
TOFU OBTAINED WITH LEMON COAGULANT AND SOYBEAN CONCENTRATE**TOFU OBTIDO COM COAGULANTE DE LIMÃO E CONCENTRADO DE SOJA***Gabriela Albuquerque Três¹**Juliana Steffens²**Clarice Steffens³*

Resumo: O objetivo deste trabalho foi desenvolver e caracterizar tofu, utilizando coagulante vegetal (5-15%) e concentrado proteico soja - CPS (0-10%), aplicando um delineamento experimental para maximizar o rendimento de tofu. Também foram avaliadas as características de acidez, umidade, sólidos totais, proteínas, sinérese e cinzas no primeiro e sétimo dias de armazenamento. No primeiro dia de armazenamento, alta porcentagem de coagulante resultou em um aumento na acidez do tofu. Alto teor de umidade foi observado em altas concentrações de coagulante sem concentrado proteico de soja. Nos sólidos totais, verificaram-se altos valores em baixa concentração de coagulante e CPS. Proteínas, sinérese e cinzas foram altas quando adicionadas baixas concentrações de coagulante e CPS. O alto rendimento foi observado no experimento com alto CPS e baixa quantidade de coagulante. No sétimo dia de armazenamento, a alta concentração de limão resultou em efeito significativo ($p < 0,05$) na sinérese e na proteína. Portanto, o tofu fabricado com coagulante vegetal (5%) e a adição de 10% de CPS apresentou altas quantidades de proteínas, cinzas e melhor rendimento, além de baixa acidez, podendo ser uma alternativa para os consumidores que procuram produtos com alto teor de proteínas.

Palavras-chaves: Limão. Soja. Tofu. Coagulante vegetal. Extrato hidrossolúvel de soja.

Abstract: *The objective of this work was to develop and characterize tofu, using vegetable coagulant (5-15%) and soy protein concentrate - SPC (0-10%) applying an experimental design to maximize the yield of tofu. Also were evaluated the characteristics of acidity, moisture, total solids, proteins, syneresis and ash in the first and seventh days of storage. In the first day of storage, high percentage of coagulant resulted in an increase in the acidity of the tofu. High moisture contents were observed in high concentrations of coagulant without soy protein concentrate. In the total solids the high values were verified in low concentration of coagulant and SPC. Protein, syneresis and ash were high when added low concentrations of coagulant and SPC. The high yield was observed in the experiment with high SPC and low amount of coagulant. On the seventh day of storage, the high concentration of lemon resulted in a significant effect ($p < 0.05$) on syneresis and protein. Therefore, tofu made with vegetable coagulant (5%) and addition of 10% SPC showed high amounts of protein, ash and better yield, as well as low acidity and could be an alternative for consumers looking for products with high protein content.*

Keywords: *Lemon. Soybean. Tofu. Vegetable coagulant. Water-Soluble Soy Extract.*

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1 INTRODUCTION

Tofu, also known as soy cheese, is an excellent source of protein and essential amino acids and has grown worldwide popularity (TSAI et al., 2006; RUI et al., 2016). It has a firm texture similar to cheese, delicate flavor, creamy aspect and white color. The manufacturing process from soy milk or called soybean water extract (SWE), is very similar to cheese made from cow milk, also having the same consistency.

The production process involves the steps of obtaining soybean milk (CIABOTTI et al., 2007), next the coagulation, which is the most critical step to obtain good texture and yield of the tofu. In the production of tofu the protein coagulation is the most important step. Some industrial coagulants used in the manufacture are salts such as calcium chloride, magnesium chloride and magnesium sulfate (HOU and CHANG, 2003; LI et al., 2015). In addition, for the coagulation can be used vegetable coagulants, such as lemon juice, that is a source of proteases and increase the acidity (MAZORRA-MANZANO et al., 2013). Acid coagulation occurred by the gradually decreases of the pH, associated with extending heating and enzyme activity, forming a homogeneous gel (NIK et al., 2011). The two major proteins in soybean are β -conglycinin and glycinin, as well as isoflavones, including daidzein and genistein in the presence of acid coagulant form different extent of aggregations and precipitate. The coagulation temperature depends on type of coagulant, were the optimum temperature range from 70 to 80 °C. With the decrease of pH during the coagulation occur a gradual release of hydrogen ions makes the pH approach the isoelectric point. Thus, the denatured soy proteins are repulsed and the protein particles get close together, and aggregation occurs, forming a homogenous gel network (O'TOOLE, 2016).

Schmidt et al. (2017) studied the influence of different coagulants (lemon, kiwi and ginger) and observed that for the production of tofu the lemon coagulant was the one that presented the best texture and yield, besides lower syneresis, using pH 2 and temperature of coagulation at 80°C. However, Ciabotti et al. (2009) state that at low solids concentrations the yield is very low, regardless of the coagulant used.

The soy protein concentrate, popularly called soybean meal, is the most used vegetable ingredient in the formulation of diets, due to its protein value (44 to 50% crude protein), adequate amino acid balance (GATLIN et al., 2007), and have as main functional characteristic solubility, since this influence in the majority of other properties, such as: gelling, foaming and emulsifying capacity (FENNEMA, 2010). Soy protein concentrate increases yield when used with other proteins, such as dairy; improving foam stability and acting as a surfactant in oil-water and air-water systems; avoid syneresis, especially if accompanied by soluble fibers; and improve rheological and texture characteristics (ZHANG et al., 2005). In addition, it can be applied industrially as a gelling agent in the production of meats and cheeses.

In this sense, the objective of this work was to evaluate different concentrations of vegetable coagulant (lemon) and solids (soybean protein concentrate) in obtaining tofu to increase yield. In addition to obtaining and characterizing the soybean water extract (SWE) from conventional soybean in relation to protein, total solids, pH, humidity and acidity. Verify moisture, syneresis, total solids and proteins, ash and acidity of each formulation from design of experiments.

2 MATERIAL AND METHODS

2.1 Soybean water extract (SWE)

The soybean used in this study were provided by Granja Três, from conventional cultivars, category S1, from the Jacutinga - RS region, 2017/2018 harvest. SWE was obtained by processing the soybean grains, according to the methodology of Benassi et al. (2011), with adaptations. Initially 150g of soybean grains were selected, sorted, weighed and washed, and then was left macerating (immersed) in 500 mL of distilled water at $\pm 25^{\circ}\text{C}$ for 16 h. After, the grains were drained and weighed and was evaluate the amount of water absorbed, by the difference in mass of grains macerated by the initial mass of grains. In the sequence, it was added distilled water at 90°C , considering water absorbed by the grain so that they complete a volume of 1200 mL, with a final ratio of 1: 8 (beans: water). Then the mixture was triturated (M. Vitroty, 1/2 HP model) for 3 min, and after the SWE was separated by vacuum filtration (Tecnal, TE-058 model) and the okara (residue) was removed. Then it separates the SSE of the okara (residue) by filtration vacuum (Tecnal, TE-058 model). Were performed analyzes of moisture, protein, pH, acidity and total solids.

2.2 Vegetable coagulant

The coagulant used for production of tofu was the lemon Taiti, which was purchased in local shop (Erechim-RS-Brazil) and the extract was obtained according to the methodology of Adetunji et al. (2008). The lemons were washed, cut and extracted the juice, to obtain 1L of juice and an equal portion of 20 mM sodium phosphate buffer (pH 7.2) (1:1 v/v) was added. After centrifugation (Centrifuge MPW®, model 351R) at 5000 g for 30 min at 4°C and vacuum filtration (Tecnal, model TE-058) was performed. The samples were stored at 4°C until the time of tofu preparation, approximately 2 h.

2.3 Elaboration of tofu

Tofu was obtained from methodology of Benassi et al. (2011), with adaptations, made de coagulation with lemon extract. 1.5L of SWE was heated to 90°C in a water bath (Marconi®, MA126 model).

After heating, the SWE was transferred to a glass vessel and cooled to the coagulation temperature (80°C), according to Schmidt et al. (2017). After coagulant (lemon) was added, adjusting the pH of the lemon extract to 2.0 with citric acid. Also was added soy protein concentrate (purchased from Cerelus local food with 40% protein), homogenized with mixer and allowed to coagulate for 40 min. The concentrations of coagulant extract (lemon) and soy protein concentrate (SPC) were evaluated by experimental design. The levels of variables were defined according to preliminary tests, and Table 1 presents the levels and variables of experimental design 2^2 . The amount of SWE was kept fixed (1.5 L). According to the matrix of the experimental design (2^2) 5 different formulations of tofu were prepared, with runs 5, 6 and 7 being the triplicates of the central point.

Table 1 - Matrix of experimental design 2^2 with independent variables (coded and real values) to obtain the tofus.

		Levels		
Independent variables	Code	-1	0*	+1
Coagulant (%)	X ₁	5	10	15
SPC (%)	X ₂	0	5	10

*Central point of the experimental design

After coagulation the mass was cut by lire, and inserted into cheese molds (500 mL), which remained for 30 min to syneresis. After the tofu was removed of the mold, and packed in plastic packages without adding water and without vacuum, and stored under refrigeration (4°C) for 7 days. During the coagulation of tofus were measured the pH and acidity.

In the tofu was carried out the analysis of moisture, protein, ash, acidity, total solids and syneresis according to the methodology of AOAC (2000), in the 1st and 7th days of storage. The yield was obtained according to Benassi et al. (2011) in the 1st day of storage.

2.4 Characterization of WSE and tofu

Moisture and total solids were determined by the gravimetric method, in a drying chamber (Fanem®, a Model 320) at 105°C for about 4 h to constant weight. Protein was determined by Kjeldahl method. Ashes were obtained by gravimetric method, after calcination in a muffle oven (Quimis), at 550°C for 6 h. The pH was determined by potentiometry (Digimed, Model DM-22), while the acidity was analyzed by titrimetric method. The syneresis of tofu was determined by the method of drainage.

2.5 Statistical analysis

The results obtained from the experimental design where used to evaluated the differences between means variance analysis (ANOVA) follow by Tukey's test using Statistic software, version 5.0, at a significance level of 95% of confidence.

3. RESULTS AND DISCUSSION

The WSE presented moisture of 93.76% (± 0.21), total solids of 6.23% (± 0.18), protein of 4.13% (± 0.02), pH of 6.39% (± 0.06) and acidity of 10.5 °D (± 0.7).

Barros and Venturini Filho (2016) found moisture of 95.2% of WSE of soybean cultivar BRS-257, provided by EMBRAPA Soybean. Already, Maia et al. (2006) obtained 93.6% for common soybeans. These values are similar to the obtained in this study.

The value of protein found in the WSE is in accordance with the Resolution RDC N. 268 of 2005 (Brasil, 2005), which provides a minimum protein of 3%. Ciabotti et al. (2009) found 3.56% of protein in common soybeans, also near to obtained in this study.

According to Lambrech et al. (1996) the pH of the WSE for production of tofu, should be between 6.4 and 6.6, value close to that obtained in this work. Martins et al. (2013) found acidity between 0.1 and 0.2% of lactic acid in of common soybeans, and the acidity of the present study was between the range studied by the authors (10.5°D-0.105% of lactic acid).

The physicochemical parameters of SWE can vary according to soybean type, grain storage time and conditions, initial moisture content, extraction procedures, water-soy ratio and extraction temperature, as well as the climate where is produced, among others.

Table 2 shows the combinations between the independent variables (lemon concentration and SPC) for each run, as well as the results obtained from the response variables (acidity, moisture, total solids, yield, protein, syneresis and ashes) for the 1st and 7th days of storage, respectively.

The acidity of tofu ranged from 1.67 to 4.21% on the 1st day and from 1.24 to 3.52% on the 7th day of storage. In the formulations developed on the 1st day, among the variables studied, it was observed that the lemon concentration had a significant positive effect ($p < 0.05$), and the interaction between the variables had a significant negative effect at a level of significance of 95% in the acid content of tofu (Figure 1a). Thus, the high concentration of lemon added to the formulations resulted in high acidity. The ions and the hydrogen ionic strength influence the water-holding capacity of the tofu (TAY et al. 2006).

Table 2. Matrix of an experimental design with the independent variables (coded and real values) and responses of physical-chemical analysis of the tofu in the 1st and 7th days of storage.

Runs	Independent Variables*			Responses					
	X ₁	X ₂	Acidy (%)	Moisture (%)	Total Solids (%)	Protein (%)	Syneresis (%)	Ash (%)	Yield (%)
1st day of storage									
1	-1 (5%)	-1 (0%)	2.11±0.35	75.21±1.16	24.78±1.19	13.71±0.18	9.80±0.21	1.53±0.12	20.40
2	1(15%)	-1 (0%)	4.21±0.25	81.21±0.06	18.78±0.02	14.13±0.07	12.00±0.05	1.57±0.16	17.57
3	-1(5%)	1 (10%)	1.67±0.46	74.71±0.31	25.28±0.24	15.63±0.11	11.50±0.02	1.88±0.08	40.17
4	1(15%)	1 (10%)	3.15±0.31	71.84±0.14	28.15±0.12	15.03±0.02	10.00±0.01	1.74±0.08	39.86
5	0(10%)	0 (5%)	3.36±0.12	72.64±0.16	27.36±0.13	14.17±0.01	10.10±0.03	1.67±0.04	24.48
6	0(10%)	0 (5%)	4.02±0.30	74.09±0.04	25.90±0.01	14.18±0.09	10.80±0.23	1.64±0.04	26.36
7	0(10%)	0 (5%)	3.90±0.09	72.45±0.62	27.54±0.41	14.09±0.07	11.20±0.34	1.66±0.02	27.30
7th Day of storage									
1	-1(5%)	-1 (0%)	2.01±0.23	70.17±0.26	29.82±0.23	13.14±0.04	1.80±0.20	1.49±0.06	-
2	1(15%)	-1 (0%)	1.24±0.16	77.82±0.29	22.17±0.24	14.18±0.03	4.10±0.50	1.53±0.07	-
3	-1(5%)	1 (10%)	1.34±0.17	72.99±0.20	27.01±0.13	15.96±0.05	2.80±0.01	1.90±0.14	-
4	1(15%)	1 (10%)	3.15±0.31	69.56±0.21	30.47±0.20	15.47±0.01	3.30±0.02	1.68±0.03	-
5	0(10%)	0 (5%)	3.45±0.06	70.57±0.40	29.42±0.32	14.07±0.15	2.31±0.03	1.70±0.10	-
6	0(10%)	0 (5%)	3.16±0.05	72.64±0.50	27.36±0.26	14.10±0.12	2.52±0.2	1.62±0.01	-
7	0(10%)	0 (5%)	3.52±0.13	70.37±0.18	29.62±0.16	14.06±0.05	2.10±0.01	1.63±0.02	-

*Independent Variables: X₁: Lemon Concentration, X₂: SPC

However, on the 7th days of storage only a significant positive effect ($p < 0.05$) was observed between the interaction of the variables (Figure 1b). This effect can be resulted from hydrolysis of neutral lipids to fatty acids and also the oxidation of fatty acids during storage (LIU and CHANG, 2008). Schmidt et al. (2017), studied tofu with lemon coagulant, not found significant differences ($p < 0.05$) between the 1st and the 7th day of storage on the acidity. According to Fasoyiro (2014), the acidity values of tofu are dependent on the coagulant used.

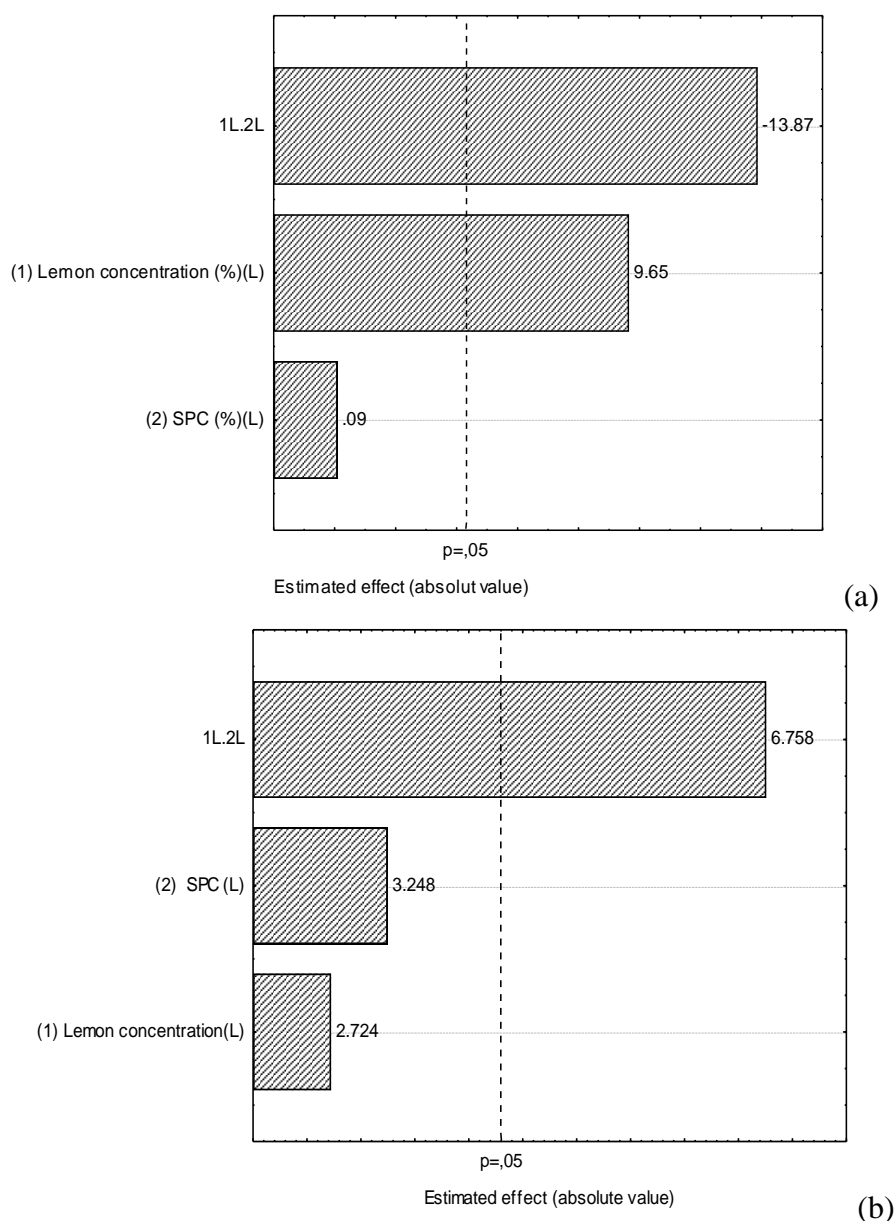


Figure 1. Pareto chart of effects of acidity on the 1st (a) and 7th (b) days of storage.

In Table 2 is observed that in run 2 (tofu with 15% of lemon extract and 0% of SPC), the highest content of moisture at 1st and 7th days of storage. The results were statistically treated by analysis of variance (ANOVA). For tofu on the 1st first and 7th days of storage is observed by the Pareto chart (Figure 2a and 2b) that the variables studied did not present a significant effect ($p > 0.05$) on the moisture content.

The use of acidic coagulant (lemon) provided a soft tofu, presenting a fragile texture due to the high moisture. This behavior was also observed by Ciabotti et al. (2009) that studied glucone- δ -lactone and lactic acid to obtain tofu and obtained 79-80% of moisture.

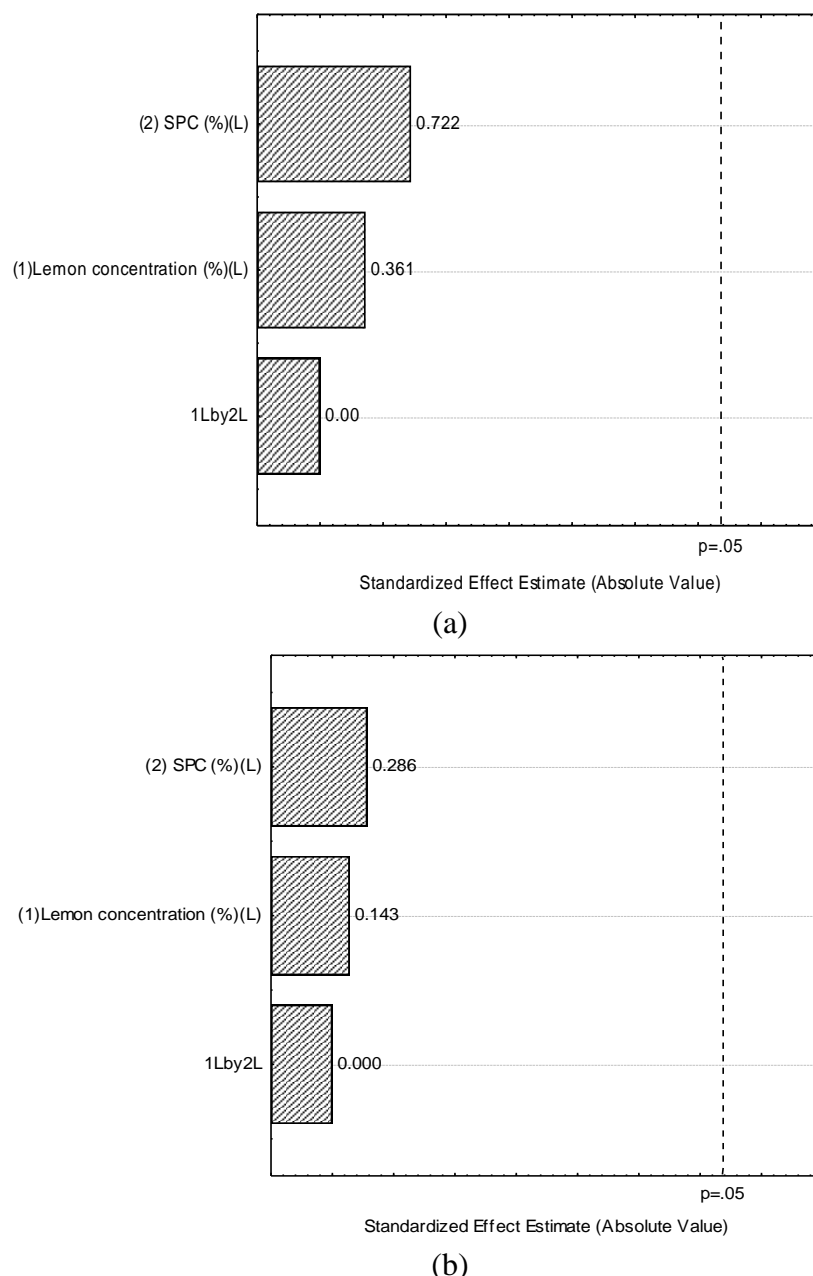
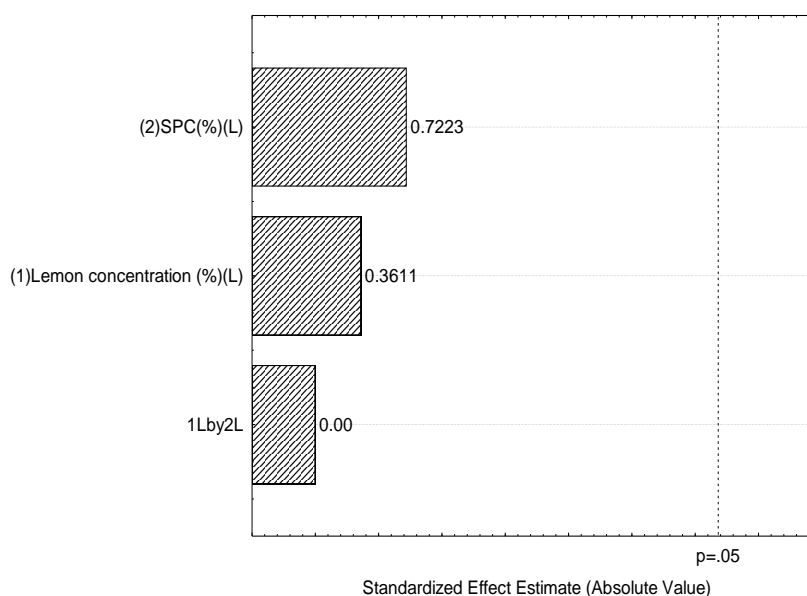


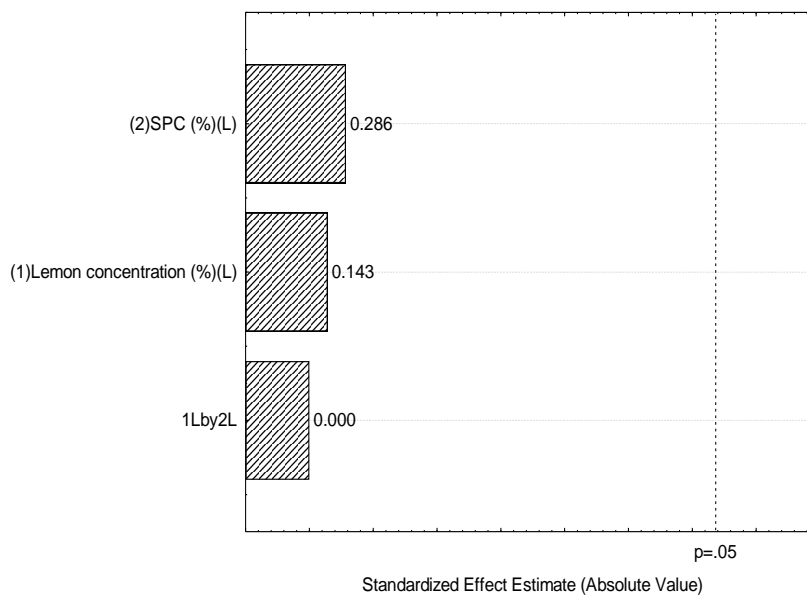
Figure 2. Pareto chart of effects of moisture on the 1st (a) and 7th (b) days of storage.

In relation to the total solids contents of the tofu, it can be observed that run 4 presented higher values on the 1st and 7th days of storage (Table 2). The results were statistically treated by analysis of variance (ANOVA). For tofu on the 1st first and 7th days of storage is observed by the

Pareto chart (Figure 3a and 3b) that the variables studied did not present a significant effect ($p > 0.05$) on total solids contents. The similar values found can be due to the use of same coagulation process (temperature and time) (HOU and CHANG, 2004). This result is very interesting for practical operation and manufacturer that can achieve high quality tofu without economic loss.



(a)



(b)

Figure 3. Pareto chart of effects of total solids on the 1st (a) and 7th (b) days of storage.

Benassi et al. (2011) studied soft tofu purchased commercially and obtained total solids content of 15.3%. It is observed that this value is lower than that obtained in the present study, which may be associated with the amount soy protein concentrate used in the tests.

The high protein content was obtained in run 3 (with 5% of lemon extract and 10% of SPC) (Table 2). This high content is very interesting, that resulted in a product with high yield due the coagulation process, when occurs the cross linking of protein molecules with the divalent cations of the coagulant. Also, the high value of protein is very interesting for the consumer

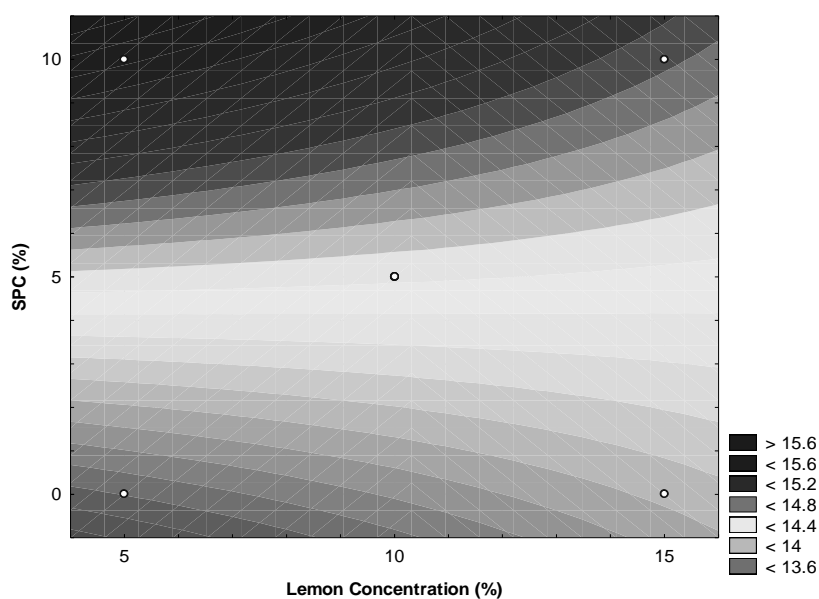
health once is the main functional component. The major proteins are 7S and 11S that have different isoelectric points, molecular weights and properties to form gel in soybean milk with the coagulant resulting in a electrostatic interactions between the cations and the proteins (YUAN et al., 2002).

Equations 1 and 2 show the first order coded models for protein in the 1st and 7th days of storage, respectively. The models were validated by variance analysis (ANOVA), with correlation coefficient of 0.92 and 0.94 and calculated F were 2 and 3 times greater that of the F tabulated, respectively. The results presented significant difference ($p < 0.05$), allowing the construction of the contour curves (Figure 4).

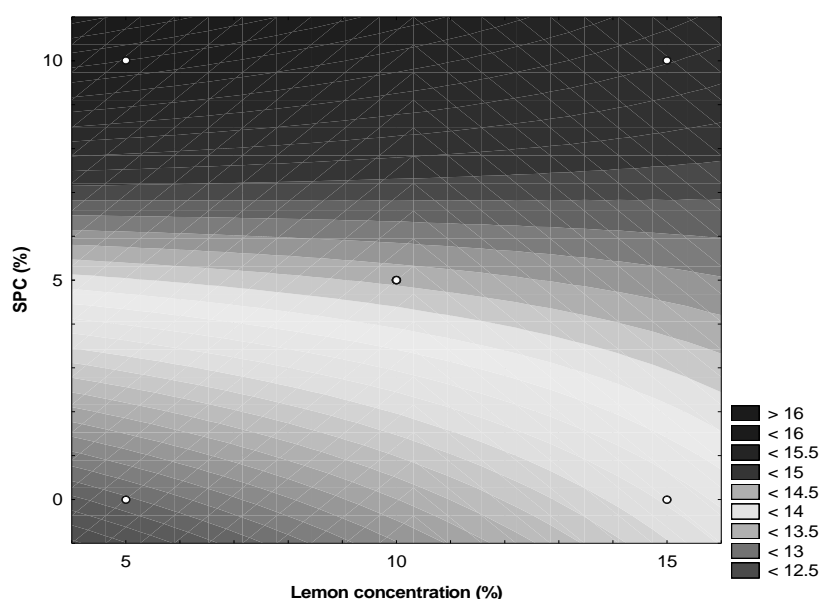
$$\text{Protein (\%)} 1^{\text{st}} \text{ day} = 14.42 - 0.363X_2 \quad (1)$$

$$\text{Protein (\%)} 7^{\text{th}} \text{ days} = 14.42 - 2.055X_2 \quad (2)$$

Where: X_2 is SPC concentration.



(a)



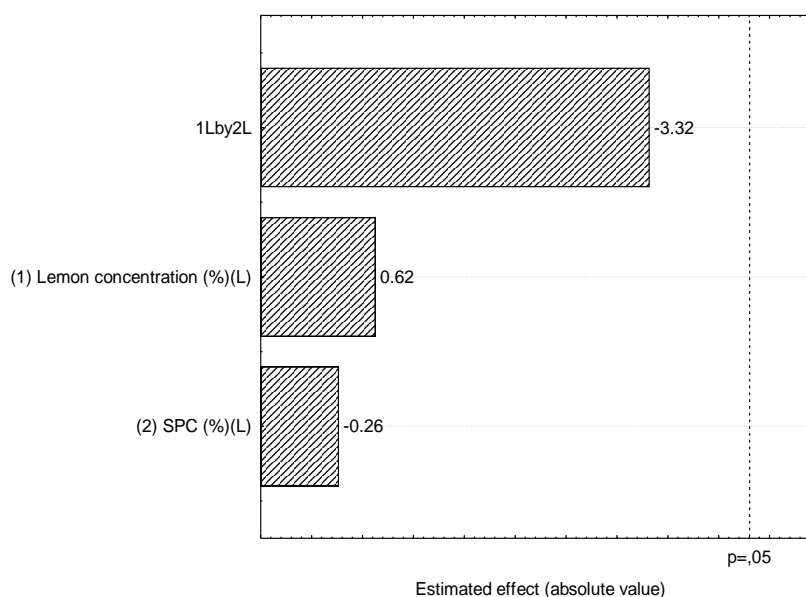
(b)

Figure 4. Contour curve for protein (%) as a function as independent variables on 1st (a) and 7th (b) days of storage.

Ciabotti et al. (2009), obtained a 9.8% of protein in tofu made with bleached common soybeans and coagulated with glucagon- δ -lactone. Schmidt et al. (2017) obtained 10.5% of protein tofu coagulated with lemon. The present study presented higher levels of protein in relation to these authors, which is associated with the addition of soy protein concentrate.

The run 2 presented the highest values for syneresis on the 1st and 7th days of storage (Table 2). Schmidt et al. (2017) also found high syneresis on the 1st day of storage in relation to the 7th of tofu coagulated with lemon. This behavior is due to the precipitation of proteins during tofu processing, causing greater serum release soon after coagulation, consequently, less serum will be released during storage. Run 2, with a higher concentration of lemon and without addition of soybean protein concentrate, presented higher syneresis at both storage times. This can be due to the fact that in the other experiments water absorption occurs due to the addition of the protein concentrate soybean, causing less serum release. In addition, the concentration of lemon provided a softer tofu mass. According to Pereira et al. (2003), the phenomenon of syneresis occurs due to the continuous rearrangements of protein molecules, leading to stress in the network and subsequent breakage of protein bonds. Several factors influence syneresis, such as incubation temperature and low pH.

In relation to syneresis of the tofu, the results were statistically treated by analysis of variance (ANOVA), and is observed on the 1st first and 7th days of storage by the Pareto chart (Figure 5a and 5b) that the variables studied did not present a significant effect ($p > 0.05$).



(a)

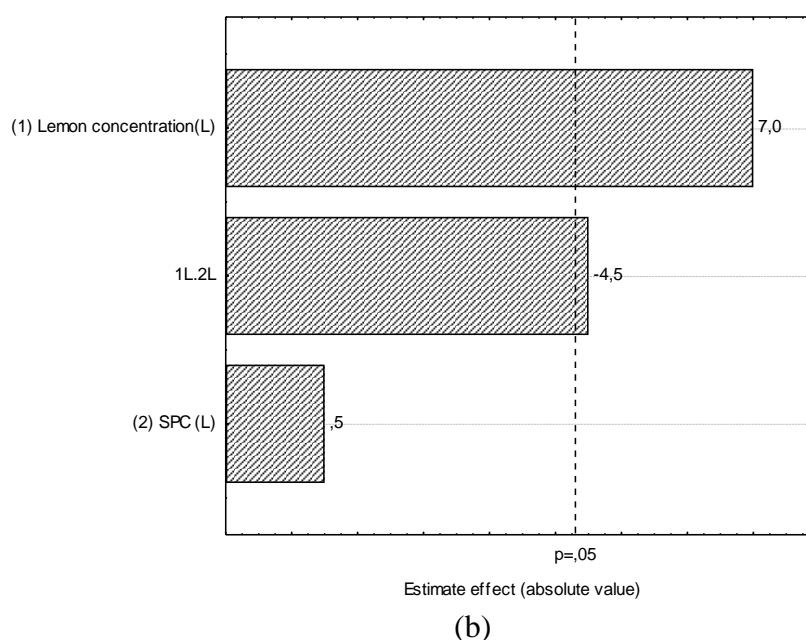


Figure 5. Pareto chart of syneresis on the 1st (a) and 7th (b) days of storage.

In relation to ash content of tofu, it can be observed that run 3 presented higher content on the 1st and 7th day of storage (Table 2). The values found in the present study are higher than those obtained by Schmidt et al. (2017) in tofu coagulated with lemon, which may be due to the addition of soy protein concentrate.

Equation 3 present the first order coded model for ash of tofu on the 1st day of storage where both lemon concentration and soy protein concentrate were significant. However, on the 7th day of storage, according to Equation 4, only the soy protein concentrate was significant. The models were validated by analysis of variance (ANOVA), presenting a correlation coefficient of 0.99 and 0.98 and calculated F of 6.02 and 2.93 times greater than the F tabulated on the 1st and 7th days of storage of tofu, respectively. Thus, it was possible to construct the contour curves for the ashes shown in Figure 6.

$$\text{Ash (\%)} 1^{\text{st}} \text{ day} = 1.67 + 0.04X_1 + 0.13 X_2 \quad (3)$$

$$\text{Ash (\%)} 7^{\text{th}} \text{ day} = 1.65 + 0.14X_2 \quad (4)$$

Where X_1 is the lemon concentration and X_2 is SPC concentration.

According to Figure 6, it is possible to verify that the highest ash content was obtained with the highest concentrations of lemon and soy protein concentrate, on the 1st and the 7th day of storage.

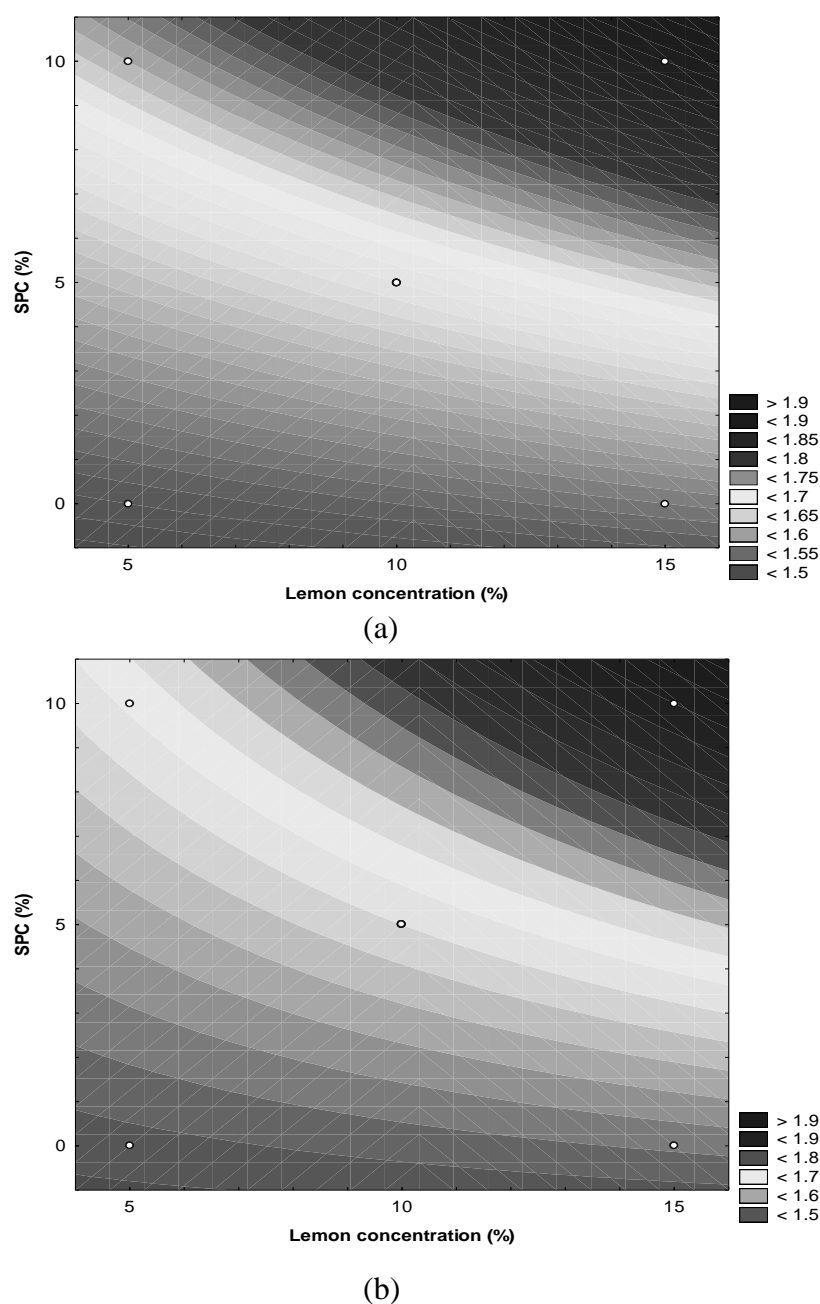


Figure 6. Contour curve for ash (%) as a function as independent variables on 1st (a) and 7th (b) days of storage.

The ash values of tofu found in the present study were similar to those obtained by Li et al. (2015) that elaborated tofu with organic soybean, coagulated with $MgCl_2$, which found 1.85%.

For the yield it was observed that run 3 presented the highest value (Table 2). The results were statistically treated by analysis of variance (ANOVA), where it was possible to obtain a first order coded model (Equation 5), which describes the yield as a function of the analyzed variables and it is observed that the concentrated soybean protein had a significant positive effect ($p < 0.05$). Was found a correlation coefficient of 0.92 and calculated F of 1.96 times greater than the tabulated F, allowing the construction of the contour curve (Figure 7).

$$\text{Yield (\%)} = 28.02 + 21.03X_2 \quad (5)$$

Where: X_2 is SPC concentration

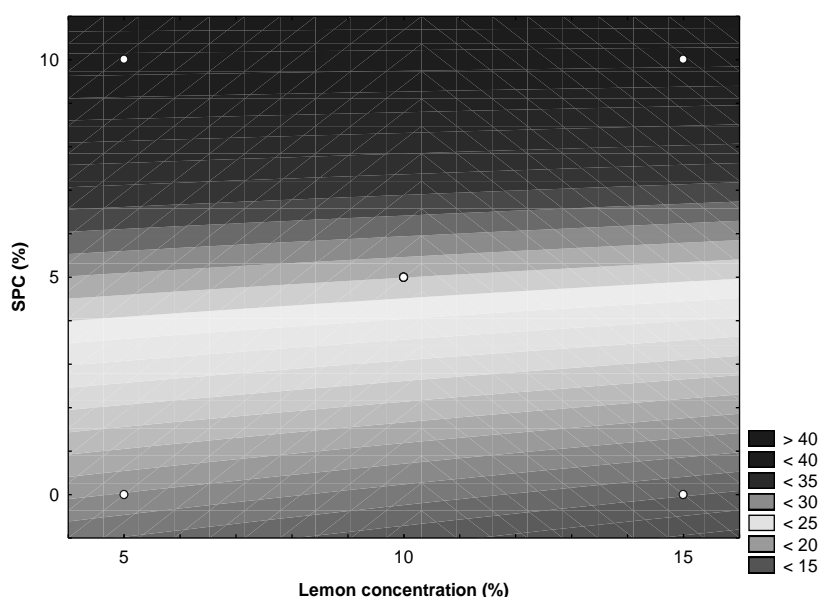


Figure 1. Contour curve for yield (%) as a function as independent variables.

The yields obtained in this study are high than those obtained by Schmidt et al. (2017), due to the addition of soybean protein concentrate, because in the tests with great addition of soybean protein concentrate resulted in high yield.

In general, comparing tofus on the 1st and 7th days of storage was observed a decrease in acidity, moisture and syneresis and increase in solids and ash, and the protein content was not altered.

Figure 8 shows the visual appearance of the tofus of the 5 runs of the experimental design, on the 1st day of storage. All samples of experiments had similar white colour. According to Hou and Chang (2004) the white color is very acceptable for tofu.

It can be observed in Figure 8 (b), run 2, that is the tofu that presented a soft aspect, which is related to the higher moisture content, this occurred due to the low cross-linking between protein molecules resulting in a product softer. The low linking loose the network encompassing, resulting in many air gaps within the network (Jayasena et al., 2014). This aspect can affect the consumers acceptability of tofu.

The tofu of run 3 (Figure 8 c) was the one with the highest yield, protein and ash content, and lower acidity on the first day of storage. The tofu from runs 4 and 5 presented a hardness visual aspect (Figure 8 d and e), that can resulted from the number of protein particles proportion and structure of 11S in the soybeans. The high amount of 11S subunits causes leads to the complete coagulation of soy-curd (SYAH et al., 2015). Regarding the visual aspect, the tofu of run 1 presented the best consistency.

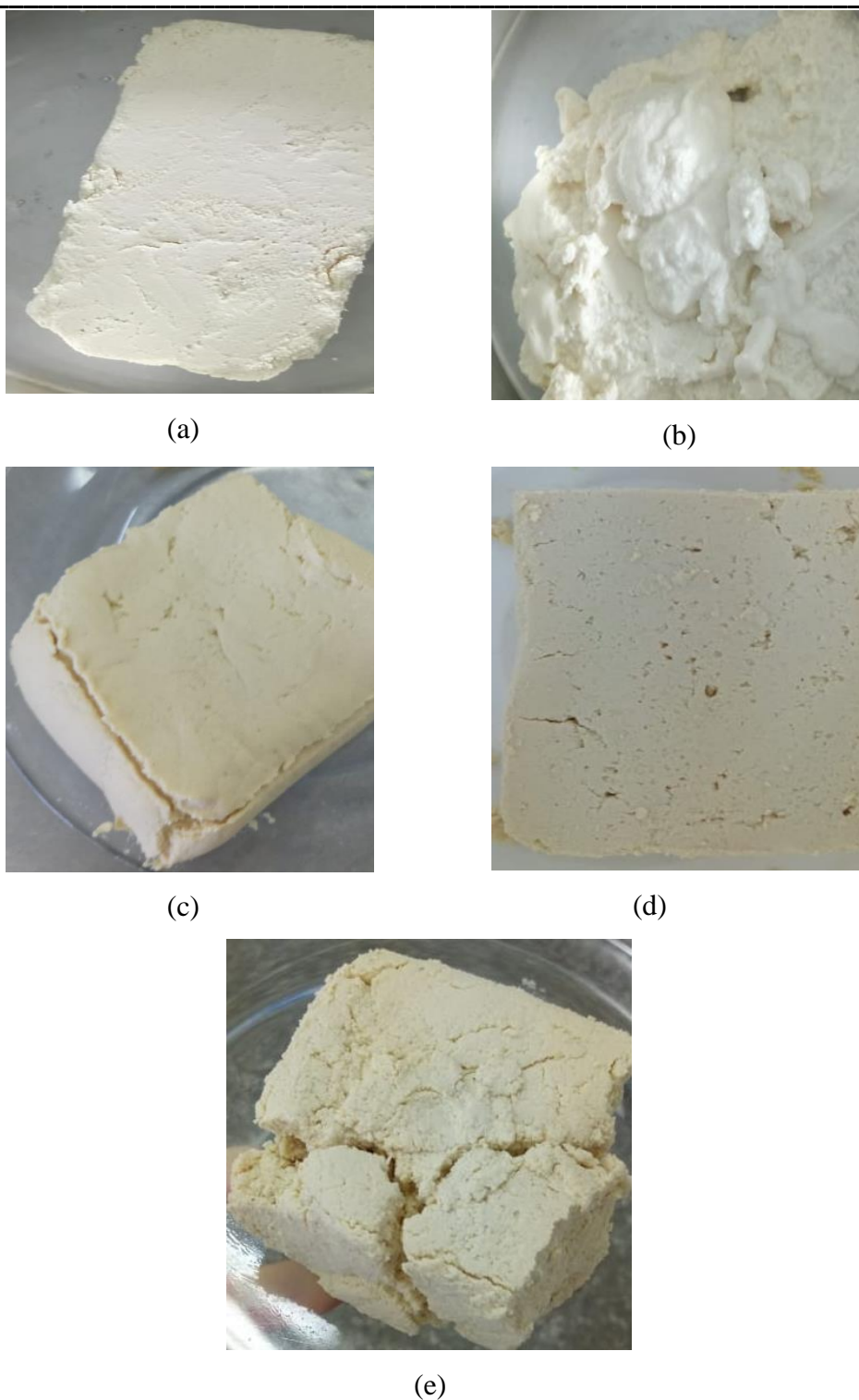


Figure 2. Visual appearance of tofus (a) run 1, (b) run 2, (c) run 3. (d) run 4 and (e) run 5.

4 CONCLUSIONS

The tofus of run 3, made with vegetable coagulant (5%) and SPS (10%) presented better results in relation to protein, ash and yield, where this formulation is recommended for

preparation of tofu, and could be an alternative for consumers looking for products with high protein content. This condition can be use by manufacturers to obtain the best quality of tofu.

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