

β -GLUCAN FROM *SACCHAROMYCES CEREVISEAE* IN SKIM YOGURT PRODUCTION

β -GLUCANA DE Saccharomyces cerevisiae NA PRODUÇÃO DE IOGURTE DESNATADO

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ABSTRACT: The demand for functional foods has increased in recent years, following a market trend in which the consumer values foods associated with health improvements. Skim milk yogurts were produced with yeast β -glucan (YBG, 0.5 and 1.0 %) isolated from *Saccharomyces cerevisiae* and compared with full fat (FFY) and skim milk (SMY) yogurt as controls. The samples were analyzed for physical, chemical, color, syneresis, culture starter count, textural, microstructure and sensory attributes. Yeast β -glucan was not able to retain the serum of yogurts during cold storage. Skim yogurt firmness increased with the addition of 0.5% yeast β -glucan, presenting a more compact microstructure. Yogurts containing yeast β -glucan had low scores by the untrained panelists in the sensorial analysis (0.5% YBG overall acceptance 5.2, FFY 6.8, SY 6.3) and low purchase intention. Thus, although similar characteristics were observed, low scores in the sensorial analysis and purchase intention were a barrier to commercializing skim milk yogurt with yeast β -glucan developed in the present study.

KEYWORDS: Non-fat dairy products. Polysaccharides. Food development. Syneresis. Micrography.

INTRODUCTION

β -glucans are polysaccharides of D-glucose monomers linked by β -glycosidic linkages, either in linear chains or multiply branched structures and the glucose units branching several ways depending on the source of origin (LAM; CHEUNG 2013). Units of β -D-glucopyranose for microbial β -glucans are linked together through β (1,3) linkages to form a long backbone, whereas side chains mostly arise through β (1,6) linkages (AHMAD *et al.* 2012). They are found in cereals, yeast, mushrooms, seaweeds and some bacteria and are a valuable source of dietary fibers. β -glucans are major structural components of the cell walls of brewers' yeast *Saccharomyces cerevisiae*, fungi and some bacteria, and may account for up to 30% of the dry weight of the yeast cell (FLEET 1991).

The health benefits of β -Glucan are related to its potential in stimulating the immune system to protect the body against viral, bacterial, and fungal infections, tumors, radiation effects, and stress-related immunosuppression (BOHN; BeMILLER, 1995; DI LUZIO *et al.* 1979; ADACHI *et al.* 1990). Antioxidant properties and free radical scavenging capabilities, increasing antibiotic effectiveness and reducing low-density lipoprotein (LDL) cholesterol level in the body, are also described (STIER *et al.*

2014; El KHOURY *et al.* 2012; Vetvicka and Vetvickova, 2009). The Food and Drug Administration (FDA) has approved β -glucan (3 g day⁻¹) to present the coronary heart disease claim (FDA, 2005). The Brazilian Health Regulatory Agency (ANVISA) also approved β -glucan as a functional food in 2008. The claim “ β -glucan (dietary fiber) helps in the reduction of cholesterol absorption. Its consumption must be associated to balanced feeding and health life habits” can be used on the label when the foodstuff contains minimum 3 g per food portion (BRASIL 2008).

The use of β -Glucan in foods promote both, health impact and is a valuable raw material to be used in the industry. Due to the specific physical properties of β -glucan, such as water solubility, viscosity, and gelation, it has been increasingly used by the food and other industries. The development of food containing this polysaccharide contribute to an important issue of the modern life, the use of food pro to the health.

Yoghurt is traditionally made by fermenting liquid milk. The belief in the beneficial influence of yoghurt on human health and nutrition has existed in many civilizations over a long period of time. There has been a great interest in fortifying yoghurt to further improve its nutritional value and health benefits (SINGH *et al.* 2012). Also, the substitution

of fat increases the benefits of the product. Thus, the objective of the present study was to evaluate skim yoghurt with added yeast β -glucan regarding its physical-chemical composition and pH, syneresis, starter microorganisms and texture throughout storage, and microscopic and sensory analysis.

MATERIAL AND METHODS

β -glucan extraction

β -glucan was extracted from spent brewer's yeast. Autolysis was conducted adding water to the spent brewer at 50 °C/24 h, centrifuging and discarding the supernatant. Extraction was made with five NaOH 1 N washes. NaOH was added and the mixture was heated to 90 °C/1 h with gentle agitation. After, the precipitated was washed with water, dialyzed against water at 4 °C/48 h and lyophilized (L 101, Liobras, São Carlos, Brazil).

Yoghurt formulation

Yeast β -glucan at concentrations of 0, 0.5 and 1% (w/v) was incorporated into skim yoghurt. Full fat yoghurt was prepared as a control sample. Skim powdered milk and full fat powdered milk were used (12% w/v) and pasteurized at 65 °C/30 min, cooled to 42 °C and 0.05% yoghurt ferment (YF-L812, Christian Hansen, Valinhos, Brazil) was added. Yeast β -glucan was added and the mixture was distributed in 50 g plastic containers. Fermentation was performed at 42 °C for four hours and stopped by cooling at 8 °C. The yoghurts were stored at 8 °C until analysis at 1, 14 and 28 days.

The four formulations were produced three times.

Physical-chemical analysis

Total solids, fat, ash and protein were analyzed according Instituto Adolfo Lutz (2008).

Colour (L^* , a^* , b^*) was measured using a Konica Minolta colorimeter (model CR-400, Osaka, Japan). In this system the L^* represents lightness, a^* red value and b^* yellowness: $+a^*$ is the red, $-a^*$ is the green, $+b^*$ is the yellow, and $-b^*$ is the blue directions. The colorimeter was calibrated using the black and the white standard tiles.

The pH was measured at room temperature with a pH-meter (Tec-3MP, Tecnal, Piracicaba, Brazil).

Syneresis

Yoghurt samples were set in centrifuge tubes and centrifuged at $1000 \times g$ for 10 min at 8 °C (5804, Eppendorf, Hamburg, Germany). The separated whey was carefully decanted using a

pipette and weighed, and the centrifuge tube was also weighed to confirm the whey removed. Syneresis was calculated as the weight of the whey separated per unit weight of yoghurt. The degree of syneresis was expressed as a percentage.

Lactobacillus bulgaricus subsp. *delbrueckii* and *Streptococcus salivarius* subsp. *thermophilus* counting

The enumeration of *L. bulgaricus* was with MRS agar (Acumedia, Lansing, USA) with pH adjusted to 5.4 and incubated under anaerobic conditions at 37 °C/72 h. M17 agar (Sigma-Aldrich, Buchs, Switzerland) and aerobic incubation at 37 °C/48 h was used for the selective counting of *S. thermophilus*.

Texture profile analyses (TPA)

TPA were performed using a Brookfield texture analyzer-CT III (Brookfield Engineering Labs, Middleboro, USA) with a TA/1000 cylindrical probe; the penetration speed was 1 mm s^{-1} , distance target was 5 mm and trigger was 15 g. For these tests, samples were taken out of the refrigerator just before the test operation. Hardness, adhesiveness, cohesiveness, and gumminess values were obtained. Five replicates were performed for each sample.

Microstructure

Samples of each yoghurt were freeze-dried in a lyophilizer. Afterwards, the samples were stuck on stubs with double-face tape and coated with gold (BALTEC SDC 050 Sputter Coater). Four fields of each sample were observed under a scanning electron microscope (FEI Quanta 200, Hillsboro, USA), operating at a voltage of 9.0 kV, and micrographs were registered under 2,400 - 10,000 x magnifications.

Sensory analysis

The sensory analysis was carried out with 93 untrained panelists recruited from the staff and students of the University. Sensory sessions were performed in individual testing booths, under white lighting. Portions of approximately 25 g of the four yoghurt formulations were served on disposable transparent polyethylene recipients, coded with randomized 3-digit numbers in a random order. The test was carried out under controlled conditions, with mineral water and cream crackers available to the panelists. The panelists rated appearance, flavor, texture, and overall acceptance. Each panelist used a nine point-hybrid hedonic scale (1 = disliked extremely; 5 = neither liked nor disliked; 9 = liked extremely). The same 93 people were asked to

indicate their purchase intention of the product using a verbal numeric scale from 1 to 5 (1= I certainly would not buy; 3 = Maybe I would buy, maybe not; 5 = I would certainly buy). The Ethics Committee of the University North of Paraná approved the study (Process No. 1.428.154/2016; CAAE: 52522415.8.0000.0108) and terms of free and enlightened participation were signed by all consumers.

Statistical analysis

All formulations were repeated three times, and each measurement was performed in triplicate. Normality of data was tested by the Lilliefors test. When this assumption was not verified, non-parametric Kruskal-Wallis tests were applied to determine significant differences ($p < 0.05$ or $p < 0.10$) using the Statistica 13.0 software. Sensorial data were compared by analysis of variance (ANOVA) followed by the Tukey test ($p < 0.05$). Comparisons were made between the yoghurt samples made from full fat and skim yoghurt controls as well as skim yoghurts with 0.5 and 1.0 % yeast β -glucan.

RESULTS AND DISCUSSION

Since 2008, brewer's yeast β -glucan is Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA, 2008). Oat and barley β -glucan are approved (3 g d^{-1}) to present coronary heart disease (CHD) claim (FDA 2013). Thus, fortification of foods with β -glucan is of great interest. β -Glucan has various physical properties, such as thickening, stabilizing, emulsification, and gelation (AHMAD *et al.* 2012), and has the potential for use in acceptable health products that offer a wide range of added health benefits.

The chemical composition of the yoghurts with or without yeast β -glucan is shown in Table 1. Total solids, protein and fat contents in the skim yoghurts did not differ significantly, while ash content differed. The ash content increased ($p < 0.05$) with increasing levels of yeast β -glucan in the yoghurts. The composition results indicated that the overall composition was not changed by the low level addition of yeast β -glucan in skimmed yoghurt. Sahan *et al.* (2008) observed similar results for fat and ash contents in non-fat yoghurts with 0, 0.25, 0.5 and 1.0% β -glucan.

Table 1. Chemical composition and color of yoghurts made with or without yeast β -glucan.

| | Full fat yoghurt | Skim yoghurt (0 % β -glucan) | Skim yoghurt (0.5 % β -glucan) | Skim yoghurt (1.0 % β -glucan) |
|-------------------------|----------------------|---------------------------------------|---|---|
| Total Solids (%) | 9.0 ^{A,B} | 8.6 ^A | 9.3 ^{A,B} | 9.8 ^B |
| Fat (%) | 3.2 | 0.0 | 0.0 | 0.0 |
| Protein (%) | 4.4 | 4.7 | 5.3 | 6.1 |
| Ash (%) | 0.6 ^b | 0.9 ^{a,b} | 0.9 ^{a,b} | 1.1 ^a |
| L | 79.36 ^a | 75.15 ^{a,b} | 75.65 ^{a,b} | 61.34 ^b |
| a* | -2.43 ^{a,b} | -2.80 ^a | -2.22 ^{a,b} | -0.68 ^b |
| b* | 7.35 ^a | 6.39 ^{a,b} | 6.73 ^{a,b} | 5.40 ^b |

Values are presented as mean; ^{a,b} Different lowercase superscript letters are significantly different by the Kruskal-Wallis test ($p < 0.05$). ^{A,B} Different uppercase superscript letters in the column indicate significant difference on different days of storage of the same formulation by the Kruskal-Wallis test ($p < 0.10$).

Table 1 shows the L*, a*, b* values obtained according to the CIE color scale. Color plays an important role in food choice for consumers. The results indicated that non-fat yoghurt with 1% yeast β -glucan was yellower, greener and darker than full fat yoghurt ($p < 0.05$). These colors could be attributed to the light brown color of yeast β -glucan. Mejri *et al.* (2014) observed similar results in non-fat yoghurt with 0 to 2% β -glucan. Considering the barely noticeable differences limit of 2.3 on a human perception scale reported by Mahy *et al.* (1994), all the samples presented visual change in their colors (ΔE), except between non-fat without and with 0.5% yeast β -glucan.

Figure 1 shows the changes in pH values in full fat and skim milk yoghurt (with and without yeast β -glucan) stored at 8 °C for 28 days. On day 1, skim yoghurts (0, 0.5 and 1.0% yeast β -glucan) presented higher pH values compared to full fat yoghurt ($p < 0.05$). At days 14 and 28, non-fat yoghurt with 1.0% yeast β -glucan presented higher pH than skim yoghurt without yeast β -glucan ($p < 0.05$) and the pH ranged from 4.48 to 5.62 on day 1 and reached 4.30 to 4.62 after 28 days storage, respectively (Figure 1). The normal pH of commercial yoghurt products ranges from 4.0 to 4.4 (SAHAN *et al.*, 2008). In the current study, the use of skim milk for yoghurt production resulted in pH levels higher than 4.4 during the 28 days of storage.

Yeast β -glucan had no influence on pH levels of skim yoghurts ($p > 0.05$).

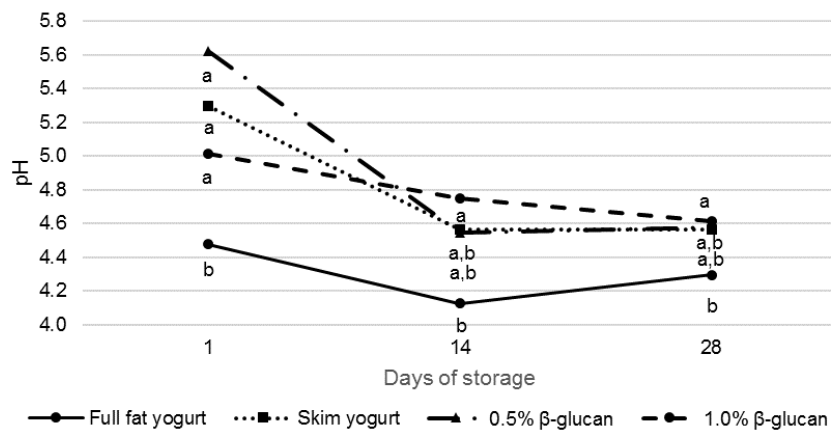


Figure 1. pH of yogurts with and without yeast β -glucan during cold storage.

Values are presented as mean; ^{a,b} Different lowercase superscript letters in the row indicate significant difference in the different formulations on the same day of storage by the Kruskal-Wallis test ($p < 0.05$).

Syneresis was higher in skim yoghurts with or without yeast β -glucan than full fat yoghurt on day 1 (Table 2). Serum expulsion is considered a primary defect in yoghurt (LUCEY, 2002). During cold storage, syneresis values were similar in skim yoghurt with 1% yeast β -glucan compared to full fat yoghurt ($p > 0.05$). The highest syneresis values were observed in skim yoghurt without β -glucan or with 0.5% yeast β -glucan ($p < 0.05$). The higher syneresis indicates a loose casein micelle network resulting in a weak gel incapable of retaining water. It was observed that β -glucan was capable of retaining this water during storage. During storage, skim yoghurts

with β -glucan presented a decrease in syneresis, similar to that observed in full fat yoghurt ($p < 0.05$), while skim yoghurt without β -glucan continued to expulse serum. Using oat β -glucan, Brennan and Tudorica (2008) observed different results, yoghurt containing 0.5% β -glucan had a syneresis level comparable with the full-fat control yoghurt while the addition of higher percentages of β -glucan significantly improved the ability of the yoghurt to retain larger amounts of serum within the structure. Sahan *et al.* (2008) observed no relation between a β -glucan composite concentrations and the whey separation levels.

Table 2. Syneresis and texture profile analysis (TPA) in yoghurts with and without yeast β -glucan during cold storage.

| Texture parameter | Day of storage | Full fat yoghurt | Skim yoghurt (0 % β -glucan) | Skim yoghurt (0.5 % β -glucan) | Skim yoghurt (1.0 % β -glucan) |
|-------------------|----------------|------------------------|------------------------------------|--------------------------------------|--------------------------------------|
| Syneresis | 1 | 32.21 ^{b,A} | 47.67 ^{a,A} | 47.92 ^{a,A} | 44.11 ^{a,A} |
| | 14 | 26.89 ^{c,A,B} | 47.19 ^{a,A} | 42.93 ^{a,b,A,B} | 34.00 ^{b,c,B} |
| | 28 | 23.43 ^{c,B} | 42.66 ^{a,A} | 36.63 ^{a,b,B} | 31.89 ^{b,c,B} |
| Firmness (N) | 1 | 1.05 ^{c,C} | 0.98 ^{b,c,B} | 1.24 ^{a,B} | 1.15 ^{a,b,B} |
| | 14 | 1.71 ^{a,b,A} | 2.02 ^{a,A} | 1.67 ^{a,b,A} | 1.63 ^{b,A} |
| | 28 | 1.28 ^{a,b,B} | 1.62 ^{a,A} | 1.38 ^{a,b,A,B} | 0.94 ^{b,B} |
| Adhesiveness (mJ) | 1 | 0.20 ^B | 0.27 | 0.11 ^B | 0.23 |
| | 14 | 0.24 ^{A,B} | 0.18 | 0.26 ^A | 0.31 |
| | 28 | 0.29 ^A | 0.25 | 0.32 ^A | 0.39 |
| Cohesiveness | 1 | 0.82 ^A | 0.89 | 0.79 ^A | 0.72 ^{A,B} |
| | 14 | 0.64 ^{a,b,B} | 0.74 ^a | 0.57 ^{a,b,B} | 0.56 ^{b,B} |
| | 28 | 0.86 ^{a,A} | 0.83 ^{a,b} | 0.75 ^{b,A} | 0.86 ^{a,b,A} |
| Gumminess (N) | 1 | 0.79 ^B | 0.61 | 0.91 | 0.69 ^B |
| | 14 | 1.17 ^{b,A} | 1.41 ^a | 1.13 ^b | 1.01 ^{b,A} |
| | 28 | 1.23 ^A | 1.30 | 1.10 | 1.00 ^{A,B} |

Values are presented as mean; ^{a,b} Different lowercase superscript letters in the row indicate significant difference in the different formulations on the same day of storage by the Kruskal-Wallis test ($p < 0.05$). ^{A,B} Different uppercase superscript letters in the column indicate significant difference on different days of storage of the same formulation by the Kruskal-Wallis test ($p < 0.10$).

L. bulgaricus and *S. thermophilus* counts varied between 3.2 and 3.0 Log cfu mL⁻¹ and 8.8 and 8.3 Log cfu mL⁻¹ from day 1 to 28, respectively (Figure 2). These are in accordance with the Brazilian legislation that establishes a minimum of 7 Log cfu mL⁻¹ for the lactic bacteria count for the fermented milk be considered as yoghurt (BRASIL

2007). In general, no effect of yeast β -glucan was observed in lactic bacteria counts. No difference was observed in *L. bulgaricus* and *S. thermophilus* counts during the 28 days of cold storage. Although β -glucan is considered a prebiotic, the added amounts did not interfere in the viability of the studied microorganisms.

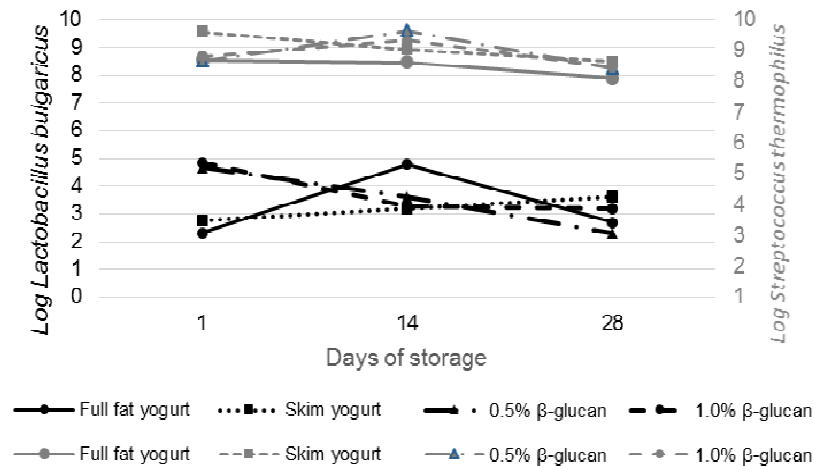


Figure 2. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* counts in yogurts with and without β -glucan during cold storage.

Values are presented as mean.

Texture means the product physical properties that come from its structural elements, which can be perceived by human senses (DOMAGALA *et al.* 2006). The main texture parameters of yoghurt are: firmness, adhesiveness, cohesiveness and gumminess (DOMAGALA *et al.* 2006). Data is presented in Table 2. Skim yoghurt firmness increased with the addition of yeast β -glucan ($p < 0.05$) on day 1. This was the sole difference observed between formulations in recently manufactured yoghurt. During cold storage, it was observed that firmness was maximum on day 14 ($p < 0.05$) for the four formulations but decreased after this peak.

Micrographs were recorded to visualize the microstructure of yoghurt supplemented with yeast β -glucan. The micrographs show some differences (Figure 3). A normal full fat yoghurt structure can be observed with a compact homogeneous structure, comprised of a network of casein micelles and fat (Figure 3A and 3B). Skim milk yoghurt without yeast β -glucan presented a casein network interspaced with void spaces that were originally occupied by whey (Figure 3C and 3D). The protein network was more open and relaxed than that of the full fat yoghurt. Yoghurts made with different yeast β -glucan concentrations showed particular

networks. A more compact microstructure was observed with increasing concentrations of yeast β -glucan (Figure 3E, 3F, 3G and 3H). Void spaces observed in skim milk yoghurt without yeast β -glucan almost disappeared when yeast β -glucan was added.

Table 3 presents the sensory scores, acceptability and purchase intention for the yoghurts. No significant effects were observed related to age with respect to liking the samples. The subjects under 20 years old represented 25.8%; 21 to 30 years old 36.6%; and over 31 years old were 18.3% of panelists. Regarding gender, the majority of panelists were woman (58.1%) (54 females; 23 males) whose reported consumption frequency of yoghurt was: daily – 9.7%; weekly – 10.8%; monthly – 21.5%; almost never – 32.3%. The addition of yeast β -glucan decreased the product acceptability compared with full fat yoghurt and skim yoghurt without β -glucan. Statistical differences ($p < 0.05$) were observed for all the parameters evaluated. Taking into account the score frequency, 51% of the panelists liked the appearance of full fat yoghurt (scores 8 and 9), while for skim yoghurt (0, 0.5 and 1.0 % yeast β -glucan) 27% of panelists gave scores 8 and 9. Around 40% of the panelists liked the flavor and texture of full fat

yoghurt, whereas only 10% liked the flavor of the skim yoghurt with yeast β -glucan. About overall acceptance, 42% liked the full fat yoghurt, 26% liked the skim yoghurt without yeast β -glucan and 10% liked the skim yoghurt with yeast β -glucan.

Regarding the purchase intention of the yoghurts, most of the respondents would not buy the skim yoghurt with yeast β -glucan.

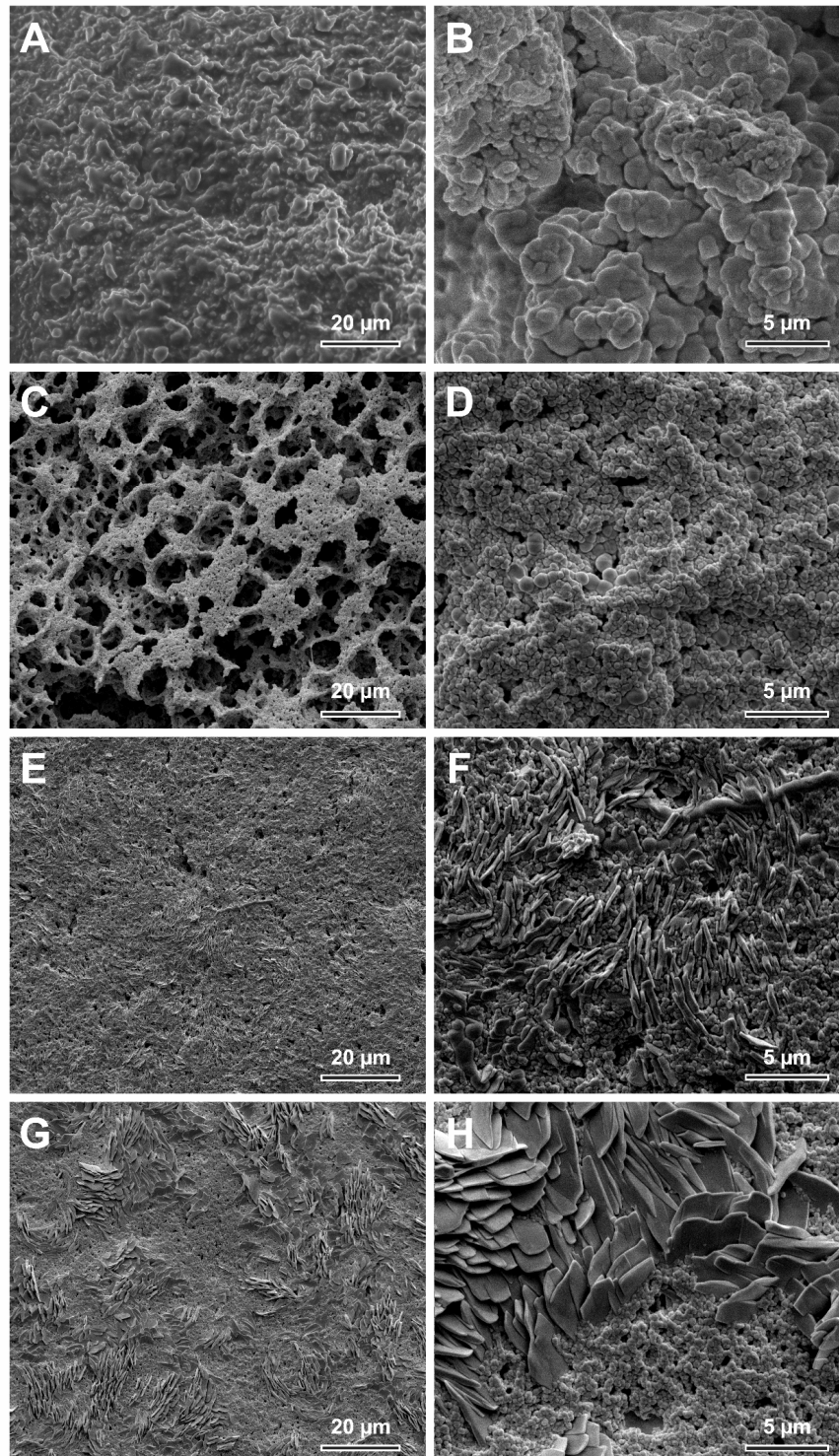


Figure 3. Scanning electron micrographs of yogurts. A and B: full fat milk; C and D: skim milk without yeast β -Glucan; E and F: skim milk with 0.5% yeast β -glucan; G and H: skim milk with yeast 1% β -glucan.

Table 3. Sensory quality, acceptability and purchase intention of yoghurts with and without yeast β -glucan.

| Sensory parameter | Full fat yoghurt | Skim yoghurt (0 % β -glucan) | Skim yoghurt (0.5 % β -glucan) | Skim yoghurt (1.0 % β -glucan) |
|--------------------|------------------|------------------------------------|--------------------------------------|--------------------------------------|
| Appearance | 7.1 ^a | 6.3 ^b | 6.1 ^b | 6.2 ^b |
| Flavor | 6.6 ^a | 5.9 ^a | 4.6 ^b | 4.3 ^b |
| Texture | 6.7 ^a | 6.2 ^{a,b} | 5.7 ^b | 5.8 ^b |
| Overall acceptance | 6.8 ^a | 6.3 ^a | 5.2 ^b | 5.0 ^b |
| Purchase intention | 3.4 ^a | 3.2 ^a | 2.4 ^b | 2.3 ^b |

Values are presented as mean; ^{a,b} Different lowercase superscript letters in the row indicate significant difference in the different formulations by the Tukey test ($p < 0.05$).

Despite of having comparable instrumental adhesiveness, cohesiveness and gumminess on day 1, in the sensory evaluation, the texture of skim yoghurt formulated with yeast β -glucan was significantly different ($p < 0.05$) from that of full fat yoghurt. The difference in instrumental firmness was associated to the texture observed in the sensory evaluation. (DOMAGALA *et al.* 2006) observed that instrumental hardness and the sensory features of yoghurt containing different levels of fat and maltodextrin had little or no relation.

Fortifying yoghurt or dairy products with β -glucan is of great interest to improve functionality and create functional foods with health benefits. The addition of yeast β -glucan to yoghurt would complement its healthy characteristics. Nonetheless, the present study has shown that fortifying skim yoghurt with 0.5 and 1.0 % yeast β -glucan produced

an unacceptable product. Regardless of its similar physical-chemical characteristics, the product presented low scores in the sensorial analysis and low purchase intent. Therefore, new technologies should be applied in order to improve the palatability of β -glucan, since the technology to obtain it is simple and the beneficial effects of this polysaccharide are proven.

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RESUMO: A demanda por produtos funcionais tem aumentado recentemente, seguindo um padrão no qual os consumidores valorizam os alimentos que são associados a melhoria da saúde. Iogurte desnatado foi produzido com β -glucana extraída de *Saccharomyces cerevisiae* e comparado com iogurte integral e iogurte desnatado. O iogurte desnatado contendo β -glucana não reteve soro durante a estocagem refrigerada. A firmeza do produto aumentou com a adição de 0,5% de β -glucana e sua microestrutura foi mais compacta. Iogurtes contendo β -glucana apresentaram menor escore na análise sensorial. Assim, embora características semelhantes tenham sido observadas, os baixos escores da análise sensorial são uma barreira para a comercialização de iogurte desnatado contendo β -glucana produzido no presente estudo.

PALAVRAS-CHAVE: Produtos lácteos sem gordura. Polissacarídeos. Desenvolvimento de alimentos. Sinérese. Micrografia.

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