

Physicochemical Characteristics and Rheological Properties of Soymilk Fermented with Kefir

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Received: 22.12.2021; Accepted: 24.01.2022; Published: 25.03.2022

Abstract: The objective of this work was to evaluate the physicochemical characteristics and rheological behavior of soymilk fermented with kefir. The titratable acidity ranged from 0.25 to 1.47 %; the pH ranged from 5.06 to 5.89 %. The soluble solids (SS) ranged from 1.82 to 5.38 Brix, while the differences between initial SS and final SS were 0.74 to 7.65 °Brix. The sedimentation percentage of the formulations ranged from 50.15 to 99.40 %. The rheological parameters for the flow behavior of the soymilk fermented with kefir were adjusted by the Herschel-Bulkley model. The 9 assays were characterized as non-Newtonian fluids because the behavior showed no linear relationship between the shear rate and shear stress. In the present work, the beverages were characterized as pseudoplastic fluids. In this work, we carry out the fermentation of soymilk with kefir without using any additional ingredients. The optimal treatment was determined, being a solution with an initial soymilk concentration of 5 g of kefir biomass (-0.5), 8 h (-1) of fermentation time, and 10 Brix of SS (+1).

Keywords: viscosity; shear stress; beverage; soybean.

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1. Introduction

Consumers have been searching for foods that, when consumed, produce metabolic, physiological, and/or health-beneficial effects in human health, known as functional foods. Bioactive compounds like vitamins C and E, carotenoids, flavonoids, phenolic acids, anthocyanins, and probiotics are present in food and contribute to the beneficial effects [1, 2]. The food industry has invested in developing products with the functional potential to keep up with this trend.

Soybean is a legume of excellent nutritional value, with high protein content (40% of them are of good digestibility), 20% of lipids, 34% of carbohydrates (mainly oligosaccharides with prebiotic potentials, such as raffinose and stachyose), and 5% of minerals [3, 4]. Soybean and its products, such as soymilk, have received attention from researchers for their nutritional value, mainly due to the presence of bioactive compounds (isoflavones) and their positive claims on human health [3]. Soy intake is associated with several health benefits, such as the

low incidence of cardiovascular and cancer diseases in Asian populations proportional to soy consumption [5-7].

Probiotics are living microorganisms that confer a health benefit when consumed in adequate amounts, including nutrient absorption and immune system functionality [8]. Kefir is a symbiotic culture that may contain potentially probiotic microorganisms such as lactic acid and acetic acid bacteria, yeast, and fungi, which are maintained by a polysaccharide structure called kefirian [9, 10]. Although kefir by itself is not considered a probiotic, it can contain probiotic microorganisms in its symbiosis [11]. Among the microorganisms that are considered probiotics that can be found in kefir are *Lactobacillus acidophilus* LA15, *Lactobacillus delbrueckii* ssp. *bulgaricus* B-30892, *Lactobacillus kefiranofaciens* M1, *Lactobacillus kefiranofaciens* DN1, *Lactobacillus lactis* WH-C1, *Lactobacillus mali* APS1, *Lactobacillus paracasei* CIDCA 8339, and *Lactobacillus plantarum* MA2 [9].

Kefir has been used as fermentation of water-based beverages such as soymilk-based beverages [9, 12-14], fruit-based beverages [15-17], vegetable-based beverages [18, 19], and nut- and almond-beverages [20], among others. By adding live microorganisms to a product, they can promote a fermentation process with a consequent decrease in pH production of organic acids and other metabolites, among other changes that can promote a change in the properties of the final product. The technological characteristics of beverages, such as rheological properties, may change during storage when kefir is added to soymilk beverages [12, 21].

Because the rheological properties correlate with product quality and sensory perception [22-24], they can be used as a tool for analyzing the viability of foods during shelf life. The rheological parameters can also be used for sizing thermal and mechanical systems of industrial equipment [25]. The objective of this work was to evaluate the physicochemical characteristics and rheological behavior of soymilk fermented with kefir.

2. Materials and Methods

2.1. Materials and soymilk fermentation with kefir.

Soybean (Siamar Natural Food, Neves Paulista, Brasil) was used in soymilk production as described by Baú, Garcia, & Ida [13]. Briefly, the soybean was immersed in water for 14 h at 5 °C (1:3 ratio, soybean:water), drained, and rinsed. The soaked soybean was mixed with distilled water using an industrial blender, and the soluble solid was adjusted according to factorial design (Assay 1 to Assay 9).

The kefir grains were obtained from local families that traditionally consumed kefir, and the biomass was increased by growth in a brown sugar solution (10:1, v/m) with a daily change of nutrient material. The kefir grains were frozen; before use, they were activated for 3 days in the same solution at 25 °C with continuous exchange of the nutrient material every 24 h [21].

2.2. Optimization of fermented soymilk with kefir.

The effects of the different fermentation processing variables on the characteristics of the fermented beverage were evaluated using a factorial design with 3 factors and 3 levels to determine the amount of kefir biomass (KB, 4, 6, and 8 g), fermentation time (FT, 8, 12 and 16 h), and soymilk concentration (SC, 6, 8, and 10°Brix), totaling 9 experiments performed twice (Table 1). The statistical analysis of the tests was conducted as described in item 2.4.

2.3. Characterization of fermented soymilk with kefir.

The pH, total titratable acidity (TA), and total soluble solids (SS, difference between initial and final contents) were realized as described by AOAC [26]. The pH was measured using a potentiometer (LUCA-210, Lucadema, Campinas, Brazil). TA was determined by titration with 0.1 N NaOH solution and expressed in mg of lactic acid per 100 mL of beverage. The SS was evaluated using a digital refractometer (model 14043, Reichert, Depew, USA) and expressed in Brix.

The percentage of sedimentation was evaluated using a Falcon tube with 2 g of samples stored refrigerated at 5 ± 1 °C for 72 hours. Sedimentation (%) was determined by the ratio between the height of the supernatant liquid and the total sample height multiplied by 100 [27].

Rheological analyses were carried out at 25°C using a controlled stress rheometer (Physica, MCR 101, Ostfildern, Alemanha) and a smooth cone-plate geometry (CP50). The samples were first placed in the Peltier plate of the rheometer and maintained at rest for 1 min before shearing. After resting, the samples were sheared at a constant rate (500/s), and the shear stress was measured. This fixed shear period was used to ensure no structural changes occurred, which could compromise the steady-state evaluations. At the same time, these results were also used to study the time-dependent rheological behavior of beverages.

After the time-dependent shear period, the flow behavior was evaluated using three shear rate ramps: 1) a linear increasing stepwise protocol (0.1 to 500/s); 2) a linear decreasing stepwise protocol (500 to 0.1/s), and 3) a linear increasing stepwise protocol (0.1 to 500/s). The last run was used to guarantee steady-state shear conditions for rheological mathematical model fitting. The product flow behavior was modeled using the Herschel-Bulkley and Ostwald-de-Waele (power-law) models [28].

2.4. Statistical analysis.

The software Statistica® version 7.0 (Statsoft Inc., USA) was used for the regression analysis of the experimental data obtained. The quality of fit of the first-order model equation was expressed by the coefficient of determination R^2 , and its statistical significance was determined by the F-test.

The main effects and the interaction between the quantitative factors (X_1X_2) were transformed (X_3) using a multilinear regression approach ($Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_nX_n$). The optimum KB, FT, and SC values were identified by applying the desirability function [29] and the simultaneous responses of the independent variables, pH, total titratable acidity (TA), soluble solids (SS), and rheological properties.

3. Results and Discussion

Table 1 shows the results obtained for pH, titratable acidity (TA), soluble solids (SS), and percentage of sedimentation for soymilk fermented with kefir. The TA ranged from 0.25 % (A1 and A8) to 1.47 % (A9), the pH ranged from 5.06 % (A9) to 5.89 % (A1). Several studies have reported that soymilk fermentation is possible when adding other ingredients, such as colostrum and fermented honey [30], inulin [21], inulin and bocaiuva pulp [12], and soy fiber [14]. Still, the literature has not reported the behavior of fermented soymilk with kefir without other ingredients, which was demonstrated in this work.

Table 1. Variables (coded and encoded) and results obtained for titratable acidity (TA), soluble solids (SS), and percentage of sedimentation of soymilk fermented with kefir.

Assay	Coded variables			Encoded variables			pH		SS (°Brix)		TA	% sedimentation
	KB	TF	SC	KB (g)	TF (h)	SC (°Brix)	pH _{final}	ΔpH	SS _{final}	ΔSS	TA _{final}	
A1	-1	-1	-1	4	8	6	5.89±0.03	0.71 ^{cd}	5.38±0.25	0.74 ^g	0.25±0.03	78.33±7.07
A2	-1	0	+1	4	12	10	5.67±0.02	0.83 ^c	3.02±0.05	7.23 ^a	0.58±0.04	96.59±3.82
A3	-1	+1	0	4	16	8	5.07±0.05	1.49 ^a	1.90±0.05	6.20 ^{bc}	0.45±0.08	90.96±14.71
A4	0	-1	+1	6	8	10	5.37±0.67	1.21 ^{ab}	3.08±0.00	6.97 ^{ab}	0.58±0.07	90.99±1.98
A5	0	0	0	6	12	8	5.48±0.05	1.13 ^{abc}	2.32±0.01	6.03 ^c	0.44±0.04	91.12±8.23
A6	0	+1	-1	6	16	6	5.14±0.03	1.44 ^a	1.82±0.02	4.19 ^d	0.49±0.06	99.40±0.66
A7	+1	-1	0	8	8	8	5.59±0.02	0.29 ^d	2.14±0.24	5.91 ^e	0.40±0.07	89.28±11.27
A8	+1	0	-1	8	12	6	5.80±0.05	0.80 ^{bc}	3.85±1.45	2.56 ^f	0.25±0.03	83.99±11.14
A9	+1	+1	+1	8	16	10	5.06±0.02	1.41 ^a	2.40±0.19	7.65 ^a	1.47±0.45	50.15±25.34

KB: kefir biomass; TF: fermentation time; SC: soymilk concentration.

The pH of the evaluated assays remained between 5.0 and 6.0 without a significant difference between them ($p > 0.05$), and the beverages can be considered slightly acidic. Under these conditions, it was demonstrated that the longer the fermentation time, the pH decreased, and consequently, the beverage's acidity increased. Beverages fermented for 8 hours (A1, A4, and A7) showed pH values between 5.37 and 5.89, while those fermented for 16 hours (A3, A6, and A9) showed pH values between 5.06 and 5.14. This decrease in pH value is considered common in fermented foods due to bacterial growth and acid production for kefir presence [16]. Fiorda *et al.* [30] reported that soymilk with colostrum and honey fermented with kefir showed decreased pH and increased titratable acidity with increasing fermentation time. Baú, Garcia, and Ida [14] reported that the pH of fermented soymilk with kefir decreased during storage from 4.4 to 4.1, and this range is considered optimal for soymilk gel formation. On the other hand, $pH > 4.5$ in fermented soymilk is considered desirable to maintain the sensory quality of the product mainly because it interferes with the product's texture and increases the phase separation of the beverage [21].

The SS ranged from 1.82 (A6) to 5.38 (A1) °Brix, while the differences between initial SS and final SS were 0.74 (A1) to 7.65 (A9) °Brix. The SS of fermented beverages using kefir biomass decreases with increasing fermentation time and/or the proportion of biomass:substrate used [18]. The highest averages of ΔBrix showed that the highest consumption of available sugars occurs with higher microbial activity. This is because sucrose is metabolized [16], and microbial growth depends on sugar catabolism [13].

With the results of the chemical analysis, it was observed that the fermentation process was active since the beverage remained in the pH range ($pH > 4.5$) and optimum growth acidity (between 0.25 and 1.47 %). In addition, consumption of soluble solids (SS) decreased its value and demonstrated substrate consumption by kefir biomass. Increased TA is associated with SS consumption, which indicates the continuity of the fermentation process by lactic acid bacteria produced during the storage period [31]. Baú, Garcia, and Ida [14] compared soybean fiber and non-fiber fermented formulations under the same storage conditions and reported that the acidity was higher in soybean fiber formulation, indicating that soy and some fibers may stimulate early microorganism growth.

The sedimentation percentage of the formulations ranged from 50.15 (A9) to 99.40 (A6) %. Higher particulate sedimentation in beverages results in lower product stability during shelf life [32, 33]. In addition, increased sedimentation negatively affects the overall appearance of beverages [21], resulting in changing consumer behavior at the time of purchase.

Adding microorganisms to soymilk in the fermentation process alters the coagulation of the proteins contained in it. The viscosity of soymilk rises due to the hydrodynamic effects of these bulky clusters [34]. The curves obtained by increasing the shear rate at steady-state (Figure 1) and the apparent viscosity of the soymilk fermented with kefir were calculated using the Herschel-Bulkley and Ostwald-de-Waele parameters (Table 2) at a shear rate of 500/s.

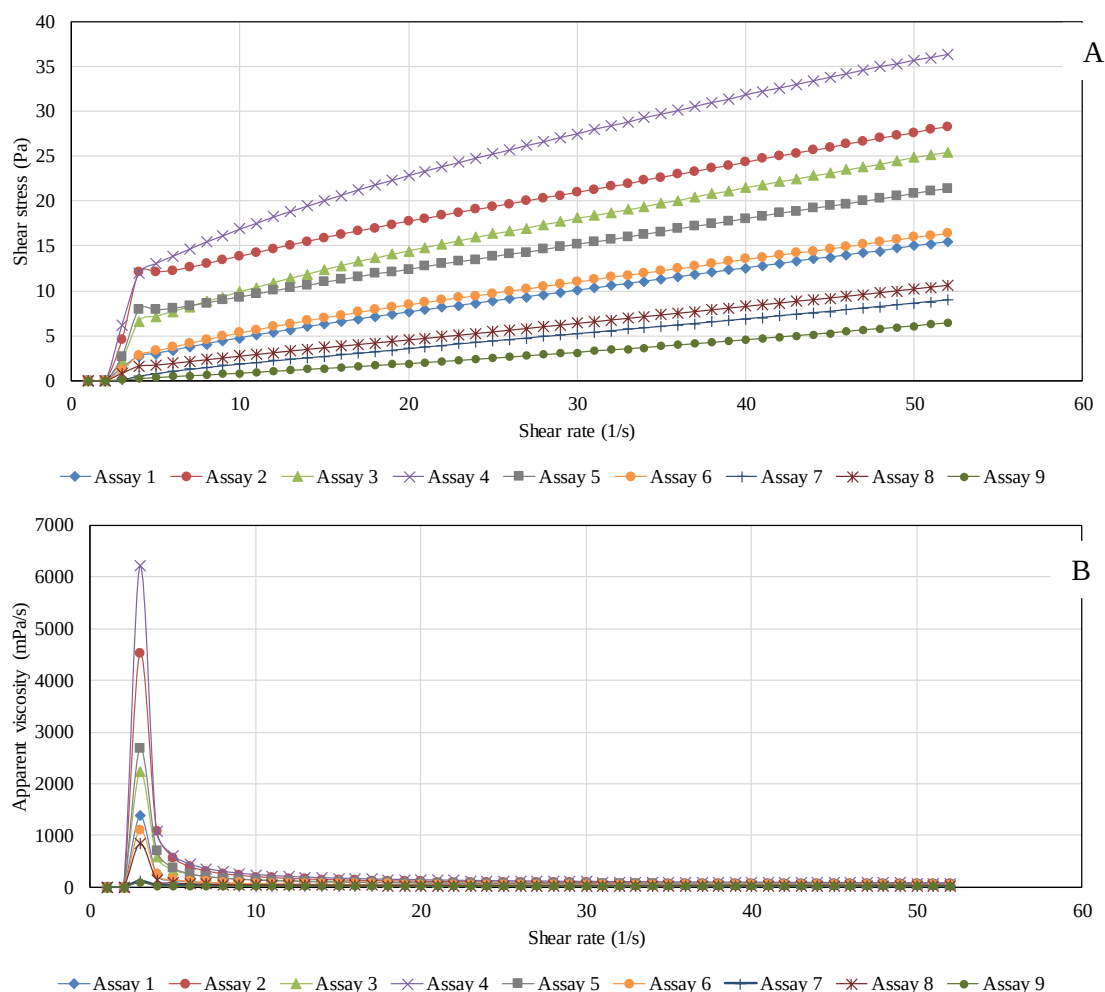


Figure 1. Relation between shear stress and shear rate (A) and apparent viscosity and shear rate (B) of fermented soymilk with kefir.

The rheological parameters for the flow behavior of the soymilk fermented with kefir fit the Herschel-Bulkley model, with higher R² and lower SE values than the Power Law. The R² values of the predicted models were higher than 0.99, indicating that they were relatively adequate for the purpose of prediction [35]. Considering these factors, the Herschel-Bulkley model was the one that correlated best with the results obtained for the soymilk fermented with kefir.

The highest and lowest values of the consistency index (k) were observed for A7 and A9 assays, respectively, while the highest and lowest values of the flow index (n) were observed for A9 and A4 assays, respectively.

Figure 1A shows the flow curves (shear stress and shear rate) of soymilk fermented with kefir at a shear rate from 0.1 to 500/s after reaching a steady state. The 9 assays were characterized as non-Newtonian fluids because the behavior showed no linear relationship between the shear rate and shear stress. In similar findings to the present study, Karaman, Yilmaz, & Kayacier [36] reported that soybean drink sweetened with honey presented a non-Newtonian flow, with a flow behavior index (n) less than unity.

In the present work, the beverages presented a decrease in apparent viscosity according to the increase of the shear rate, being characterized as pseudoplastic, with flow behavior index (n) less than one ($n < 1$). The lowest n value found was for A2 (0.3342), and this value gradually increased to A9 (0.8478), close to results related by Pang, Luo, Li, Zhang, & Liu [37] for soymilk gels with different types of hydrocolloids (0.42 to 2.89). Lower viscosity (3-72 Pa/s) and pseudoplastic behavior were demonstrated by İçier *et al.* [38] for fermented soy milk with added apple juice but with a higher n value than the present work.

Table 2. Rheological parameters obtained by the Power Law (LP) and Herschel-Bulkley (HB) models of fermented soymilk with kefir.

Assays	PL						HP					
	k (mPa/s)	n	R ²	P	SE	χ ²	k (mPa/s)	n	R ²	P	SE	χ ²
A1	0.37	0.59	0.99	5.47	0.04	0.00	0.10	0.79	1.00	1.61	0.01	0.17
A2	3.27	0.33	0.96	5.12	0.00	0.00	0.10	0.84	0.97	3.25	0.03	17.76
A3	1.32	0.47	0.99	4.26	0.01	0.00	0.34	0.66	1.00	2.93	0.01	2.26
A4	3.26	0.38	0.98	4.05	0.00	0.00	0.51	0.64	1.00	1.48	0.02	5.40
A5	1.71	0.39	0.96	5.98	0.01	0.00	0.06	0.89	0.98	3.66	0.03	8.40
A6	0.48	0.56	0.99	4.34	0.00	0.00	0.19	0.70	1.00	2.14	0.01	0.23
A7	0.06	0.79	1.00	3.68	0.03	0.00	0.05	0.82	1.00	2.40	0.01	0.00
A8	0.15	0.68	0.99	7.83	0.10	0.00	0.02	0.98	1.00	3.92	0.07	0.19
A9	0.04	0.85	0.98	15.05	0.14	0.00	0.01	0.99	0.99	6.87	0.06	0.00

Similar behavior was demonstrated for A9, where the lowest shear stress (0.08) and the highest ΔBrix (7.65) were found. Shear stress values and soluble solids were close to each other for A1 and A6, A7 and A8, and A3 and A5 (Table 1).

Statistical analysis of experimental design showed that the only significant dependent variable (response) was pH ($R^2 = 95.25\%$ and $R_{adj} = 90.51\%$). Equation 1 presents the mathematical modeling for this variable.

$$pH = 5,45 - 0,09I^2 - 0,26T + 0,14T^2 - 0,12C \quad (\text{Eq.1})$$

This equation indicates that increasing inoculum and temperature and decreasing soluble solids concentration in soymilk within the studied range increases the pH of the final fermented beverage. Figure 2 shows the contour plot of pH response

Combining the parameters of low pH, high soluble solids in fermented soymilk, and high viscosity of fermented beverage and using the desirability tool, the present work found the experimental assay that would be responsible for these responses. The optimal treatment was determined, being a solution with an initial soymilk concentration of 5 g of kefir biomass (-0.5), 8 h (-1) of fermentation time, and 10 °Brix of SS (+1) (Figure 3). Using the rheological parameters (low pH, high soluble solids, and high viscosity) to designate the optimal treatment, we obtained conditions that best meet the consumer's expectation and preference (high viscosity, high soluble solids, and low pH) [39], when compared to optimization as a function of pH, total soluble solids, and sedimentation percentage reported by dos Santos *et al.* [21].

This experimental design used vertex points that seem to have led to the excess of the fermentation process, making the production of beverage impossible since the kefir biomass remained strongly adhered to the fermented which was already in solid-state.

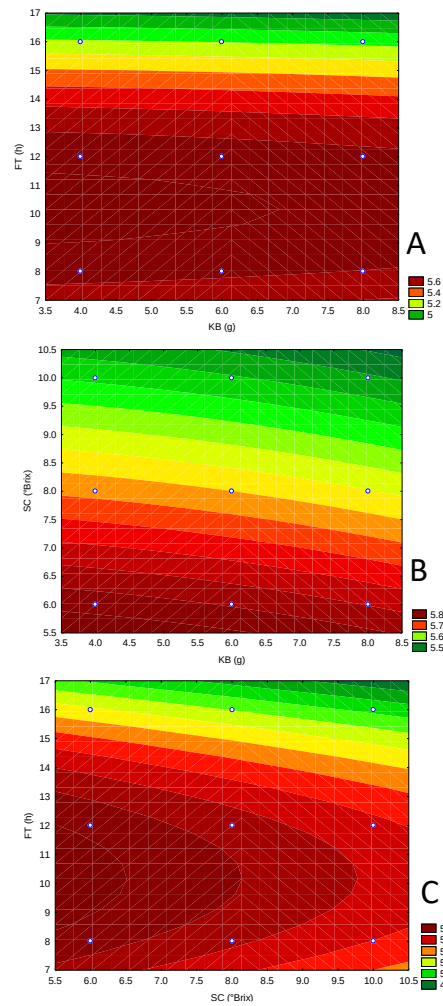


Figure 2. Contour plot of pH of the fermented soymilk with kefir: FT x KB (A), SC x KB (B), and FT x SC (C). Fermentation time (FT); kefir biomass (KB); and solid concentration (SC).

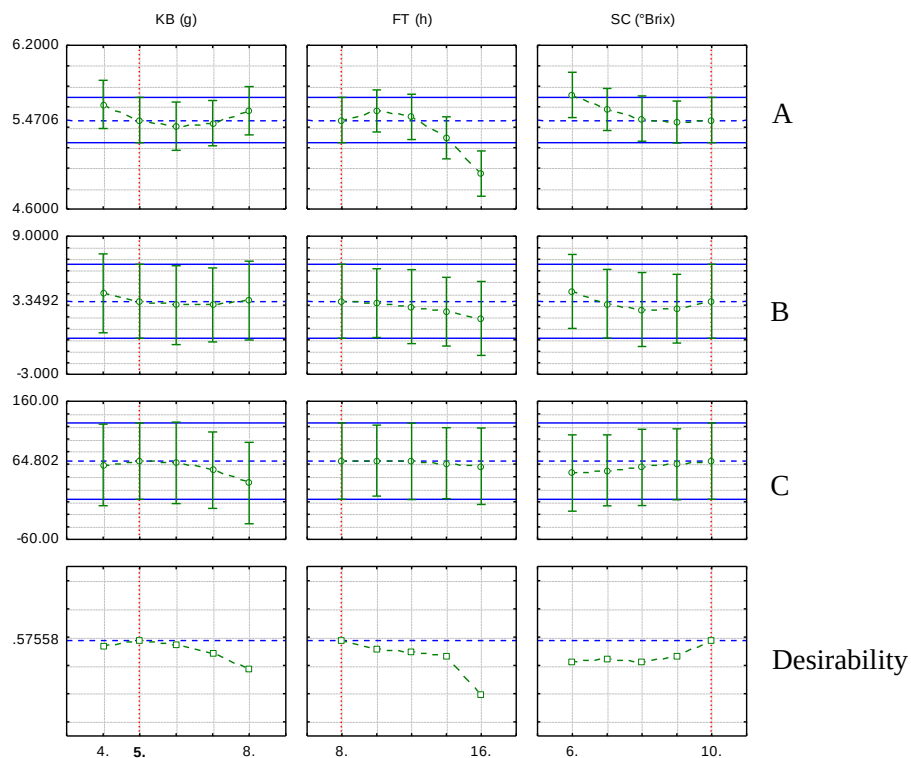


Figure 3. Responses of desirability-related characteristics of fermented soymilk with kefir: (A) pH; (B) soluble solids in fermented soymilk; (C) viscosity.

4. Conclusions

It is concluded in the present study that the fermented soymilk beverage with kefir has pseudoplastic rheological behavior independent of soymilk concentration used, kefir biomass ratio, and fermentation time. The flow behavior index (n) remained less than one gradually increased with the increase of harmlessness. It can be concluded that the rheological model that best fits the experiment is the Herschel-Bulkley which has the closest linear correlation coefficient, which means greater linearity between the analyzed variables.

Funding

This research was funded by CNPq, grant number 469104/2014-7.

Acknowledgments

The authors acknowledge the financial support of CNPq, CAPES, and the Instituto Federal Goiano.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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