using a pachymeter (Starrett ™ model Universal Series 125), in triplicate, for each type of grain studied. The weight of the viable sprouts (from 50 g of grains) was determined with precision scale (0.001 g) and the results expressed in g.

Instrumental color was determined using a colorimeter (Minolta CR400), with light source D65, expressing the results in the Lab color space (L*a*b*). Equipment calibration was carried out using a standard white plate. The samples were placed in a Petri dish (9 cm diameter) using the amount of sample necessary to cover completely the bottom of the dish. All the measurements were carried out in triplicates.

For the chemical analysis of humidity and protein, the sprouts were previous freezing in ultra-freezer (Indrel IUT 355D) at -80 °C, and after lyophilized (Edwards®, Modulyon model) at -40 °C for 72 h. Later, the sprouts were milled in a Cuisinart® grinder (model DCG-20BKN), and stored in plastic bottles and kept in a refrigerator (10 °C). Humidity was determined by gravimetric method at 105 °C for 4 h, until constant weight (AOAC, 2000). The total protein was determined by Kjeldahl method, according to methodology AOAC (2000).

2.4 STATISTICAL ANALYSIS

The results of the physical and chemical analyses were subject to Tukey's test at 5 % significance for comparison between the means. Principal component analysis (PCA) between the parameters length, weight, humidity and protein were applied to examine the interrelationships of the experiments. All statistics analysis were performed using Statistic 5.0 (StatSoft Inc®, USA) software.

3. RESULTS AND DISCUSSION

3.1 PHYSICAL AND PHYSIOLOGICAL QUALITY OF SOYBEAN

The soybeans of cultivar BRS 216 and of 3 lines (A, B and C) were evaluated in relation to quality for sprouts production, which are the germination percentage and the weight of 100 grains (Table 1).

TABLE 1: GERMINATION AND WEIGHT OF 100 SOYBEANS (INITIAL AND END) OF CULTIVAR BRS 216 AND OF 3 LINES (A, B AND C) USING DIFFERENT ULTRASONIC POWER.

Cultivar	Power	Germination (%)	Initial Weight (g)	End Weight (g)	Yield (%)
	10 W	83 [C] ±0.60	9.91 [A] ±0.65	37.80 [B] ±0.41	73.78
BRS 216	50 W	86 [B] ±0.56	8.91 [A] ±0.55	36.96 [B] ±0.75	75.89
	90 W	88 [A] ±0.86	9.06 [A] ±0.35	41.92 [A] ±0.30	78.39
	10 W	91 [C] ±0.90	9.91 [A] ±0.70	40.00 [B] ±0.80	75.23
Line A	50 W	94 [B] ±0.53	8.81 [A] ±0.76	39.18 [B] ±0.67	77.51
	90 W	96 [A] ±0.48	9.56 [A] ±0.68	43.58 [A] ±0.72	78.06
	10 W	68 [C] ±0.47	14.80 [A] ±0.87	34.68 [C] ±0.43	57.32
Line B	50 W	70 [B] ±0.65	12.66 [A] ±1.40	36.71 [B] ±0.87	65.51
	90 W	78 [A] ±0.85	13.15 [A] ±0.86	44.90 [A] ±0.38	70.71
	10 W	79 [C] ±0.96	12.84 [A] ±0.75	43.77 [B] ±0.53	70.66
Line C	50 W	82 [B] ±0.39	11.31 [A] ±0.93	41.52 [C] ±0.70	72.76
	90 W	84 [A] ±0.43	12.21 [A] ±0.73	48.83 [A] ±0.65	74.99

Means (± standard deviations) followed by same letters on columns (same cultivar) represents no significant difference at 5% level (Tukey's test).

The germination test is used to evaluate the physiological quality of the grains and allows knowing the germination potential of a batch under favorable conditions, that is, suitable conditions for each type of grain (CARVALHO and NAKAGAWA, 2000). The line A presented the highest percentage of germination (96 % to 90 W), differing statistically from the others ultrasonic powers ($p < 0.05$) used for this line. It was verified that with the increase of the ultrasonic power increase the germination, probably this is due ultrasonic waves travel through the product increase the mass transfer promoting the hydration. This also affects the biological system accelerating the germination.

After germination, all grains submitted to 90 W presented statistically greater weight and yield, than those with 50 and 10 W. The lines B and C present greater weight, indicating larger size of the grains. However, the cultivar BRS 216 and line A presented lower values of weight, but a higher yield of sprouts. Taking into account that smaller grains are desirable for the production of sprouts, cultivar BRS 216 and line A are more suitable for this purpose. In addition, the grains must be of quality, with high physical purity, without contamination (VIEIRA and LOPES, 2001).

3.2 SPROUTS CHARACTERIZATION

Table 2 shows the results of the viable sprouts of BRS 216 and line A, B and C, germinated for 4 days, applying different ultrasound power before germination.

According to the Table 2, the viable sprouts with high weight (for each 50 g of soybean), were the line A, followed by BRS 216, line C and B, with statistical differences ($p < 0.05$) between the cultivars. A significant difference ($p < 0.05$) was observed for the same cultivar in different ultrasonic powers, as well as in the different cultivars using the same ultrasound power. Oliveira et al., (2013) produced soybean sprouts from cultivar BRS 216, using 25 g of soybean grains, with five days of germination obtained 35.73 g of viable sprouts; with six days 43.12 g, and seven days 64.19 g, regardless of irrigation frequency each 12 h, 27.5 ± 5.1 ℃ of temperature and relative humidity (53.5 ± 16.5 %). Thus, it can be seen that BRS 216 sprouts, germinated for 4 days showed much higher weight than those obtained by Oliveira et al., (2013) with 5 days of germination, indicating that in the present work there was a significant weight increment with using ultrasonic power.

TABLE 2: WEIGHT (G) AND LENGTH (CM) OF SOYBEANS SPROUTS OF CULTIVAR BRS 216 AND OF 3 LINES (A, B AND C) USING DIFFERENT ULTRASONIC POWERS.

Cultivar	Ultrasonic power		
	10 W	50 W	90 W
Weight (g)			
BRS 216	190.71 [Cb] ± 1.2	207.42 [Bb] ± 1.4	231.47 [Ab] ± 1.4
Line A	216.90 [Ca] ± 1.5	222.30 [Ba] ± 1.6	237.87 [Aa] ± 2.1
Line B	117.17 [Cd] ± 1.7	144.96 [Bd] ± 2.2	170.74 [Ad] ± 1.2
Line C	170.25 [Cc] ± 1.1	183.60 [Bc] ± 1.7	199.94 [Ac] ± 1.7
Length (cm)			
BRS 216	12.3 [Ab] ± 1.4	6.9 [Ba] ± 1.2	11.4 [Aa] ± 1.3
Line A	10.7 [Bb] ± 1.2	9.1 [Ba] ± 1.4	14.0 [Aa] ± 2.0
Line B	10.8 [Ab] ± 1.9	9.2 [Aa] ± 2.0	7.5 [Bb] ± 1.1
Line C	15.8 [Aa] ± 1.2	8.4 [Ba] ± 1.4	9.0 [Bb] ± 1.6

Means (± standard deviations) followed by same uppercase letters on the line and lower case letters on columns represents no significant difference at 5% level (Tukey's test).

Cantelli *et al.* (2017), evaluating the weight of soybean sprouts under the same conditions of the present study, also using cultivar BRS 216 and lines A, B and C of the 2014/2015 crop, but without application of ultrasound power, obtained values of 140.93; 214.36; 60.70 and 67.62 g, respectively. Thus, comparing the results with the present study, the application of ultrasound power before the germination increased the product weight.

The soybean sprouts from Line C presented a longer length using 10 W, differing statistically (p<0.05) of the others (Table 2). At 50 W, there was no significant difference (p> 0.05) between the cultivars. Using 90 W the BRS 216 and line A were the ones that presented greater length differing statistically (p <0.05) of the others. Comparing the lengths of the sprouts in different powers, it is verified that the same did not follow a trend among them, presenting different behaviors, characteristics of each cultivar. Figure 1 shows the lengths of soybean shoots on the 4th day of germination.

FIGURE 1. ILLUSTRATIVE IMAGE OF THE LENGTH (CM) OF SOYBEANS SPROUTS FROM CULTIVAR BRS 216 AND 3 LINES (A, B AND C).

Oliveira *et al.* (2013) found length values of 7.74, 11.11 and 13.77 cm for soybean sprouts from cultivar BRS 216, germinated for five, six and seven days, respectively, with irrigation frequency of 12 h. Vilas Boas et al., (2002), studying the average length of soybean sprouts, observed values ranging from 4.30 cm (3 days of germination) to 9.80 cm (6 days of germination). The differences in weight and length found among the soybean sprouts studied in the present work are expect, since they are different lines, which present different genetic characteristics.

The protein and humidity contents, in the soybean sprouts from cultivar BRS 216 and lines A, B and C, are present in Tables 3. The sprouts with the highest protein value were those from cultivar BRS 216, for all ultrasonic power studied, presenting a significant difference ($p < 0.05$) of the other cultivars. These differences in protein content can be attributed to the genetic differences between soybean cultivars, the stage of development in which the grains were harvested and the climatic conditions (Silva, 2009). According to the Brazilian Food Composition Table - TACO (2011), soybean has around 36 % of protein content, black beans (21.3 %), peas in pods (7.5 %) and peanuts (27.2 %), in this way the soybean highlights in relation to the other leguminous in relation to protein.

TABLE 3: VALUES OF PROTEIN (G/100G) AND HUMIDITY (G/100G) OF SOYBEANS SPROUTS FROM CULTIVAR BRS 216 AND 3 LINES (A, B AND C) USING DIFFERENT ULTRASONIC POWERS.

Cultivar	Ultrasonic power		
	10 W	50 W	90 W
Protein (g/100g)			
BRS 216	40.7 [Ba] ± 0.4	42.1 [Ba] ± 1.1	43.8 [Aa] ± 0.1
Line A	38.1 [Bb] ± 1.5	38.3 [Bb] ± 0.3	42.5 [Ab] ± 0.8
Line B	37.4 [Bb] ± 2.1	38.1 [Bb] ± 0.2	40.6 [Ac] ± 0.4
Line C	38.4 [Ab] ± 1.1	38.5 [Ab] ± 0.7	39.0 [Ac] ± 1.4
Humidity (g/100g)			
BRS 216	78.5 [Bb] ± 0.6	79.2 [Ba] ± 0.4	80.4 [Ab] ± 0.2
Line A	75.4 [Cc] ± 0.1	80.4 [Ba] ± 0.9	81.8 [Aa] ± 0.3
Line B	72.7 [Ad] ± 1.1	73.4 [Ac] ± 0.1	75.1 [Ac] ± 2.3
Line C	77.0 [Bc] ± 2.3	78.4 [Bb] ± 1.0	80.9 [Aa] ± 1.2

Means (± standard deviations) followed by same uppercase letters on the line and lower case letters on columns represents no significant difference at 5% level (Tukey's test).

Ultrasonic power of 10 and 50 W not have significant difference on the protein content, in the same cultivar. With the increase to 90 W, a significant difference ($p < 0.05$) was observed among cultivars, except for line C. These differences in the protein content can be explained by the traveling of ultrasonic wave through the soybean, which causes the expansion and compression of the medium (MIANO *et al.* 2016a). The acoustic cavitation can change the product structure that resulted in high protein content.
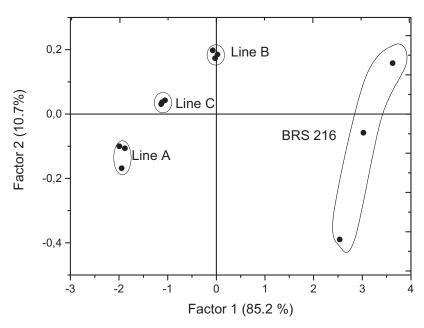
The increase content of protein in the germinated soybean occurs due to protein enzymatic synthesis or the change in composition following the degradation of other constituents (Martinez et al., 2011; Barcelos et al., 2002).

With the application of ultrasound power in the soybean germination is observe the trend of increasing humidity content with increased of ultrasonic power (Table 3). Line A presented

high humidity content for 50 W and 90 W with values of 80.4 and 81.8 %. This result can be explained, because during the germination process the hydration of the grains occurs in order to promote the growth of the plant (VILLELA *et al.* 2007). The ultrasound process could to affect the porosity of the soybean, increases the water intake by pumping the water into the tissues and by unblocking the pores (MIANO *et al.* 2017). According to Miano et al., (2016b) related that the ultrasonic waves facilitating the acoustic cavitation and the formation of micro cavities, thus causes a change in the pores size or shape that improve the mass transfer.

According to PCA analysis (Figure 2) showed that the first and second principal components explained, respectively, 85.2 % and 10.7 % and influenced the separation of the cultivars. Evaluating the parameters of weight, length, protein and humidity in soybean sprouts, this analysis could classify the cultivars. These groupings were due to the fact of genetic differences of the cultivars, since the germination conditions were identical.

FIGURE 2. PRINCIPAL COMPONENT ANALYSIS OF WEIGHT, LENGTH, PROTEIN AND HUMIDITY IN SOYBEAN SPROUTS.

The results of color of the soybean sprouts germinated for 4 days, using irrigation frequency every 12 h, are present in Table 4. It was evaluated the color coordinates, such as brightness L* (lighter or darker sample), a* (red and green difference) and b* (blue and yellow difference).

TABLE 4: COLOR OF SOYBEANS SPROUTS FROM CULTIVAR BRS 216 AND 3 LINES (A, B AND C) USING DIFFERENT ULTRASONIC POWERS.

Color	BRS 216			LineA			Line B			Line C		
	10 W	50 W	90 W	10 W	50 W	90 W	10 W	50 W	90 W	10 W	50 W	90 W
L*	45.4	55.21	57.63	63.12	50.12	56.57	63.42	63.24	56.26	29.95	51.55	60.09
a*	-0.88	-2.24	-3.99	-5.35	-4.21	-10.26	-9.32	-5.87	-2.43	-1.48	-0.72	-2.55
b*	19.96	24.22	20.94	23.13	26.73	36.38	27.06	22.49	24.57	10.32	16.92	24.65

L *: lightness or brightness, ranging from black (0) to white (100), a *: coordinated chromaticity ranging from green (-) to red (+), and b *: coordinated chromaticity ranging from blue (-) to yellow (+).

By evaluating the brightness (L*) of the sprouts it can be verified that Line B presented a higher index of luminosity with application of 10 and 50 W, followed by Line A with 10 W (Table 4). High indices of luminosities can make the sprouts more attractive to consumers, since they have a coloration tending to white.

Regarding the parameter a * it was verified that all the soybean sprouts showed a coloring tending to green, and parameter b * tending to yellow. For these parameters, the line A with 90 W showed the high value, which characterizes better color for sprouts production.

4 CONCLUSIONS

In the germination process, all the cultivars presented an increase in the germination, with the increase of ultrasound power where the line A presented greater potential. After the 4 days of germination the line A presented a higher weight of viable sprouts in relation to the quantity of grains used in the process.

Regarding the color parameter a * it was verified that all the soybean sprouts showed a coloring tending to green, and for the parameter b * tending to yellow. For these parameters, the Line A at 90W showed the high value, characterizing this line with better color characteristics for sprout production.

For the protein and moisture content, all the cultivars in the different ultrasonic powers had an increase in protein and moisture content. The results of the work subsidize the possibility of recommendation for commercial cultivation of line A and the use the ultrasound to help the germination in sprout production.

RESUMO

MELHORA NA PRODUÇÃO DE BROTOS DE SOJA COM POTÊNCIA ULTRASSONICA

O presente trabalho objetivou a aplicação de diferentes potências de ultrassom (10, 50 e 90W) para avaliar a germinação e características dos brotos de soja a partir da cultivar BRS 216 e de 3 linhagens (A, B e C). No teste de germinação a linhagem A foi o que apresentou maior porcentagem de germinação (96% para 90 W). A cultivar BRS 216 e a linhagem A apresentam valores menores de peso e maior rendimento de grãos germinados. Os brotos viáveis que apresentaram o maior peso (para cada 50g de grãos de soja) foram: a linhagem A seguida pela BRS 216, linhagem C e B, com diferenças estatísticas (p<0,05) entre si. Nas linhagens B e C verificou-se que apesar de ter ocorrido a germinação, os brotos não apresentaram um bom desenvolvimento. Deste modo, para cada 50 g de soja da cultivar BRS 216 e Linhagens A, B e C a produção de brotos viáveis foi de 231,47; 237,87; 170,74 e 199,94g, respectivamente, para potência de 90W. Em relação ao comprimento dos brotos de soja, utilizando a potência de 10W, a Linhagem C apresentou maior comprimento. A Linhagem A com aplicação de 90W apresentou melhores características de cor. O maior valor proteico encontrado foi na cultivar BRS 216, para todas as potências aplicadas. Com este trabalho pode-se concluir que a linhagem A seria a mais indicada para produção de brotos independentemente da potência aplicada.

PALAVRAS CHAVES: CULTIVAR, GERMINAÇÃO, PROTEÍNA, POTENCIA.

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ACKNOWLEGMENTS