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Use of soy as a source of protein in low-fat yogurt production: microbiological, functional and rheological properties

Uso da soja como fonte de proteína na produção de iogurte desnatado: propriedades microbiológicas, funcionais e reológicas

Uso de la soja como fuente de proteínas en la producción de yogur desnatado: propiedades microbiológicas, funcionales y reológicas

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Abstract

The objective of this study was to evaluate the effect of different protein sources incorporated into milk to produce low-fat yogurt (LF-yogurt). Five treatments were used: YC – yogurt control (without protein supplementation), YM – yogurt with milk powder, YWP – yogurt with whey protein, YSP – yogurt with soy protein, and YSF – yogurt with soy flour. Microbiological counts of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, pH, lactic acid and changes in rheological and structural properties (firmness, color, microstructure and syneresis) were followed throughout 28 days of storage. Antioxidant capacity (DPPH and ABTS assay) and total phenolic compounds were also analyzed. Addition of different ingredients influenced *S. thermophilus* and *L. bulgaricus* CFU counts. LF-yogurts supplemented with milk powder, soy flour and soy protein were firmest, and yogurts supplemented with milk powder and soy flour had the least syneresis. LF-yogurts with soy flour were darker, redder and yellower, contained more polyphenols and exhibited higher antioxidant activity. Thus, supplementation of LF-yogurt with soy is interesting since it confers functional activity to the yogurt and also modify its technological properties.

Keywords: Scanning electron microscopy; Soy protein isolate; Milk; Whey protein; Texture.

Resumo

O objetivo desde estudo foi avaliar o efeito de diferentes fontes de proteína incorporadas ao leite para produzir iogurte desnatado. Foram utilizados cinco tratamentos: YC - iogurte controle (sem suplementação proteica), YM - iogurte com leite em pó, YWP - iogurte com proteína de soro de leite, YSP - iogurte com proteína de soja e YSF - iogurte com farinha de soja. Contagens microbiológicas de *Streptococcus thermophilus* e *Lactobacillus bulgaricus*, pH, acidez e mudanças nas propriedades reológicas e estruturais (firmeza, cor, microestrutura e sinérese) foram acompanhadas ao longo de 28 dias de armazenamento. A capacidade antioxidante (ensaio DPPH e ABTS) e os compostos fenólicos totais também foram analisados. A adição de diferentes ingredientes influenciou a contagem de UFC de *S. thermophilus* e *L. bulgaricus*. Os iogurtes suplementados com leite em pó, farinha de soja e proteína de soja foram os mais firmes, e os iogurtes suplementados com leite em pó e farinha

de soja tiveram menos sinérese. Os iogurtes com farinha de soja ficaram mais escuros, mais vermelhos e mais amarelos, continham mais polifenóis e exibiam maior atividade antioxidante. Assim, a suplementação do iogurte com soja é interessante, pois confere atividade funcional ao iogurte e também modifica suas propriedades tecnológicas.

Palavras-chave: Microscopia eletrônica de varredura; Proteína isolada de soja; Leite; Proteína do soro de leite; Textura.

Resumen

El objetivo de este estudio fue evaluar el efecto de diferentes fuentes de proteínas incorporadas a la leche para producir yogur bajo en grasa. Se utilizaron cinco tratamientos: YC - yogur control (sin suplementación proteica), YM - yogur con leche en polvo, YWP - yogur con proteína de suero, YSP - yogur con proteína de soja y YSF - yogur con harina de soja. Los recuentos microbiológicos de *Streptococcus thermophilus* y *Lactobacillus bulgaricus*, pH, acidez y cambios en las propiedades reológicas y estructurales (firmeza, color, microestructura y sinéresis) fueron monitoreados durante 28 días de almacenamiento. También se analizó la capacidad antioxidante (ensayo DPPH y ABTS) y compuestos fenólicos totales. La adición de diferentes ingredientes influyó en el recuento de FQ de *S. thermophilus* y *L. bulgaricus*. Los yogures suplementados con leche en polvo, harina de soja y proteína de soja fueron los más firmes, y los yogures suplementados con leche en polvo y harina de soja tuvieron menos sinéresis. Los yogures con harina de soja se volvieron más oscuros, más rojos y más amarillos, contenían más polifenoles y exhibieron una mayor actividad antioxidante. Así, resulta interesante la suplementación del yogur con soja, ya que confiere actividad funcional al yogur y también modifica sus propiedades tecnológicas.

Palabras clave: Microscopía electrónica de barrido; Proteína de soja; Leche; Proteína de suero; Textura.

1. Introduction

Yogurt products are a popular food across the world, mainly due to being associated with a healthy diet, practicality and varied forms of consumption (Hekmat & McMahon, 1997).

Aiming to reduce disease risks, the search for healthy eating options has increased. Thus, the demand for healthy products has increased, especially low-fat ones which include dairy products. However, fat plays an important role in food, contributing to the texture, appearance and flavor.

In addition to the presence of live microorganisms and the nutritional value, rheological and texture properties are important for consumer acceptability, and dairy products such as low-fat yogurt generally have a brittle texture, with more syneresis (Lee & Lucey, 2010), during transport and storage.

In order to prevent these defects and ensure appropriate texture, dairy manufactures usually modify (increase) the total solids content through the use of stabilizers or milk ingredients (Matumoto-Pintro, Rabiey, Robitaille, & Britten, 2011), a standard practice in yogurt manufacture being the addition of skim milk powder. Thus, proteins are interesting ingredients for foods due to their possible impact on rheological properties as well as their nutritional value (Jose, Pouvreau, & Martin, 2016).

Soy is considered a functional food, rich in protein, fiber, minerals and vitamins, with biologically active phytochemicals which offer health benefits such as antioxidant activity, reducing the risk of heart disease and lowering cholesterol (Goodin et al., 2007; Malenčić, Popović, & Miladinović, 2007; Tripathi & Misra, 2005). However, many people do not consume soy in its in natura form, it being necessary to create alternatives to introduce it into consumers' diet without changing their eating habits. In addition to the nutritional characteristics of soybean, its gel formation capacity is an important functional property, and the proportion of proteins influences the gel structure (Malaki Nik, Alexander, Poysa, Woodrow, & Corredig, 2011).

Regarding different proteins, a protein derived from milk, whey protein, is one that has different properties, acting as a foaming agent, presenting greater solubility and emulsifying properties (Ortega, Romero, Muro, & Riera, 2015) and which also can be used in food as a source of protein.

Therefore, the aim of this study was to verify the effects of soy (flour and protein), compared with supplementation with other protein sources (milk powder and whey protein) commonly used by the food industry, on the structural properties (syneresis, texture, microstructure) and functionality (survival of microorganisms and antioxidant activity) of low-fat yogurt during storage.

2. Material and Methods

This study brings the effects of different sources of protein application in low-fat yogurt and was done based on previous studies carried out by the same research group as (Matumoto-Pintro et al., 2011; Vital et al., 2015), and developed according to scientific norms

(Pereira et al., 2018).

2.1. Material

Folin-Ciocalteu reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium persulfate, 2,2- Azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS) and sodium carbonate were from Sigma Aldrich (USA). MRS and M17 culture media and peptone water were from Himedia (USA). Milk powder (CONFEPAR, Londrina - Paraná, Brazil), soy flour – BRS 257 (EMPRAPA, Paraná, Brazil), soy protein - Isoflavon (Terra Verde, Maringá – Paraná, Brazil), whey protein (Terra Verde, Maringá – Paraná, Brazil). For the yogurt production, was used a culture (YOG-03, BV- Bela Vista, Brazil), with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*.

2.2. Soy flour preparation

Soybeans were toasted at 120 °C for 20 min, then crushed into a fine powder, sieved (50 mesh) and stored in opaque flasks until use.

2.3. Total phenolic compounds and antioxidant activity

2.3.1. Bioactive compounds extraction

Ingredients (milk, whey protein, soy protein, soy flour) and yogurts (1:10 w/v) were mixed with acetone (50%), homogenized for 15 min and centrifuged for 10 min (3000 rpm). For analysis, the supernatant was used.

2.3.2. Total phenolic content

The total phenolic content (TPC) of ingredients and yogurt was measured, with modifications: 125 µL of supernatant, 125 µL of Folin–Ciocalteu reagent (1:1 deionized water) and 2250 µL of sodium carbonate (28 g/L) were mixed following that order. After that, the samples were kept in the dark for 30 min at 25 °C. The absorbance was read at 725 nm (Evolution™ 300 spectrophotometer, Thermo Scientific). A standard curve with gallic acid was used (0 to 300 mg/L), and the results are presented as gallic acid equivalent (GAE) per

gram of sample (Singleton & Rossi, 1965).

2.3.3. ABTS assay

The ABTS assay was done with modifications (Re et al., 1999): 7 mM ABTS and 140 mM potassium persulfate were mixed (5 mL and 88 µL, respectively) to form the ABTS·+ solution. This solution was placed in the dark for 16 h at 25 °C. After that, the ABTS·+ radical was diluted in ethanol (absorbance of 0.70). The capacity of samples to scavenge the radical (%) was read at 734 nm. Forty microliters of each sample and 1960 µL of ABTS·+ solution were mixed and kept at rest for 6 min when the reading was taken. The absorbance was read at 515 nm. The scavenging activity was calculated as:

ABTS radical scavenging (%)=(1-(A_(sample t)/A_(sample t=0))*100

A sample t=0: sample absorbance at time zero;

A sample t: sample absorbance after 6 min.

2.3.4. DPPH assay

The DPPH assay was done with modification: 150 µL of sample and 2850 µL of DPPH solution (60 µM in methanol) were mixed and kept at rest for 30 min. The absorbance was read at 515 nm (Li, Hydamaka, Lowry, & Beta, 2009). 150 µL of samples and 2850 µL of DPPH solution (60 µM in methanol) was mixed and remained in rest during 30 min. The absorbance was read at 515 nm. The scavenging activity was calculated as:

DPPH activity (%)=(1-(A_(sample t)/A_(sample t=0))*100

A sample t=0: sample absorbance at time zero;

A sample t: sample absorbance after 30 min.

2.4. Yogurt production with protein supplementation

A starter culture was made for yogurt production. For its preparation, sterilized skim milk powder was used (12%, w/v); it was inoculated with the culture (0.1%) and incubated until reaching pH 5.3 at 41 °C. Before yogurt production, heat treatment was applied to milk (90 °C for 3 min). Five treatments were used: YC – yogurt control (without protein supplementation), YM – yogurt with milk powder, YWP – yogurt with whey protein, YSP – yogurt with soy protein, and YSF – yogurt with soy flour. After the heat treatment, the milk

was cooled (41 °C), then the starter culture was added (3% v/v) and the mixture was put into its respective tubes for analyses. After that, the tubes were incubated also at 41 °C until the pH reached 4.6. The majority fermentation process was ended by cooling (4 °C) and samples were stored also at 4 °C until analysis. Different periods of storage were evaluated (1, 7, 14, 21 and 28 days).

Reconstituted milk and water were mixed, and the final concentration of protein was 4.2%. The ingredients were added in order to achieve a final protein concentration of 6%, and control low-fat yogurt (LF-yogurt) remained with 4.2% protein. All ingredients were added to milk before the heat treatment, except the whey protein, which was added when the temperature reached 41 °C.

2.5. pH and acidity

The pH during fermentation and storage was verified with a digital pH meter (mPA-210, Tecnopon). To determine the titratable acidity, LF-yogurt (10 g) was mixed with distilled water (10 mL) and titrated with sodium hydroxide (0.1 M) until reaching a pH of 8.3. Acidity was calculated as follows:

Titratable acidity (g lactic acid/100g LF-yogurt) =Vxfx0.9/m

where V is the volume (mL) of sodium hydroxide (0.1 M) used in the titration, m is the mass of yogurt (g), 0.9 is the lactic acid conversion factor, and f is the molarity of sodium hydroxide. (ISO, 1997).

2.6. Microbiological counts

M17 and MRS culture media were used to quantify *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, respectively. The plates were incubated at 37 °C under anaerobic conditions for 48 h for *S. thermophilus* and at 37 °C for 72 h for *L. bulgaricus* (IDF, 1997). The results are expressed as colony-forming units (CFU) per gram of LF-yogurt.

2.7. Analysis of LF-fat yogurt texture

The firmness of yogurt was analyzed using a texturometer (Brookfield CT III texture analyzer) with a cylindrical probe (TA4/1000). The distance target was 5 mm, the speed penetration was 1 mm/s, and the trigger force was 15 g (Vital et al., 2015).

2.8. Evaluation of susceptibility to syneresis

To evaluate syneresis, yogurts (25 g) were prepared in Falcon tubes. For the analysis, yogurt was centrifuged at 2200 rpm for 10 min at 4 °C (Robitaille et al., 2009). Syneresis was calculated in relation to the percentage of whey released due to the centrifugation.

2.9. Color evaluation

Color was evaluated by CIELAB scale using a colorimeter (Minolta Chroma Meter CR-400), and D65 illuminant was used as reference. L* (white, 100; black, 0), a* (+, red; −, green) and b* (+, yellow; −, blue) parameters were measured.

2.10. Microstructure

First the LF-yogurts were lyophilized, then fixed on aluminum stubs and covered with gold (SCD 050 Sputter Coater, Baltec). Photos were taken at 15 kV using a Shimadzu Superscan SS-550 scanning electron microscope (SEM) (Matumoto-Pintro et al., 2011).

2.11. Statistical analysis

In the experiment, each analysis was performed in triplicate and the experiment was repeated four times. The data were analyzed by analysis of variance – general linear model (GLM) – using SPSS software (v.23.0), and means and standard deviation are presented. Types of LF-yogurt and storage time were considered fixed effects in a factorial design. When significant differences were found, a Tukey test was performed ($p = 0.05$).

3. Results

3.1. Polyphenol content and antioxidant activity of the protein ingredients used in LF-yogurt production

The TPC of SF, SP, WP and milk was 2.55, 1.19, 0.42 and 0.41 mg GAE/g, respectively (Table 1). In relation to the DPPH radical scavenging assay, the values were 80.68%, 40.20%, 10.15 and 10.79% for SF, SP, WP and milk, respectively. The ABTS assay

also demonstrated the same behavior as for the other methodologies applied, and SF showed the highest radical scavenging value (42.41%).

Table 1 – Total phenolic content and antioxidant activity (DPPH and ABTS radical scavenging capacity) of ingredients and yogurt at day one.

Ingredients	Total phenolic content (mg GAE[5]/g)	DPPH (%)	ABTS (%)
M[1]	0.41 ± 0.01^{C}	10.79 ± 0.98^{C}	6.10 ± 0.38^{C}
WP[2]	0.42 ± 0.01^{C}	10.15 ± 1.39^{C}	5.96 ± 0.19^{C}
SP[3]	1.19 ± 0.23^{B}	40.20 ± 7.30^{B}	21.21 ± 4.50^{B}
SF[4]	2.55 ± 0.61^{A}	80.68 ± 0.41^{A}	42.41 ± 0.38^{A}
Yogurt	Total phenolic content (mg GAE[5]/g)	DPPH (%)	ABTS (%)
YC[6]	0.65 ± 0.02^{B}	25.58 ± 0.25^{C}	11.86 ± 0.29^{B}
YM[7]	0.61 ± 0.02^{B}	27.26 ± 0.49^{BC}	13.35 ± 0.48^{B}
YWP[8]	0.54 ± 0.01^{B}	25.41 ± 0.33^{C}	11.59 ± 0.10^{B}
YSP[9]	0.72 ± 0.08^{B}	29.76 ± 1.23^{B}	13.41 ± 0.19^{B}
YSF[10]	1.06 ± 0.06^{A}	32.89 ± 0.41^{A}	19.65 ± 1.15^{A}

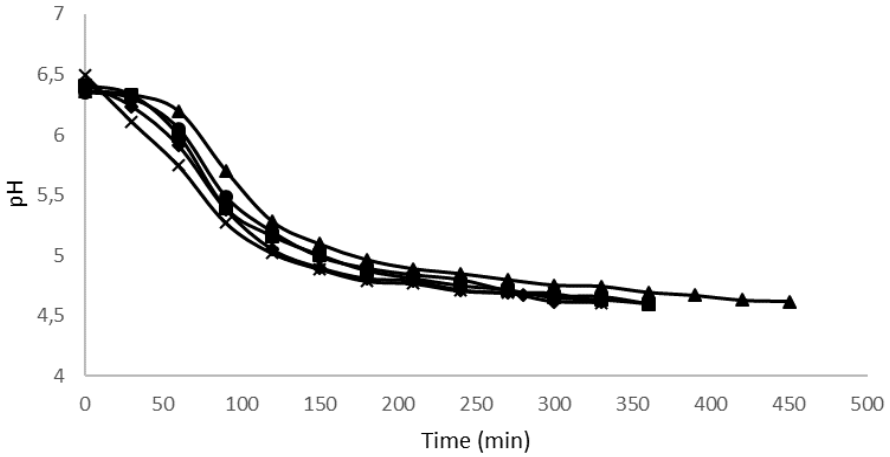
Means with different uppercase letters in the same column are significantly different ($p<0.05$). Results are expressed as mean ± standard deviation. [1]M – milk; [2] WP – whey protein; [3] SP – soy protein; [4]SF – soy flour. [5]GAE – gallic acid equivalent. [6]YC – Control yogurt, without supplementation; [7]YM – yogurt with milk supplementation; [8]YWP – yogurt whey protein supplementation; [9]YSP - yogurt with soy protein supplementation; [10]YSF – yogurt with soy flour supplementation. Source: Authors.

In Table 1 it is important to note the differences in the content of polyphenols and antioxidant activity of the ingredients used as sources of proteins and the yogurts made with them.

3.2. LF-yogurt fermentation

During fermentation, lactic acid is produced by *S. thermophilus* and *L. bulgaricus* and thus the pH decreases. LF-yogurt supplemented with milk powder presented the lowest counts for both microorganisms and the longest time to reach the ideal pH (Figure 1).

Figure 1. Decreasing in pH during fermentation of yogurt supplemented with different protein sources. YC – Control yogurt, without supplementation; YM – yogurt with milk supplementation; YWP - yogurt with whey protein supplementation; YSP - yogurt with soy protein supplementation; YSF - yogurt with soy flour supplementation.



Source: Authors.

3.3. *S. thermophilus* and *L. bulgaricus* cell counts, pH and acidity

Counts of both microorganisms, *S. thermophilus* and *L. bulgaricus*, decreased with storage for all treatments; however, on addition of ingredients, significant differences in the microorganism populations were observed (Table 2). In general, LF-yogurt supplemented with milk showed the lowest count during storage, and YSF and YSP the highest.

Table 2 – Effect of yogurt supplementation with different protein sources on pH, acidity (g lactic acid/100g) and microorganisms count (10^{-8} CFU) during 28 days of storage.

		Storage time				
	Yogurt	1	7	14	21	28
pH						
	YC	4.60 ± 0.02^{Aa}	4.46 ± 0.08^{Bab}	4.37 ± 0.02^{Bb}	4.38 ± 0.05^{Bb}	4.20 ± 0.10^{Ac}
	YM	4.60 ± 0.03^{Aa}	4.57 ± 0.02^{Aab}	4.52 ± 0.04^{Abc}	4.49 ± 0.04^{Ac}	4.21 ± 0.02^{Ad}
	YWP	4.60 ± 0.04^{Aa}	4.49 ± 0.04^{Abab}	4.38 ± 0.11^{Bb}	4.40 ± 0.02^{ABb}	4.20 ± 0.04^{Ac}
	YSP	4.55 ± 0.05^{Aa}	4.44 ± 0.04^{Bab}	4.41 ± 0.01^{Abb}	4.40 ± 0.03^{ABb}	4.21 ± 0.10^{Ac}
	YSF	4.59 ± 0.05^{Aa}	4.50 ± 0.02^{ABab}	4.42 ± 0.02^{ABb}	4.43 ± 0.08^{ABb}	4.24 ± 0.12^{Ac}
Acidity						
	YC	1.03 ± 0.03^{Ad}	1.14 ± 0.01^{Ac}	1.18 ± 0.02^{Abc}	1.22 ± 0.01^{Ab}	1.31 ± 0.01^{Aa}
	YM	1.03 ± 0.03^{Ad}	1.08 ± 0.01^{Bc}	1.11 ± 0.01^{Bc}	1.18 ± 0.01^{Bb}	1.33 ± 0.03^{Aa}
	YWP	1.02 ± 0.02^{Ad}	1.14 ± 0.01^{Ac}	1.18 ± 0.02^{Ab}	1.22 ± 0.02^{ABb}	1.33 ± 0.02^{Aa}
	YSP	1.07 ± 0.03^{Ad}	1.16 ± 0.02^{Ac}	1.16 ± 0.01^{Abc}	1.23 ± 0.01^{Ab}	1.36 ± 0.04^{Aa}
	YSF	1.05 ± 0.01^{Ad}	1.13 ± 0.01^{Ac}	1.15 ± 0.01^{Abc}	1.18 ± 0.02^{Bb}	1.32 ± 0.02^{Aa}
L. bulgaricus						
	YC	18.20 ± 0.26^{BCa}	14.00 ± 3.18^{BCb}	13.43 ± 0.30^{ABbc}	11.63 ± 1.69^{ABbc}	10.43 ± 0.70^{CDc}

	YM	15.50 ± 0.70^{Da}	10.53 ± 2.50^{Cb}	10.96 ± 0.75^{Bb}	10.06 ± 0.73^{Bb}	9.56 ± 0.65^{Db}
	YWP	17.16 ± 1.16^{CDa}	13.96 ± 1.12^{BCb}	13.40 ± 0.45^{ABbc}	12.00 ± 2.19^{ABbc}	11.33 ± 1.15^{BCc}
	YSP	20.83 ± 1.57^{Aa}	16.96 ± 0.65^{ABb}	14.43 ± 2.72^{Abc}	13.13 ± 1.32^{Ac}	12.43 ± 0.86^{ABc}
	YSF	19.73 ± 1.33^{ABa}	18.80 ± 1.32.^{Aa}	15.83 ± 0.45^{Ab}	14.16 ± 0.35^{Abc}	13.46 ± 0.65^{Ac}
S. thermophilus						
	YC	19.86 ± 1.05^{ABa}	19.03 ± 1.10^{ABa}	15.90 ± 1.73^{Bb}	13.63 ± 0.51^{Bbc}	12.06 ± 2.30^{Bc}
	YM	15.43 ± 3.31^{Ba}	14.50 ± 2.43^{Ca}	12.73 ± 1.01^{Ca}	8.90 ± 0.40^{Cb}	7.63 ± 0.30^{Cb}
	YWP	20.70 ± 0.65^{Aa}	16.96 ± 1.36^{BCb}	16.53 ± 2.12^{Bb}	13.46 ± 1.79^{Bc}	11.43 ± 0.75^{Bc}
	YSP	23.13 ± 2.55^{Aa}	21.36 ± 1.01^{Aa}	20.83 ± 0.66^{Aa}	17.20 ± 2.26^{Ab}	16.30 ± 0.52^{Ab}
	YSF	23.73 ± 3.40^{Aa}	21.10 ± 0.52^{Aab}	20.60 ± 0.20^{Aab}	19.50 ± 1.03^{Ab}	17.60 ± 2.66^{Ab}

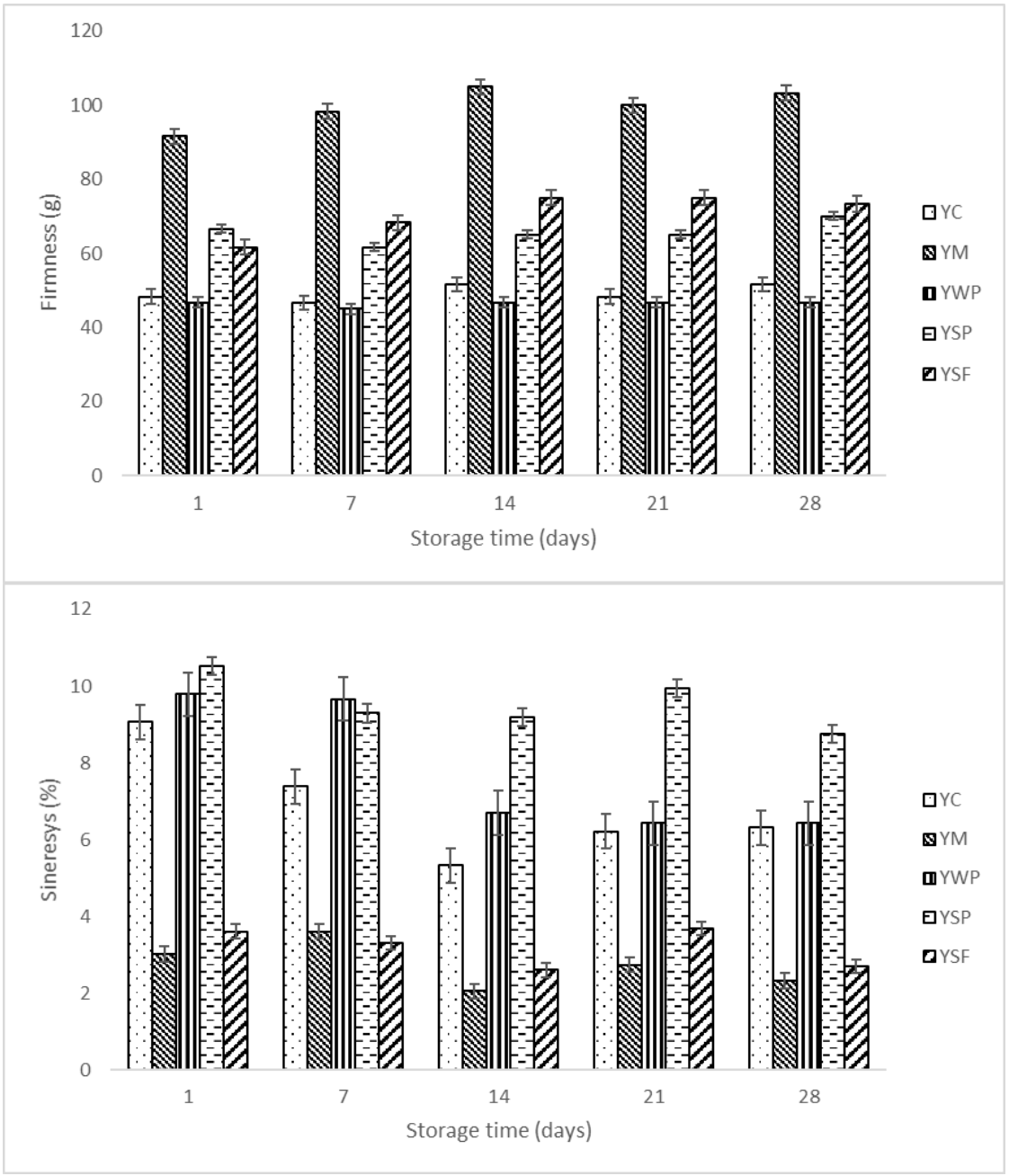
Means with different lowercase letters in the same line are significantly different ($p<0.05$). Means with different uppercase letters in the same column are significantly different ($p<0.05$). Results are expressed as mean±standard deviation. YC – Control yogurt, without supplementation; YM – yogurt with milk supplementation; YWP – yogurt whey protein supplementation; YSP - yogurt with soy protein supplementation; YSF – yogurt with soy flour supplementation. Source: Authors.

Table 2 shows the behavior of the microorganisms added in the production of the yogurt during the storage period, as well as the pH and acidity of the yogurt over the days.

3.4. Changes in texture, syneresis and structural properties

During storage, LF-yogurts YC and YWP were the least firm. LF-yogurt YM was firmest, followed by YSF after day 7. YSP and YSF showed similar values during storage and regarding the 21 days evaluated, no differences was observed in LF-yogurt firmness ($p > 0.05$). In the present study, addition of milk powder increased LF-yogurt firmness and reduced syneresis (Fig. 2). On day 1, control yogurt, with whey protein and soy protein, presented more syneresis ($p < 0.05$) (Figure 2). After the 14th day, YC and YWP showed less syneresis than 1 and 7 days of storage and stabilized. LF-yogurts YM and YSF showed the least syneresis. Only LF-yogurts YC and YWP showed differences ($p < 0.05$) in syneresis during storage.

Figure 2 - Effect of different protein sources addition on the firmness and syneresis of low fat yogurt during shelf life. YC – Control yogurt, without supplementation; YM – yogurt with milk supplementation; YWP - yogurt with whey protein supplementation; YSP - yogurt with soy protein supplementation; YSF - yogurt with soy flour supplementation.



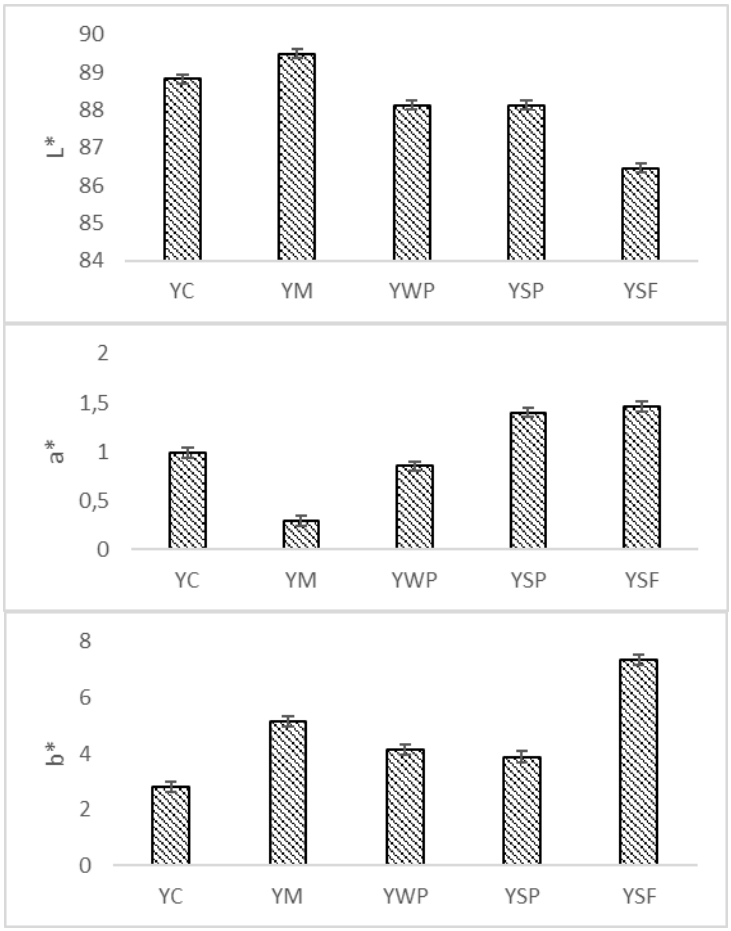
Source: Authors.

In Figure 2 the behavior of the release of yogurt serum (syneresis) and its texture are presented.

3.5. Color measurement

In relation to L* values, LF-yogurt supplemented with soy flour had the lowest L* value, and yogurt with milk powder the highest. In relation to redness (a* values), YSP and YSF yogurts presented the highest values. Regarding b* values (yellowness), YSF presented the highest value (Figure 3).

Figure 3. Effect of milk, whey protein, soy protein and soy flour supplementation on yogurt color: L* (Lightness), a* (redness) and b* (yellowness). YC – Control yogurt, without supplementation; YM – yogurt with milk supplementation; YWP - yogurt with whey protein supplementation; YSP - yogurt with soy protein supplementation; YSF – yogurt with soy flour supplementation.



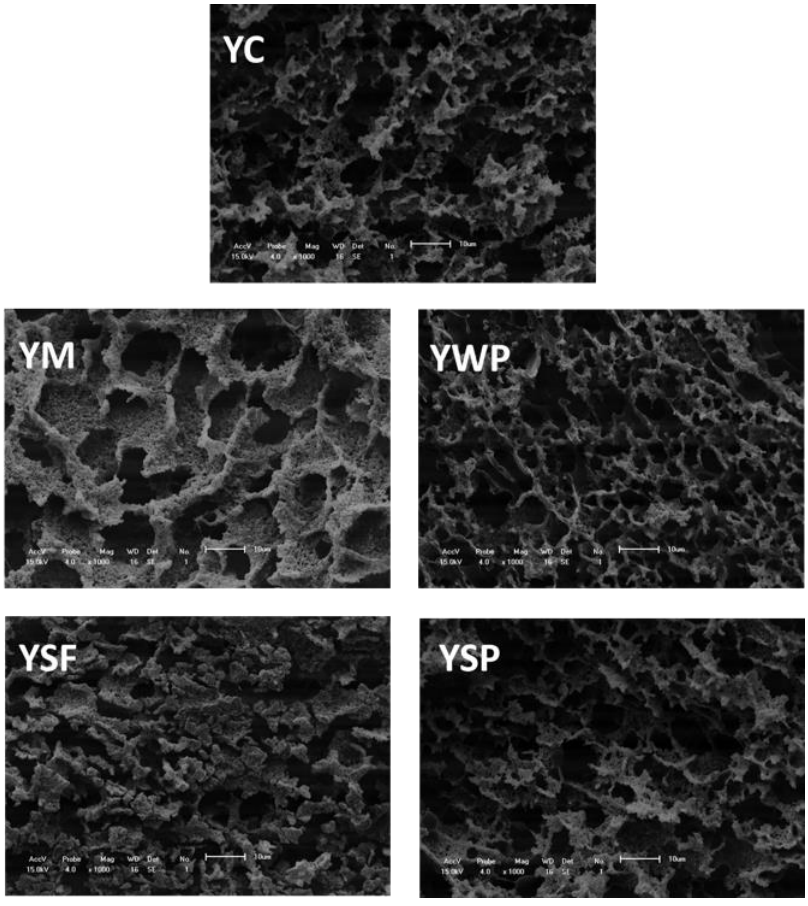
Source: Authors.

Figure 3 shows the difference in color caused by the addition of different sources of protein.

3.6. Microstructure of yogurt

The microstructures of LF-yogurts are presented in Figure 4. Particular networks were observed for yogurts. Control yogurt, YWP and YSP showed a branched network, with void spaces. A more compact structure was observed in YM and YSF. Although the YM structure still presented void spaces, most of the network had become denser, with smaller open spaces. These void spaces observed in YC, YWP, YM and YSP almost disappeared when YSF was added.

Figure 4. Scanning electron microscopy (SEM) images of yogurt with different protein sources. YC – Control yogurt, without supplementation; YM – yogurt with milk supplementation; YWP - yogurt with whey protein supplementation; YSP - yogurt with soy protein supplementation; YSF - yogurt with soy flour supplementation.



Source: Authors.

Through electron microscopy analysis it is possible to clearly observe the differences in the structure of each yogurt caused by the addition of different sources of protein, as shown

in Figure 4.

3.7. Phenolic content and antioxidant potential of LF-yogurt supplemented with different sources of protein

Yogurts supplemented with SF exhibited higher TPC and antioxidant activity than YC and other supplemented LF-yogurts ($p < 0.05$). The polyphenol content in LF-yogurts ranged from 0.54 to 1.06 mg GAE/g, and the antioxidant activity from 25.41% to 32.89% for DPPH radical scavenging and from 11.59% to 19.65% for ABTS radical scavenging.

4. Discussion

Malenčić, Cvejić, and Miladinović (2012) evaluated the polyphenol content of colored soybean seeds and found values between 2.68 and 6.22 mg GAE/g extracted with 70% aqueous acetone. Malenčić et al. (2007) evaluated the polyphenol content of colored soybean seeds and found values between 2.68 and 6.22 mg GAE/g extracted with 70% aqueous acetone. Some authors evaluated the antioxidant capacity of soybean and found values for DPPH radical scavenging ranging from 22.87% to 48.17% (1:50 w/v). These differences in TPC and antioxidant activity may be due to different factors such as production location, climate of the region, how the processing done, storage and the solvents used for extraction and methodologies; however, the soy products presented higher TPC and antioxidant activity than milk products, which was already expected, due to the bioactive compounds present in soy.

Regarding yogurt fermentation, the time necessary for yogurt to reach the ideal pH (4.6) were influenced by the different ingredients added (Fig. 1) and the growth of both microorganisms was a little greater in LF-yogurts with soy products (Table 2), which might be related to the shorter incubation time for these samples. This may be related to the prebiotic effect of soy components, as they contain galactooligosaccharides (GOS) (non-digestible carbohydrates) such as raffinose (Espinosa-Martos and Rupérez, 2006). The ability of fiber to accelerate milk acidification in yogurt production has also been shown. (McCann, Fabre, and Day, 2011). Some studies have already demonstrated the positive effect of prebiotics on growth of Lactobacillus strains (Donkor, Nilmini, Stolic, Vasiljevic, and Shah, 2007).

Regarding pH and acidity during storage, a significant difference in the pH decrease was observed (Table 2); however, at the end of storage, no differences in acetic acid production were found.

Related to the changes in texture, firmness is an important parameter which was significantly changed ($p < 0.05$) by supplementation of yogurt with protein (Fig. 2). Reduced firmness, low viscosity, liquid consistency and syneresis are the major defects of yogurt (Domagała, Wszołek, Tamime, and Kupiec-Teahan, 2013). To avoid these defects, industries add stabilizers or milk ingredients to increase the total solids content as alternatives. (Matumoto-Pintro et al., 2011). LF-yogurt can present different problems like syneresis (whey separation) (John A. Lucey, 2004; McCann et al., 2011), thus gel formation is very important.

Due to a weak gel, yogurt might lose the ability to maintain the serum phase in its network, which leads to syneresis. Both the rearrangement of proteins and syneresis may happen during storage (Everett and McLeod, 2005; J. A. Lucey, 2002); however, these effects can be solved/reduced by increasing total solids (Matumoto-Pintro et al., 2011), as observed in this study with the addition of milk powder (M).

In relation to YSF, polyphenols and proteins have a significant affinity, and may form soluble complexes that might grow and form sediments. Most authors suggest that protein–polyphenol complexes are formed by weak interactions; however, a hydrogen bond could complement these interactions, stabilizing the complexes (Charlton et al., 2002; Oliveira et al., 2015). These stable polyphenol–protein complexes may be responsible for the reduced syneresis in yogurts supplemented with SF, as observed by Vital et al. (2015) in LF-yogurt supplemented with an aqueous extract of *Pleurotus ostreatus*. Stable complexes may reduce protein rearrangement during shelf life, making the networks more stable, maintaining the serum in the system and consequently reducing syneresis.

In addition, some authors cite the ability of soy proteins (such as glycinin and β-conglycinin) to form gels, and that denaturation of protein might be a prerequisite for gel formation, by applying heat treatment to soy before gelation (Malaki Nik et al., 2011). In this study, the milk with ingredients underwent heat treatment before processing (90 °C for 3 min), which may have supported gel formation in this yogurt. As happens with polyphenol–protein interaction, denatured soy proteins can form aggregates, forming a gel network, and hydrogen bonding during cooling plays an important role in increasing gel firmness (Malaki Nik et al., 2011; Renkema and van Vliet, 2002), as can be observed for LF-yogurt with SF (Fig. 2).

Yogurt color showed the same behavior over the period evaluated; thus, only differences between treatments are presented. The color was influenced specially by the soy characteristics, making samples with soy darker, redder and yellower. Gomes da Costa et al., 2020 also observed that a yogurt enriched with protein were also darker than the control yogurt without protein enrichment.

Related to the microstructure, the YSF type of arrangement may lead to less rearrangement of proteins and therefore reduced susceptibility to syneresis.

Finally, yogurts supplemented with SF had higher TPC and antioxidant activity, results in agreement with the characterization of the ingredients (Table 1) where soybean flour also presented the highest polyphenol content and antioxidant activity.

5. Final Considerations

S. thermophilus and *L. bulgaricus* multiplication was greater in LF-yogurts with soy ingredients (flour and protein), followed by those with whey protein and the LF-yogurt control, and finally LF-yogurt supplemented with powdered milk. LF-yogurts supplemented with milk powder, soy flour and soy protein were firmest, and LF-yogurts supplemented with soy flour and milk presented the least syneresis. Different structures were observed by SEM, which were closer and denser for yogurt with soy flour. The LF-yogurts supplemented with soy flour exhibited higher antioxidant activity and contained more phenolic compounds. Supplementation of LF-yogurt with soy as a source of protein is interesting since it may confer functional activity on the yogurt and also modify its technological properties.

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