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SOY YOGURT FORTIFIED WITH IRON AND CALCIUM: STABILITY DURING THE STORAGE

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■ABSTRACT: The objective of this study was to prepare a soy yogurt, fortified with microencapsulated $FeSO_4.7H_2O$ (12mg of iron/l) and calcium citrate (600mg of calcium/l), and evaluate the stability of the final product during the storage at 10°C. The soy yogurt without addition of iron and calcium was used as control. Analysis of these samples was done once a week, during 28 days, for: pH, titratable acidity, rheological properties (viscosity and consistency), sensory characteristics (acceptance test) and enumeration of viable cells (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*). The pH, titratable acidity, viscosity, consistency and acceptance test data were submitted to analysis of variance and Tukey's test. During the storage was observed a decrease in the pH and increase in the titratable acidity, due to the lactic cultures to be continue viable (10^7CFU/g) in the product. The fortified yogurt exhibited lower viscosity, but this parameter did not change significantly ($p \leq 0{,}05$) during the storage time. The acceptance test results showed that the control and fortified samples did not exhibit significant differences ($p \leq 0{,}05$), during the studied period, in relation to the all sensory attributes evaluated. In conclusion, the present work enabled the development of a iron and calcium fortified soy yogurt, stable during 28 days at 10°C, that could be used in the prevention and control of mineral deficiencies in general population.

■KEYWORDS: Soy yogurt; iron; calcium; microencapsulation; storage; stability.

INTRODUCTION

Iron deficiency is one of the most severe and important nutritional deficiencies in the world today. All age and economic groups are affected, but young children and women of reproductive age have increased requirements for iron, placing them at major risk of deficiency and related adverse consequences. [1,19]

Iron deficiency adversely affects the cognitive performance, behavior, and physical growth of children, immune status, physical capacity and work performance of all age groups and increases perinatal risks for mothers and neonates.[20]

Strategies for prevention of iron deficiency include food fortification, that exhibit an adequate benefit-to-cost. The key to success of fortification process is the choice of vehicle (food or beverage) and iron source. Fortification vehicle must be low cost, being accessible to whole population, and to exhibit high nutritional value.[8]

Soybean and its derivates represent an excellent source of high quality protein, with low content of saturated fat and a great amount of dietary fiber and bioactive components like the isoflavones.[11] In addition, soymilk and its fermented products constitute an alternative for lactose intolerant people. In this way, soy yogurt could be a suitable vehicle for iron fortification, since it has high nutritional value and low-cost. However, calcium content of soymilk is lower that found in cow's milk, being necessary the addition of this mineral to improve its nutritional properties.

The selection of an appropriate source of iron and calcium is important for the product final quality. Ferrous sulfate is a bioavailable iron source being soluble in neutral and/or acidic aqueous environments but that may cause sensorial changes, reducing product acceptability and shelf life.[7] In addition, soy components could react with iron and impaired your bioavailability. On the other hand, the tendency for calcium to coagulate the protein and cause gelation of soy beverages during the storage may difficult the fortification process.[21]

Developing a fortification technology that makes the bioavailable iron and calcium sources more compatible with the food vehicle remains a challenge. In previous studies we obtained a soy yogurt fortified with microencapsulated ferrous sulfate or with calcium citrate with adequate sensorial and technological characteristcs. [15, 16, 17] However, the effect of simultaneous fortification of soy yogurt with iron and calcium did not be studied. The purpose of the present work was to prepare a soy yogurt, fortified with iron and calcium, and verify the viability and acidifying activity of starter culture, rheological properties and sensorial characteristics of this product during the storage period.

MATERIAL AND METHODS

Experimental Design

Soy yogurts were manufactured with 1.2 mg of iron and 60.0 mg of calcium per 100 ml or without

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minerals addition (control sample). Iron and calcium was measured at the end of the experiment. Titratable acidity, viable cells, viscosity, consistency and sensory properties were determined at days 0, 7, 14, 21 and 28. The pH was measured during the fermentation time and at days 0, 7, 14, 21 and 28.

Soymilk, Chemicals and Bacterial Strain

Whole soybean (*Foscarim* variety) was boiled at 95°C for 5 minutes and the hydrated beans were drained and triturated. Soymilk was separated from insoluble residue by centrifugation and filtration (nylon filter). The extract was diluted (soy extract/ water ratio = 1:6), homogenized and pasteurized.

Soy yogurt was fortified at a concentration of 1.2mg Fe/100ml with microencapsulated ferrous sulfate. To prepare this compound, ferrous sulfate hydrate ($FeSO_4.7H_2O$) was encapsulated with partially hydrogenated lecithin (Epikuron 200 SH, Lucas Meyer Ind. Com. Exp. Ltda).[17] Calcium citrate (60.0mg Ca/100ml, obtained from Montedison Farmacêutica) was used for calcium fortification. Microencapsulated ferrous sulfate and calcium citrate was chosen as a suitable source for soy yogurt fortification in a preliminary study based on its sensory, chemical and bioavailability properties. [5,7,13,15,16,17]

Streptococcus thermophilus and *Lactobacillus delbruechii* subsp. *bulgaricus* were obtained from commercial culture (Rich®, Chr. Hansen, importated by Chr. Hansen, Valinhos, SP, Brazil).

Soy Yogurt Manufacture

Soy yogurt was manufactured at UNISOJA (Development and Production Unit for Soybean Derivatives) in the Food and Nutrition Department of the School of Pharmaceutical Sciences, UNESP at Araraquara (SP, Brazil), following the method described by Rossi et al.[9] Soy yogurt composition was presented in the Table 1. Soy milk, soy oil, lactose, iron and calcium were homogenized and heated to 70°C. Sucrose, skin milk and gelatin were added and pasteurized (95°C for 5 minutes) after prior homogenization. Each batch was cooled at 42°C and the culture containing *Streptococcus thermophilus* and *Lactobacillus delbruechii* subsp. *bulgaricus* were added. Inoculated mixes were poured in plastic recipients and incubated at 42°C until the product reached pH 4.5. The products were storage at 10°C during the storage time (28 days).

Acidity Measurement

The pH change was monitored by a pHmeter (Micronal model 320). Titratable acidity was determined by titrating a sample with 0.1 N NaOH and expressed as % lactic acid (% La).[6]

Viable Cell Counts

Each batch of finished soy yogurt was checked microbiologically by counting viable cells of *S. thermophilus* and *L. delbruechii* subsp. *bulgaricus* on two specific culture media - M17 and MRS agars, respectively (Difco, Detroit), at days 0, 7, 14, 21 and 28. The colonies were counted and the unit expressed as colony-forming units (CFU) per gram.

Viscosity and Consistency Determination

Viscosity of soy yogurts was determined at 4°C using an axial viscosimeter (Waake model VT 02) and the consistency was measured from the distance (cm) rum during 10 seconds in a Bostwick consistometer.[10]

Iron and Calcium Quantification

Iron and calcium concentrations were determined by atomic absorption spectrofometry (spectrofotometer - Perkin Elmer model 3110), λ = 248.3 nm and 422.7 nm, respectively.[15,16]

Sensory Evaluation

A panel of 30 persons aged between 20 and 40 performed this study. The attributes evaluated were aroma, color, flavor and overall acceptance. The panelists evaluated the acceptability of each each samples using an unstructured hedonic scale (9 cm) with ends anchored, “I dislike very much” and “I like very much”. The samples were presented at random and individually evaluated.[14]

Table 1 – Composition of soy yogurt fortified, or not, with iron and calcium per 100 mL.

Ingredients	Control	Iron and calcium fortified
Soy milk (ml)	82.0	82.0
Soy oil (ml)	2.6	2.6
Lactose (g)	2.0	2.0
Sucrose (g)	10.0	10.0
Skin milk (g)	3.5	3.5
Gelatin (g)	0.3	0.3
Iron (mg)	0.0	1.2
Calcium (mg)	0.0	60.0

Statistical Analysis

Titratable acidity, pH, viscosity, consistency, iron and calcium levels and sensory acceptability were tested by analysis of variance (ANOVA) and the means were compared across groups or times by Tukey test. All analyses were carried out with the SAS statistical package and the significant differences were determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Microencapsulation technique has being used to separate sensitive or incompatible ingredients, avoiding the nutritional interaction and undesirable changes in the sensory characteristics.[3, 17, 23] In the present study, we used microencapsulated ferrous sulfate to place a physical barrier around the iron to reduce color and flavor changes and enhance it bioavailability.

Fortification of soy drinks with calcium is difficult because soy protein is very sensitive to calcium ions. In addition calcium may reduce the iron absorption in function of source and amount of this mineral present in the product. In this study, the source of fortification was calcium citrate in reason of its bioavailability, compatibility with iron sources and sensory properties.[5, 13, 16]

pH Change During Fermentation Time

Appropriate acid concentration is one the important factors ensuring quality of fermented soymilk.[4] The initial pH of all soymilk samples, after inoculation, was 6.58 – 6.70. Non-fortified (control) and iron and calcium fortified soy yogurt, prepared in the present study, required 4.5 and 5.15 h to reduce pH to the 4.4 - 4.5 range, respectively, indicating that the starter culture was capable to produce enough acids in a suitable time period and that the fortification do not make the fermentation process unviable (Figure 1).

Acidity Measurement

Changes in pH and titratable acidity of products during the storage are presented in Table 2. A significant decrease in pH was noted in soy yogurt and iron and calcium fortified soy yogurt during the storage. The main decrease of pH values was observed on 7th and 14th days of the storage for control and fortified soy yogurt, respectively. After 14 days, the pH values were maintained constant until, at least, 21th day of the experiment. On 28th day, pH of fortified yogurt (4.19 ± 0.03) was statistically higher than that of control yogurt (4.10 ± 0.02). The initial titratable acidity of control soy yogurt (0.87 ± 0.01% La) was higher than of fortified product (0.85 ± 0.01% La). Titratable acidity increased on storage and on 28th day it reached 1.01 ± 0.01 and 1.00 ± 0.01% La for control and fortified yogurt, respectively. The acidity variations observed during the storage of the soy yogurts were considered normal, since the temperature reduction did not completely interrupt the lactic culture metabolism.

Viable Cell Counts

Viability of mixed starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbruechii* subsp. *bulgaricus* decreased as the storage time increased

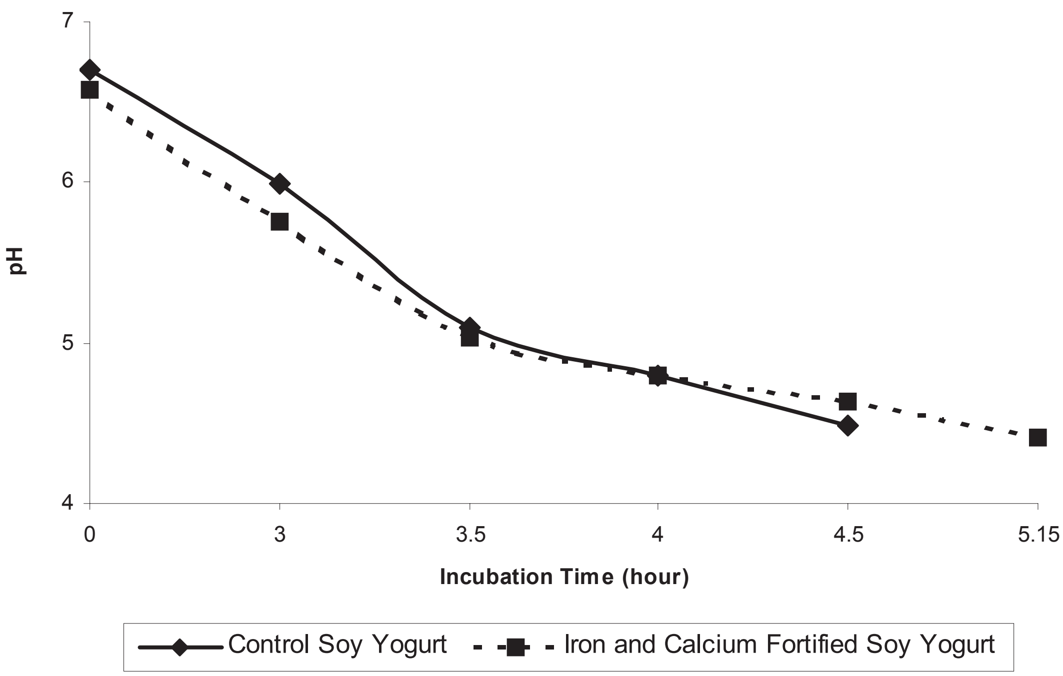

FIGURE 1 – pH changes during the fermentation time of the control soy yogurt and iron and calcium fortified soy yogurt.

Table 2 – pH and titratable acidity of control and iron and calcium fortified soy yogurt during the storage at 10°C.

Storage Time (days)	Control Soy Yogurt		Fortified Soy yogurt	
	pH	Titratable acidity (% lactic acid)	pH	Titratable acidity (% lactic acid)
1	4.47 ± 0.01^{a}	$0.87 \pm 0.01^{a*}$	4.45 ± 0.02^{a}	$0.85 \pm 0.01^{a*}$
7	4.35 ± 0.03^{b}	0.90 ± 0.01^{b}	4.39 ± 0.02^{a}	0.89 ± 0.01^{b}
14	4.21 ± 0.03^{c}	0.96 ± 0.01^{c}	4.25 ± 0.02^{b}	0.94 ± 0.01^{c}
21	4.17 ± 0.02^{cd}	0.98 ± 0.01^{c}	4.22 ± 0.03^{bc}	0.97 ± 0.01^{d}
28	4.10 ± 0.02^{e}*	1.01 ± 0.01^{d}	4.19 ± 0.03^{bcd}*	1.00 ± 0.01^{e}

Values are mean ± SD. Statistical comparison of times: Means in the same column with different superscript letter are significantly different ($p<0.05$). Statistical comparison of groups: Means in the same line with * are significantly different ($p<0.05$).

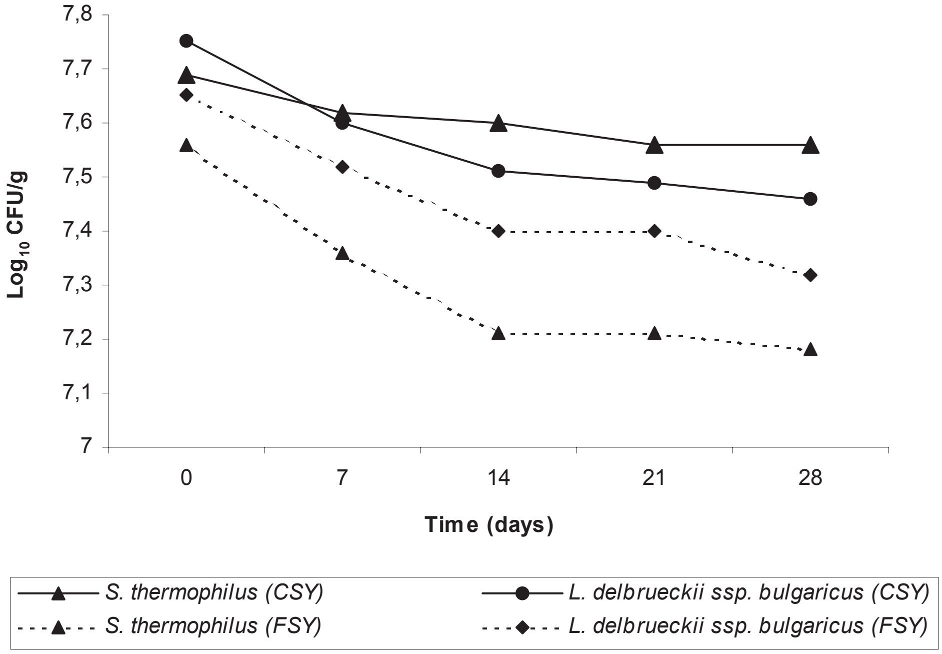

FIGURE 2 – Viability of *Streptococcus thermophilus* and *Lactobacillus delbruechii* subsp. *bulgaricus* of control soy yogurt (CSY) and iron and calcium fortified soy yogurt (FSY) during storage at 10°C.

and it was higher in control soy yogurt during all the experimental period (Figure 2).

Greater decrease in viable cell counts, mainly *S. thermophilus*, was found for fortified soy yogurt during the storage, indicating that iron and calcium addition may change the viability of this culture. The viable population of *Streptococcus thermophilus* was reduced by 0.13 and 0.38 log CFU/g in control and fortified soy yogurt, respectively, after 28 days of storage at 10°C. A population reduction of *Lactobacillus delbruechii* subsp. *bulgaricus* in the control sample was 0.29 log CFC/g and in the fortified sample was 0.33 log CFC/g, during the experimental period. Yousef & Rusli[22] demonstrated that *Streptococcus thermophilus* was inhibited more noticeably than *Lactobacillus delbruechii* subsp. *bulgaricus* during production of calcium-fortified yogurt. Others authors did not observed differences in the count of *S. thermophilus* and *L. delbruechii* subsp. *bulgaricus* between yogurts fortified with iron.[12] However, in this study the soy yogurt was fortified with both, iron and calcium, and the mineral sources were different.

Viscosity and Consistency

Storage had not any significant effect on viscosity and consistency of the soy yogurt fortified or not with iron and calcium. The average consistency did not vary between the samples. However, viscosity of iron and calcium fortified soy yogurt was lower than control soy yogurt ($p<0.05$), during all the storage period (Table 3). Early study showed reduction in soy yogurt viscosity with the addition of the same iron source alone.[15] Another work found increase in viscosity of soy yogurt fortified with calcium citrate.[16] The results showed that, despite the differences on rheological properties, the control and iron and calcium fortified soy yogurt presented a stable gel with characteristic of natural-set yogurt.

Iron and Calcium Concentrations

Table 4 shows iron and calcium content of the control and fortified soy yogurts. The fortified product exhibited an increase of 233.9% and 72.5% on iron and calcium concentration, respectively.

The results indicate that 200ml of fortified soy yogurt would provide more than 10% of the recommended dietary allowance of iron and calcium for infants, children

Table 3 – Viscosity and consistency of control and iron and calcium fortified soy yogurt during the storage at 10°C.

Storage time (days)	Viscosity (cP)		Consistency (cm/ 10s)	
	Control	Forfified	Control	Fortifed
1	2466.7 ± 28.9^{aA}	2100.0 ± 0.0^{aB}	4.8 ± 0.3^{aA}	5.3 ± 0.3^{aA}
7	2466.7 ± 57.7^{aA}	2100.0 ± 10.0^{aB}	4.8 ± 0.3^{aA}	5.3 ± 0.3^{aA}
14	2500.0 ± 5.0^{aA}	2133.3 ± 28.9^{aB}	4.7 ± 0.3^{aA}	5.2 ± 0.3^{aA}
21	2500.0 ± 5.0^{aA}	2133.3 ± 57.7^{aB}	4.7 ± 0.3^{aA}	5.2 ± 0.3^{aA}
28	2533.0 ± 2.5^{aA}	2133.3 ± 28.9^{aB}	4.7 ± 0.3^{aA}	5.0 ± 0.0^{aA}

Values are mean ± SD. Statistical comparison of times: Means in the same column with different superscript minuscule letter are significantly different ($p<0.05$). Statistical comparison of groups: Means in the same line with different superscript majuscule letter are significantly different ($p<0.05$).

Table 4 – Iron and calcium concentrations of the control soy yogurt and iron and calcium fortified soy yogurt, after 28 days of storage at 10°C.

Parameters	Control Soy yogurt	Fortified Soy Yogurt
Amount of iron added (mg/100ml)	0	1.20
Measured iron (mg/100ml)	0.53	1.77
Percent of iron increase compared to control	-	233.9
Amount of calcium added (mg/100ml)	0	60.0
Measured calcium (mg/100ml)	84.3	145.4
Percent of calcium increase compared to control	-	72.5

Table 5 – Sensory evaluation of control (CSY) and iron and calcium fortified soy yogurt (FSY) during the storage at 10°C.

Storage Time (days)	Sample	Aroma	Color	Flavor	Overall acceptability
1	CSY	6.37 ± 1.49^{a}	6.58 ± 1.67^{a}	6.36 ± 1.88^{a}	6.54 ± 1.66^{a}
1	FSY	5.94 ± 1.26^{a}	6.43 ± 1.53^{a}	6.14 ± 2.01^{a}	6.23 ± 1.89^{a}
7	CSY	6.49 ± 1.50^{a}	6.49 ± 1.56^{a}	6.13 ± 1.98^{a}	6.19 ± 1.78^{a}
7	FSY	6.47 ± 1.32^{a}	6.62 ± 1.62^{a}	5.97 ± 2.22^{a}	5.95 ± 1.99^{a}
14	CSY	6.49 ± 1.45^{a}	6.49 ± 1.70^{a}	6.13 ± 1.97^{a}	6.19 ± 1.85^{a}
14	FSY	6.47 ± 1.35^{a}	6.62 ± 1.55^{a}	5.97 ± 2.17^{a}	5.95 ± 1.95^{a}
21	CSY	5.84 ± 1.67^{a}	6.87 ± 1.41^{a}	5.93 ± 2.04^{a}	6.12 ± 1.70^{a}
21	FSY	6.18 ± 1.43^{a}	6.55 ± 1.53^{a}	6.15 ± 1.87^{a}	6.25 ± 1.81^{a}
28	CSY	6.29 ± 1.64^{a}	6.55 ± 1.48^{a}	6.02 ± 2.01^{a}	6.33 ± 1.72^{a}
28	FSY	6.05 ± 1.34^{a}	6.43 ± 1.71^{a}	5.84 ± 2.23^{a}	6.09 ± 1.96^{a}

Values are means ± SD. Means in the same column with different superscript minuscule letter are significantly different ($p<0.05$).

and adults.[2] The final calcium content of the fortified soy yogurt was comparable to or higher than those verified in bovine milk.[18]

Sensory Evaluation of Soy Yogurts

The results of sensory evaluation are summarized in Table 5. Overall, the sensory scores of the control and fortified soy yogurts were higher than 6.0 on a nine-point scale. The addition of iron and calcium and the storage period did not significantly affect the sensory scores for all evaluated attributes. This results are in agreement with earlier, which showed that the addition of iron (microencapsulated ferrous sulfate) or calcium (calcium citrate) do not interfere in the sensory properties of soy yogurt.[15,16]

CONCLUSION

Addition of iron (1.2mg Fe/100ml – microencapsulated ferrous sulfate) and calcium (60.0mg Ca/100ml – calcium citrate) to soy yogurt did not have notable effect on sensory attributes, consistency and titratable acidity. The storage during 28 days at 10°C had no significant effect on the rheological and sensory properties of iron and calcium fortified and control soy yogurt, despite the observed changes on the pH, titratable acidity and viable count cells. In conclusion, the present work enabled the development of a iron and calcium fortified soy yogurt, stable during 28 days at 10°C, that could be used in the prevention and control of mineral deficiencies in general population.

CAVALLINI, D. C. U.; ROSSI, E. A. Iogurte de soja fortificado com ferro e cálcio: estabilidade durante a estocagem. **Alim.Nutr**., Araraquara, v.20, n.1, p. 7-13, jan./mar. 2009.

■RESUMO: O objetivo desse trabalho foi preparar e avaliar a estabilidade de um "iogurte" de soja fortificado com $FeSO_4.7H_2O$ microencapsulado (1,2mg de ferro /100 ml) e citrato de cálcio (60,0 mg de cálcio/ 100ml), durante o período de estocagem a 10°C. O "iogurte" de soja sem adição de ferro e cálcio foi utilizado como controle. As amostras foram avaliadas uma vez por semana, durante 28 dias, em relação a: pH, acidez titulável, propriedades reológicas (viscosidade e consistência), características sensoriais (teste de aceitação) e contagem de células viáveis (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*). Os resultados das determinações de pH, acidez titulável, viscosidade, consistência e teste de aceitação foram submetidos à análise de variância e teste de médias de Tukey. Durante a estocagem observou-se redução do pH e aumento da acidez titulável no "iogurte" de soja fortificado ou não com ferro e cálcio, em decorrência da manutenção da viabilidade das culturas láticas no produto (10^7CFU/g). O "iogurte" de soja fortificado apresentou as menores médias de viscosidade, sendo que esse parâmetro não diferiu significativamente (p≤0,05) durante o tempo de estocagem. Os resultados do teste de aceitação mostraram que as amostras controle e fortificada não exibiram diferenças significativas em relação a todos os atributos avaliados, durante o período do estudo. Em conclusão, o presente trabalho possibilitou o desenvolvimento de um "iogurte" de soja fortificado com ferro e cálcio, estável durante 28 dias a 10°C, que poderia ser utilizado na prevenção e controle de deficiências minerais na população em geral.

■PALAVRAS-CHAVE: Iogurte de soja; ferro; cálcio; microencapsulação; estocagem; estabilidade.

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