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Canning of vegetable-type soybean in acidified brine: Effect of the addition of sucrose and pasteurisation time on color and other characteristics

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ABSTRACT

The objective of this work was to evaluate the effect of adding sucrose and pasteurisation time of the canning vegetable-type soybean in acidified brine on the color of grains by using central composite design rotational (DCCR) 2^2. Chemical, physical and microbiological characteristics of the canned grains of vegetable-type soybean in acidified brine were evaluated, and the results were compared with *in natura* grains and canning of vegetable-type soybean without sucrose. The optimum conditions for the formulation of the canned vegetable-type soybean grains in acidified brine were established, after validation of the proposed model, as being 3.43 g 100 mL^{-1} sucrose, 6 g 100 mL^{-1} $NaCl_2$, 0.29 g 100 mL^{-1} $CaCl_2$ and citric acid with pH 3.9 and a thermal processing time of 10 min. Commercial sterility was achieved in all the canned vegetable-type soybean. The *in natura* grains showed higher hardness and green colors when compared with the canning vegetable-type soybean with and without sucrose probably due the thermal processing that caused softening of cells wall and loss color. The addition of sucrose in the acidified brine contributed to maintain the desirable color of canning vegetable-type soybean grains. The canned in acidified brine presented a high content of glucose and fructose and low content of sucrose and stachyose when compared with *in natura* soybean grains. The thermal processing canned vegetable-type soybean in acidified brine with addition sucrose promoted an increase in isoflavone glycosides content, reduced the malony-glycosil content and was unable to convert the isoflavones to aglycones.

1. Introduction

Soybean pods harvested at the R6 stage of seed development are traditionally known as vegetable-type grains, which are completely developed but are still green or immature (Ferh et al., 1971; Rao et al., 2002). In Japan, they are called edamame, and the grains are consumed as an appetizer after cooking the pods *in natura* in boiling salt water for 3 min (Masuda, 1991). In Brazil, vegetable-type soybean is not well known, and to expand its utilization, some factors must be considered such, as production, harvest, storage, processing and the potential market (Mentreddy et al., 2002; Obatolu and Osho, 2006).

Grains of vegetable-type soybean can be directly consumed by cooking pods or grains *in natura*; however, an alternative form of packaging is canning, which would guarantee availability throughout the year (Fellows, 2002; Mozzoni et al., 2009a,b). This product *in natura* or canned presents a mild and sweet flavor, soft texture and superior nutritional quality (Konovsky et al., 1994); it is rich in protein, vitamins A, C, B1 and B2, minerals such as K, P and Ca, fiber and isoflavones (USDA, 2008).

To process canned grains of vegetable-type soybean, thermal treatment is needed to control microorganisms, to reduce antinutritional factors and to inactivate the lipoxygenases enzymes that are responsible for the production of off-flavors and that affect color and texture (Barcelos et al., 1999; Mozzoni et al., 2009a,b). Green coloring, which is a parameter that indicates the quality of vegetable-type soybean, can be reduced during heat processing.

A variety of additives have been used to improve the color and texture of canned food (Mozzoni et al., 2009a,b). Sugars, such as, sucrose, at high levels prevent the development of microorganisms by reducing water activity. At low concentrations, such as those used in canned foods, such sugars enhance color, providing firmness and promote a mild acidic taste (Bibek and Bhunia, 2007).

In canned foods, pH must be controlled. If the pH level is greater than 4.5, the canned food should be sterilized, and if the pH level is less than 4.5, it should be subjected to pasteurisation (Abbatemarco and Ramaswamy, 1994).

The objective of this work was to evaluate the effect of adding sucrose and of pasteurisation time of the canning vegetable-type soybean grains in acidified brine on the color of grains by using

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Table 1
Coded and real levels of independents variables used in central composite design 2^2 factorial with 4 axial points.

Variables	Levels				
	−1.41	−1	0	+1	+1.41
X_1 = g sucrose in the brine	0.10	0.45	1.30	2.15	2.50
X_2 = minutes pasteurisation	10	14	25	36	40

central composite design rotational (DCCR) 2^2. The optimum canning vegetable-type soybean in acidified brine was to evaluate the chemical, physical and microbiological characteristics and to compare with the canned vegetable-type soybean without sucrose and grains *in natura*.

2. Materials and methods

2.1. Soybean pods

Soybean pods of the BRS 267 cultivar, harvested at the R6 stage of seed development, were supplied by Embrapa-Soybean, Londrina/Parana, Brazil. After harvesting, the pods were bleached in boiling water for 3 min, packed in plastic bags, sealed and frozen at −18 °C.

2.2. Preparation of the canned grains of vegetable-type soybean in acidified brine

Soybean pods were thawed rapidly by immersion in boiling water, and the grains were manually removed to be used for the preparation of the canned in acidified brine at pH 3.9. The grains were sanitized in a solution of NaClO that was prepared according to Krolow (2006). The sanitizing solution was drained, and the grains were rinsed in running water. Grain portions with a mass of 210 g were placed in 355 mL glass bottles with the addition of 100 mL of brine. The brine was composed of constant proportions of sodium chloride (6 g 100 mL^{-1}) that were established experimentally, calcium chloride (0.29 g 100 mL^{-1}) according to Mozzoni et al. (2009a,b), citric acid to achieve a pH of 3.0 according to Bellergard et al. (2005) and different proportions of sucrose according to the experimental design (see Table 1). The bottles were closed with metal caps and pasteurized in boiling water at different times according to Table 1. After quickly cooling with water, the glass bottles were stored in closed cardboard boxes at room temperature for 30 days.

2.3. Effect of sucrose and pasteurisation time on the color of canned grains of vegetable-type soybean in acidified brine

To evaluate the effect of the addition of sucrose and of the time of pasteurisation on the color stability of the canned grains of vegetable-type soybean in acidified brine, a Central Composite Rotational Design (CCRD) with 2^2 factorial, 4 axial points and 4 replicates at the central point was used, with a total of 12 randomized experiments. Table 1 presents the code and the real levels of the independent variables: X_1 (sucrose concentration in the brine) and X_2 (pasteurisation time in minutes) for the CCRD.

After 30 days of storage, the response functions associated with the canned with acidified brine grains were determined: Y_1 = degrees of chromatic hue canned vegetable-type soybean grains. The software program Statistica version 7.0 (StatSoft, Inc., 2004) was used for multiple regression analysis and to create the response surface model. The model for each response was expressed in the form of Eq. (1), where Y = response, x_1 and x_2 = levels of the coded variables, β = estimated coefficients on the response surface and e = pure error:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1{}^2 + \beta_{22} x_2{}^2 + \beta_{12} x_1 x_2 + e \qquad (1)$$

2.4. Model validation and characterization of the canned grains vegetable-type soybean in acidified brine

2.4.1. Model validation

After analyzing the response surface obtained from the CCRD and the optimum conditions of the experiment, the proposed model was validated by performing four replicates under the optimum conditions. Upon validation of the optimum conditions of the variables studied, a formulation was developed for the canned grains of vegetable-type soybean in acidified brine and with sucrose, and a control containing only sodium chloride, calcium chloride and citric acid was created. The canned vegetable-type soybean in acidified brine and with sucrose and the control were stored under the same conditions previously described for 30 days. After this storage period, isoflavones, sugars, hardness, color and chemical composition were determined. The results, the means of three replications, were compared with those for the grains *in natura* of vegetable-type soybean and with the control (canning vegetable-type soybean without sucrose) through analysis of variance (ANOVA) and Tukey's test at 5% probability by using the software program SAS (Statistical Analysis System), version 8.2 (SAS Institute, 2001).

2.4.2. Microbiological characterization

The commercial sterility test to guarantee the microbiological control of canning was conducted as described by Silva et al. (2007). After 30 days, each canned with acidified brine was open aseptically and incubated anaerobically and aerobically in the Thermoaciduran Broth (TAB), Malt Extract (ME) and Man Rogosa & Sharpe Broth (MRS) at 35 and 55 °C for 5 days. The qualitative results were visually evaluated by the turbidity of the medium and gas formation.

2.4.3. Chemical characterization

Grains *in natura* of vegetable-type soybeans and canning vegetable-type soybean with sucrose in acidified brine and without sucrose were lyophilized (Liobras, L-101) and ground using an analytical mill (Black and Decker, model CBG100W) to obtain a finely powder (35 mesh). The chemical composition was determined by using the methods described by the Instituto Adolfo Lutz (2008). The total carbohydrate content was determined by the difference from the other constituents. The results were expressed in g 100 g^{-1} of grains of vegetable-type soybean. The isoflavones were extracted according to Carrão-Panizzi et al. (2002) and quantified by the method of Berhow (2002) using a liquid chromatograph (HPLC) (Model 2690, Waters, USA) with a reverse phase column ODS C18 (YMC-Pack ODS-AM S-5mm, 120 a, with a diameter of 4.6 mm and length 250 mm) and a diode array detector (model 996, Waters, USA) adjusted to a wavelength of 254 nm. A linear binary gradient system with methanol, trifluoroacetic acid and ultrapure deionized water was used for separation. The initial gradient was 20%, reached 80% at 35 min and returned to 20% at 40 min. The mobile phase flow rate was of 1 mL min^{-1}, and the temperature during the race was kept constant at 25 °C. Quantitation was performed with the external standard calibration curves of daidzin, genistin, glycitin, daidzein, genistein, glycitein, malonyldaidzin, malonylgenistin, malonylglycitin, acetyldaidzin, acetylgenistin and acetylglycitin purchased by Sigma Chemicals Co. (St. Louis, E.U.A.), and the results were expressed as g 100 mg^{-1} vegetable-type soybean. Sugar contents of the samples was determined following the protocol described by Masuda et al. (1996) and Mandarino et al. (2000), using an ionic exchange chromatograph (ED 50, Dionex Bio

LC, USA) equipped with an amperometric detector, AgCl electrode (ED 50, Dionex Bio LC, USA), amperometric gold cell and sample self injector. A CarboPac PA 10 column (250 mm length × 4 mm internal diameter and 5 μm particles) was used to separate the sugars. The analyzes were adopting the system isocratic analysis and a 50 mmol L^{-1} NaOH solution as mobile phase at a 1.0 mL min^{-1} flow rate at 25 °C. Quantitation was performed using the external standard curves for calibration-standard sugars (Wako Mark, Japan), and the results were expressed in g 100 g^{-1} of vegetable-type soybean. All reagents used were analytical grade HPLC.

2.4.4. Physical characterization

Grain hardness was evaluated using a Texturometer Stable Micro Systems (TA-XT2i). The samples were compressed to 25% of their initial height with a force of 0.05 N using a compression cycle at a constant speed of 1 mm s^{-1} with aluminum cylinder probe (P 25L with 2.5 diameter). The color measurement was performed using a camera (Canon EOS Digital Rebel XT) with a lens focal length of 50 mm and fixed digital images with a resolution of 3456 × 2304 pixels, according to Oliveira et al. (2003). The digital images were obtained using the BMP extension in Microsoft® Paint Program, version 6.0. Then, the digital images were converted from BMP (read pixel by pixel color) values into RGB (red–green–blue) using the program developed by Darrigues et al. (2008) and these values were converted to the CIELAB system by the program version Conversion Munsell version 4.01, obtaining the parameters L^* (lightness), a^* (red–green component) and b^* (yellow–blue component). To assess the color of the soybean grains, the hue parameter [$H^* = \text{arctang}\ (b^*/a^*)$] and cylindrical coordinates that indicate the location of the color in a diagram were used, where an angle of 0° indicates pure red, an angle of 90° indicates pure yellow, an angle of 180° indicates pure green, and an angle of 270° indicates pure blue (Lawless and Heymann, 1998).

3. Results and discussion

3.1. Effect of sucrose addition and pasteurisation time on the color stability of the canned grains of vegetable-type soybean in acidified brine

The variable X_1 (g sucrose in the brine) showed a significant linear positive effect ($\beta_1 = 0.32$) at a 5% significance level on the response function Y_1 (degrees hue of the canned in acidified brine). The linear and quadratic effects of variable X_2 (time pasteurisation) on Y_1 were significant and negative ($\beta_2 = -0.41$, $\beta_{22} = -0.38$, $p \le 0.05$) because the quadratic effect of x_1 ($\beta_{11} = 0.04$) and interaction x_1x_2 ($\beta_{12} = -0.017$) was not significant at a 5% probability. Considering only the significant variables, the following mathematical model was developed: $Y_1 = 92.37 + 0.32x_1 - 0.41x_2 - 0.38x_2^2$. The lack-of-fit of the model was not significant (at 95%), and 89.35% (R^2) of the experimental data was properly adjusted to the model. From the regression coefficients, it can be estimated that when X_1 (g sucrose in brine) increases from −1 to +1, Y_1 (degrees hue of canned grains) increases, which indicates that the canned grains approach a green color. With the increase in the variable X_2 (pasteurisation minutes) from −1 to +1 or from −1.41 to +1.41, Y_1 (degrees of hue canned grains) decreases, indicating that a yellow hue is approached, which is undesirable for the canned grains vegetable-type soybeans.

The response surface (Fig. 1) shows a region where Y_1 is maximum and equal to 93° of chromatic hue, although this lies outside the range of investigated variables. Meanwhile, X_2 has a negative effect on Y_1, and because the smaller this variable is the lower the amount of energy spent is, a shorter pasteurisation is established: −1.41 (10 min). Considering a Y_1 value of 93° of chromatic hue, it was estimated that the variable X_1 can be equal 2.5 g or 3.43 g of sucrose per 100 mL of brine, which also lies outside of the investigated range, though it is suitable to be added to brine.

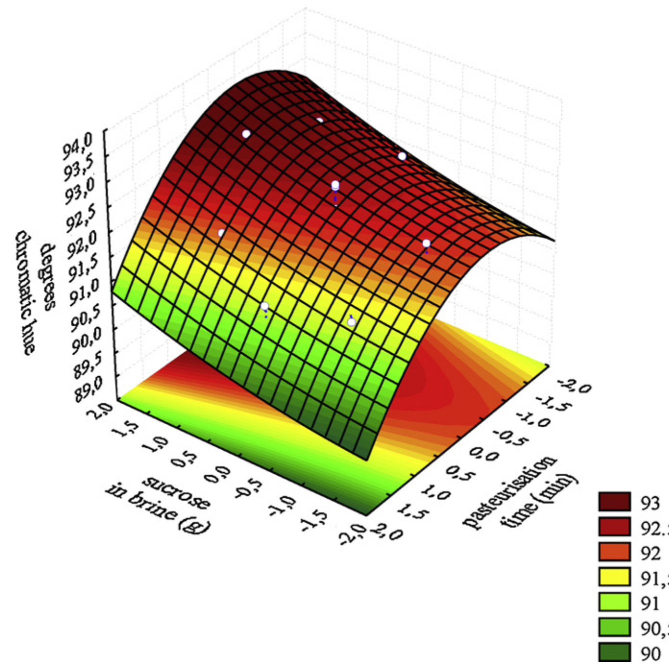

Fig. 1. Model of response surface for acid canned of vegetable-type soybean as a function of X_1 (g of sucrose in brine) and X_2 (pasteurisation time minutes).

The model was performed in quadruplicate after stabilizing X_1 to 3.43 g of sucrose per 100 mL of acidified brine and X_2 to 10 min of pasteurisation, with a $\hat{Y}_1$ value of 93° of chromatic hue for the canned grains. The response obtained experimentally (Y_1) was 93.48° of chromatic hue of canned grains of vegetable-type soybean. The error related to the model was 0.51%, indicating that the experimental results were adjusted accordingly to the model. Thus, the conditions for the formulation of canned grains of vegetable-type soybean in acidified brine were established with sucrose (3.43 g 100 mL^{-1}), $NaCl_2$ (6 g 100 mL^{-1}), $CaCl_2$ (0.29 g 100 mL^{-1}) and citric acid (to pH 3.9) and a thermal processing time of 10 min.

3.2. Microbiological analysis and physical–chemical characterization of the canned grains of vegetable-type soybean in acidified brine

After validating the model (0.51% of error) and establishing the optimum conditions for the canned grain formulation, the canned grains of vegetable-type soybean in acidified brine were prepared for microbiological, chemical and physical analysis, and the results were compared with those for the vegetable-type soybean grains *in natura* and the control the canned vegetable-type soybean grains without sucrose.

The canned grains in acidified brine and with added sucrose are commercially sterile and therefore can be consumed without presenting a health risk to consumers.

The highest value for hardness was observed in soybean grains *in natura* (73.53 N) (Table 2). Thermal processing may soften the grains by hydrolyzing the pectin, gelling the starch, partially solubilizing the hemicellulose, and distending the cell wall and the protoplasmic plant cell layer (Fellows, 2006). Mozzoni et al. (2009a,b) did not observe differences in texture between raw (*in natura*) grains of vegetable-type soybeans and grains submitted to 5 min of heating processing. Additionally, the authors verified a decrease in green color intensity after 5 min of cooking compared

Table 2
Hardness and hue chromatic degree of acid canned[a], canned without sucrose[b] and vegetable-type soybean grains *in natura*.[c]

Samples	Hardness (*N*)	Hue chromatic degree
Acid canned	59.13[b d]	93.50[b]
Canned without sucrose	56.97[b]	92.33[c]
Grains *in natura*	73.53[a]	121.16[a]

[a] Brine with $NaCl_2$, $CaCl_2$, citric acid and sucrose.
[b] Brine with $NaCl_2$, $CaCl_2$, citric acid and zinc acetate.
[c] Brine with NaCl, CaCl and citric acid.
[d] Means followed by the same letters in the columns do not differ by Tukey's test at 5% probability.

with the raw (or *in natura*) samples. No differences were observed between the canned grains in acidified brine and with sucrose (59.13 N) and those canned without sucrose (56.97 N). According to Wang and Chang (1988), the quality of canned vegetables is related to the texture, color and enzyme activity of the product. A variety of additives have been used to improve these characteristics of canned foods. In this study, the addition of sucrose (3.43 g 100 mL^{-1}), $NaCl_2$ (6 g 100 mL^{-1}), $CaCl_2$ (0.29 g 100 mL^{-1}) and citric acid (to pH 3.9) to the brine and thermal processing for 10 min allowed the desirable color of the canned of vegetable-type soybean grains to be maintained when compared with the color of the control without sucrose. Determining the optimum heat processing conditions for canned vegetable-type soybean grains guarantees that the color, texture and sugar levels, the most important attributes affecting the quality of this product, will be maintained. A pH level of 3.9 can also influence the hardness of the grains, where a pH level below 4.6 is used to maintain hardness (McGlynn et al., 1993). The canned in acidified brine and with sucrose showed more intense hue when compared with the canned grains without sucrose, indicating that the addition of sucrose contributed to the maintenance of the grains' green color. Compared to the color of the *in natura* grains, the value of this parameter for the canned grains (with sucrose and without sucrose) was higher, suggesting that heat treatment canning reduced the hue of vegetable-type soybean grains due to chlorophyll degradation during processing (Damodaran et al., 2010). Green color and texture are the most important quality attributes of canned vegetable-type soybean grains (Mozzoni et al., 2009a,b). The loss in green color can be caused by the conversion of chlorophyll into pheophytin after the subtraction of Mg^{+2} under heat and acid treatment (Von Elbe and Schwartz, 1996). The added sucrose to contribute to maintain the desirable color of the canned vegetable-type soybean grains in acidified brine.

A decrease in the concentration of lipids was observed after the thermal processing of vegetable-type soybean grains (Table 3), most likely due to the applied temperature and pH, which may cause the hydrolysis of triacylglycerol. The increase in ash content may be due to the absorption of chloride and sodium added to the canned grains (Damodaran et al., 2010). The carbohydrate content did not differ between the canned in acidified brine with sucrose and the control (without sucrose) and was higher in the canned grains than in the grains *in natura*. The addition of sucrose to the canned grains in acidified brine promoted an increase in the carbohydrate content relative to that of the vegetable-type soybean grains *in natura*.

Table 3
Chemical composition of acid canned[a], canned without sucrose[b] and vegetable-type soybean grains *in natura*.[c]

Samples	Lipids	Ash	Proteins	Carbohydrates
Acid canned	20.27[b d]	8.16[a]	35.80[b]	37[a]
Canned without sucrose	20.10[b]	8.60[a]	36.80[b]	35.30[a]
Grains *in natura*	23.03[a]	5.43[b]	38.76[a]	33[b]

[a] Brine with $NaCl_2$, $CaCl_2$, citric acid and sucrose.
[b] Brine with $NaCl_2$, $CaCl_2$, citric acid and zinc acetate.
[c] Brine with NaCl, CaCl and citric acid.
[d] Means followed by same letters in the columns do not differ by Tukey's test at 5% probability; results expressed in g 100 g^{-1} on dry basis.

Table 4
Isoflavones content of acid canned[a], canned without sucrose[b] and vegetable-type soybean grains *in natura*.[c]

Isoflavones	Acid canned[a]	Canned without sucrose[b]	Grains *in natura*[c]
Daidzin	5.73[b d]	6.40[a]	2.20[c]
Glycitin	3.10[a]	3.63[a]	2.40[b]
Genistin	5.60[a]	6.46[a]	3.43[b]
Malonyl daidzin	10.66[b]	11.23[b]	17.36[a]
Malonyl glycitin	7.06[b]	6.40[c]	11.43[a]
Malonyl genistin	10.13[c]	10.63[b]	14.93[a]
Daidzein	0.03[a]	nd	nd
Total	42.13[b]	44.66[b]	51.83[a]

[a] Brine with $NaCl_2$, $CaCl_2$, citric acid and zinc acetate.
[b] Brine with $NaCl_2$, $CaCl_2$, citric acid and sucrose.
[c] Brine with NaCl, CaCl and citric acid.
[d] Means followed by same letters in the lines do not differ by Tukey's test at 5% probability; results express in g 100 g^{-1} on dry basis; nd = not detected.

The highest total concentration of isoflavone glucosides was observed in the canned grains both with sucrose and without sucrose compared with the grains *in natura*. This suggests that the thermal processing of vegetable-type soybean grains promoted an increase in the content of isoflavone glycosides (daidzin, genistin and glycitin) and reduced that of malonyl-glycosils (malonyldaidzin, malonylglycitin and malonylgenistin) (Table 4). The reduction in the levels of malonyl-glycosides can be attributed to the cleavage of ester groups to form malonyl daidzin and genistin during the heat treatment (Carrão-Panizzi et al., 2003). The acetyl forms were not detected in canned grains of vegetable-type soybean due to the wet heat treatment. These forms of isoflavones can be detected by the decarboxylation of malonyl during dry heat treatment and extrusion (Mahungu et al., 1999). The canned vegetable-type soybean in acidified brine with sucrose showed 0.03 g 100 g^{-1} of the daidzein and not detected this form in the canned without sucrose and the *in natura* grains. The genistein and glycitein forms were not too detected. This indicated that the thermal processing was not converted the glycosides isoflavone to aglycone. The canned grains with and without sucrose showed a lower total isoflavones content when compared to grains *in natura*, most likely due to the migration of isoflavones to the brine and because the heating process can affect the conjugated forms of isoflavones (Grün et al., 2001).

The highest glucose and fructose content were observed in the canned grains in acidified brine and with sucrose and in the control (without sucrose) (Table 5). The grains *in natura* showed the highest

Table 5
Sugar content of acid canned[a], canned without sucrose[b] and vegetable-type soybean grains *in natura*.[c]

Sugar	Acid canned[a]	Canned without sucrose[b]	Grains in natura[c]
Glucose	0.23[a d]	0.26[a]	0.07[b]
Fructose	0.27[a]	0.27[a]	0.10[b]
Sucrose	0.97[c]	1.53[b]	4.67[a]
Stachyose	0.73[c]	2.43[b]	5.00[a]
Total	2.20[c]	4.43[b]	9.8[a]

[a] Brine with $NaCl_2$, $CaCl_2$, citric acid and zinc acetate.
[b] Brine with $NaCl_2$, $CaCl_2$, citric acid and sucrose.
[c] Brine with NaCl, CaCl and citric acid.
[d] Means followed by same letters in the lines do not differ by Tukey's test at 5% probability; results express in g 100 g^{-1} on dry basis.

content of sucrose, which is the most important sugar of vegetable-type soybean and is associated with a sweet taste (Masuda, 1991; Liu, 1999). In canned grains, however, thermal processing in acid promoted the hydrolysis of sucrose. The stachyose content differed among treatments, and the highest content was observed in grains *in natura*. The fact that the highest total sugar level was also observed in grains *in natura* suggests that even with the addition of sucrose heat treatment promoted the solubility of sugars in the cooking water of the canned grains. Mozzoni et al. (2009a,b) demonstrated that the sucrose content initially increased after 5 min of processing when compared with the sucrose content of raw or *in natura* vegetable-type soybean grains and that the oligosaccharide content did not change with processing time.

4. Conclusion

The optimum conditions for the formulation of the canned in acidified brine grains were established after validating the proposed model as being 3.43 g 100 mL^{-1} sucrose, 6 g 100 mL^{-1} $NaCl_2$, 0.29 g 100 mL^{-1} $CaCl_2$, citric acid with pH 3.9 and a thermal processing time of 10 min. Commercial sterility was achieved in all the canned with acidified brine grains. The added sucrose to contribute to maintain the desirable color of the canned vegetable-type soybean grains in acidified brine. The canned grains of vegetable-type soybeans in acidified brine showed high glucose and fructose content and low sucrose and stachyose content when compared with grains *in natura.* The thermal processing of vegetable-type soybean grains in acidified brine and with the addition of sucrose promoted an increase in the content of isoflavone glycosides and a decrease in the content of malonyl-glycosils and was not to convert the isoflavone glycosides to aglycones.

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