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Assessment of fat content influence over greek yogurt protossymbiotic culture

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Abstract

Greek yogurts have high concentrations of proteins and fats, which results in constant increase in their consumption in the Brazilian market. A product with higher nutritional contents allows it to have greater added value, which generates an increase in interest on the part of Brazilian dairy industries. This work aimed to evaluate the influence of the high concentration of lipids and the presence of flavorings over lactic acid bacteria (LAB) count from Greek yogurts of different brands. Results obtained showed the variation of titratable acidity and LAB count of samples used. Sample T1 showed lower microbial growth ($1x10^3$ CFU/g) and higher total fat content (6.7%) compared to the others. There was variation between brands regarding BAL count ($p<0.05$), showing variation between the different dairy products from different brands sampled. Regarding presence or absence of flavoring, there was no difference ($p>0.05$) between the products sampled. Implementation of a specific legislation for the product would culminate in the standardization and, possibly, improvement in the global quality of Greek yogurts sold in the country.

Keywords: Fat. Fermented milk. Lactic acid bacteria.

Avaliação da influência do teor de gordura sobre a cultura protossimbiótica de iogurte grego

Resumo

Iogurtes grego apresentam concentrações elevadas de proteínas e gorduras o que resulta em um aumento constante de seu consumo no mercado brasileiro. Um produto com maiores teores nutricionais permite que este tenha maior valor agregado, o que gera um aumento de interesse por parte dos laticínios brasileiros. Assim, este trabalho objetivou avaliar a influência da concentração mais alta de lipídeos e a presença de corantes e aromatizantes sob a contagem de bactérias ácido láticas (BAL) utilizando marcas distintas de iogurte grego. Os resultados obtidos mostraram a variação de acidez titulável e crescimento de BAL das amostras utilizadas. A amostra T1 apresentou menor crescimento microbiano ($1x10^3$ UFC/g) e maior teor de gordura total (6,7%) em comparação às demais. Observou-se variação entre as marcas no que diz respeito à contagem de BAL ($p<0,05$), mostrando variação entre os produtos dos distintos laticínios amostrados. No que diz respeito à presença ou não de corantes e aromatizantes, não se observou diferença

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(p>0,05) entre os produtos amostrados. A implementação de uma legislação própria para o produto culminaria na padronização e, possivelmente, melhora na qualidade global de iogurtes gregos comercializados no país.

Palavras-chave: Bactérias ácido láticas. Gordura. Leite fermentado.

Introduction

Greek yogurt is one of the several products available classified as fermented milk. With that said, according to Brazilian legislation, fermented milk is defined as a product without the addition of other food substances, obtained by coagulation and reduction of milk, or reconstituted milk, pH; added or not by other dairy products, by lactic fermentation through action of cultures of specific microorganisms. The same legislation defines yogurt as a product whose fermentation occurs with protossymbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* that can be accompanied, in a complementary way, by other lactic acid bacteria (Brasil, 2007). However, there is no specific standard that determines the composition and quality of Greek yogurt.

Bacteria *Lactobacillus delbrueckii* and *Streptococcus thermophilus* are extremely important for the microbial fermentation of milk to occur, as they are capable of producing lactic acid. As a result of the production of this acid, there is a reduction in the pH and coagulation of milk proteins, determining technological aspects of yogurt. The sensory characteristics are due to the junction of volatile compounds, namely acids, ketones, alcohols, esters, hydrocarbons and aldehydes acquired throughout the fermentation process (Dan *et al.*, 2017; Nagaoka, 2019).

The inclusion of components and techniques, such as the addition of powdered milk and/or desorption technique - which consists of removing whey by centrifugation or cloth bags - result in an increase in the volume of solids, with emphasis on proteins and fats, delivering to Greek yogurt its known characteristics of firm texture, full-bodied appearance and differentiated palatability (Varnam *et al.*, 1995).

Addition of cream is one of the techniques for increasing solids in Greek yogurt, however, studies carried out by Ramos *et al.* (2009) concluded that the addition of cream resulted in lower values in physical characteristics such as: reduction of stickiness, firmness and adhesiveness. This fact can be explained by interaction of the fat present in cream with other yogurt components. On the other hand, not adding cream makes the food matrix, particularly the protein fraction, more rigid; which is also not very desirable.

Microbial multiplication can be directly influenced according to the availability of water, energy/nitrogen sources such as sugars/carbohydrates, complex proteins (which will suffer the action of proteases and consequent release of free amino acids) and lipids, the latter being used only by a small class of microorganisms. In addition, vitamins and minerals (such as sodium, potassium, calcium and magnesium) are essential for enzymatic reactions (Pinto *et al.*, 2019).

Pinto *et al.* (2019) also report that the presence of essential oils (such as clove and cinnamon eugenol), of fruits containing organic acids and essential oils, in addition to milk substances (such as immunoglobulins, lactoferrins and lysozymes) may have a delaying and/or inhibitory effect on the multiplication of microbiota present in yogurt.

Thus, this work aims to evaluate, at different fat levels, the growth of lactic acid bacteria (protosymbiotic culture) in Greek yogurts with different flavors, strawberry and traditional.

Material and methods

In the present study, three different brands of traditional and strawberry flavored Greek yogurt were used, totaling six samples, within the expiration date, purchased from the same supermarket located in the municipality of Formiga, Minas Gerais. The experiment was carried out at the Microbiology Laboratory of the Centro Universitário de Formiga – UNIFOR-MG.

The lactic acid bacteria (LAB) count was quantified following the method described by the International Dairy Federation (IDF, 1988), where *Lactobacillus delbrueckii* is a thermophilic microorganism that forms lenticular colonies of 1mm to 3mm in diameter in De Man, Rogosa and Sharpe (MRS) medium and *Streptococcus* thermophiles, which is a thermophilic microorganism that forms lenticular colonies with a diameter of 1mm to 2mm. Decimal serial dilutions of Greek yogurt samples in sterile saline (0.9% NaCl) were performed. Subsequently, 1ml of each of the selected dilutions of each sample were added to sterile Petri dishes, being overlaid with 20ml of MRS agar melting medium, with subsequent incubation, after solidification of the medium, at 37ºC for 48 hours, thus executing the pour plate technique. Results were expressed in colony forming units - CFU/g.

To determine the titratable acidity, 10ml of yogurt was transferred to an Erlenmeyer flask, added with five drops of 1% phenolphthalein solution and titrated with

N/9 sodium hydroxide solution (alkaline solution known as Dornic solution) (Farias *et al.*, 2016).

GraphPad Prism 6.0 program (GraphPad Software, San Diego, California, USA) was used to perform all statistical analyses. Unpaired t test (or One-Way ANOVA) and Turkey's post-test were used with significance of 5% ($p<0.05$) to compare the means of samples of different brands and flavors.

Values of total fat of each sample were obtained in their respective packages.

Results and discussion

The total fat content (g/100g) of each Greek yogurt sample is described in Tab. 1, from values informed on the packaging of each product. According to Normative Instruction No. 46, from livestock and agricultural department (MAPA), whole yogurts must contain a minimum of 3.0% of fat content (Brasil, 2007), thus, all samples are within the parameters recommended by Brazilian legislation.

Table 1 – Total fat content of each sample containing 100g

Sample	Commercial presentation	Fat content (%)
Brand 1	Traditional (T1)	6,7
Brand 1	Strawberry (S1)	6,1
Brand 2	Traditional (T2)	4,7
Brand 2	Strawberry (S2)	4,6
Brand 3	Traditional (T3)	5
Brand 3	Strawberry (S3)	4,2

Estimates indicate that the per capita consumption of yogurt in Brazil presented an increase of 100% compared to 20 years ago (Barros *et al.*, 2020) which highlights the need for a specific legislation for Greek yogurts, since the lack of standardization of its composition appears to have great influence on the final product.

Amaral *et al.* (2016) evaluated physicochemical compositions of different brands of Greek yogurt and obtained values that also confirm the influence of composition variation between brands regarding titratable acidity, fat matter and other analyzed parameters.

Li *et al.* (2020) obtained results where different fat contents in cheeses fermented by *Lactobacillus rhamnosus* B10, *Streptococcus thermophilus* B8, *Weissella* confused B14 and *Lactobacillus helveticus* B6 influenced physical, chemical and biological parameters. Results found by Ferreira *et al.* (2021) were similar regarding the activity of fat content when performed with yogurts, confirming fat influence over microbial growth in different dairy matrixes.

Total count of lactic acid bacteria present in each product is shown in Fig. 1. It was possible to observe that all samples do not meet the microbiological standard defined by Normative Instruction No. 46, where it is recommended that the total count of lactic acid bacteria (CFU/g) should be at least $1x10^7$ CFU/g ($Log_{10}=7$) for fermented milks classified as yogurts (Brasil, 2007).

It is possible to observe that the T1 sample has the highest fat content (6.7%) and obtained the lowest BAL growth in MRS medium ($1x10^3$ CFU/g).

Ferreira (2005) states that for an effective growth of a dairy culture, the type and quality of the substrate used is of paramount importance, since fatty solids, in addition to minerals, will probably influence in culture growth.

Studies led by Martinovic *et al.* (2016) obtained results regarding the reduction in the count of *Lactobacillus* sp. and *Propionibacterium* sp. in semi-hard cheeses when compared in terms of fat content, where the cheese with 10% fat content had a reduction of only 1-2 Log_{10} of CFU/g while the cheese with 28% of fat content had a Log_{10} reduction of 2- 3 CFU/g. This fact can be explained by the fact that low fat cheese has more water activity (Aw) and, thus, presents more favorable conditions for microbial growth.

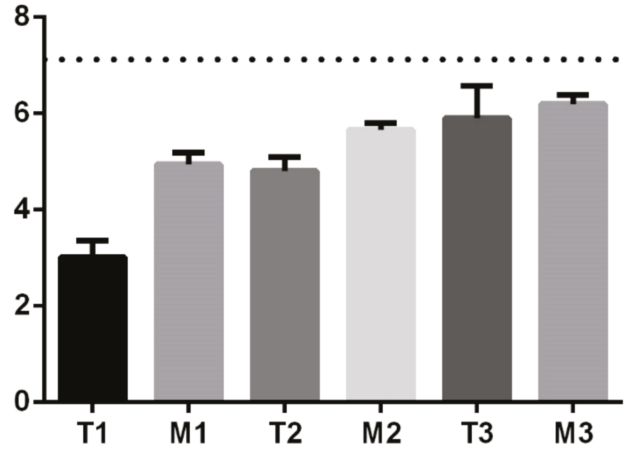
Results obtained for titratable acidity (g of lactic acid/100g of product) are shown in Fig. 2. Following Normative Instruction No. 46 for fermented milks classified as yogurt, the recommended titratable acidity standard must be between 0.6 to 1.5g of ac. lactic/100g (Brasil, 2007). With that said, it is possible to conclude that two samples are outside the standard defined by Brazilian legislation.

Based on Fig. 2, it is noteworthy that samples S2 and T3 presented higher values of lactic acid, 1.56

and 1.66 respectively; values that are out of what is recommended by legislation.

Prolonged storage can generate less stability in Greek yogurt, mainly in terms of acidity. This statement is explained due to the continuous fermentation process (lactic acid production) of *starter* cultures (Nascimento *et al.*, 2016; Gengatharan *et al.*, 2017). Lima (2011) shows that product storage temperature directly influences microbial multiplication along with metabolic reactions, such as acidification.

Figure 1 – Mean total lactic acid bacteria count (Log_{10} CFU/g ± SD) in MRS agar of Greek yogurts of different brands and flavors

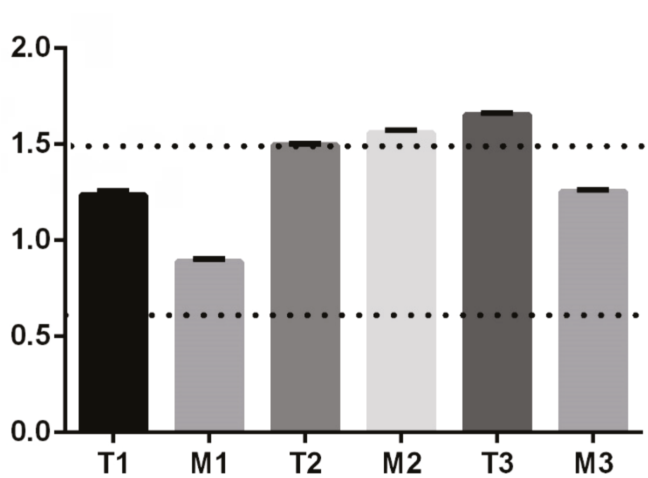


Caption: SD=standard deviation, T=traditional flavor, M=strawberry flavor. Different numbers indicate different brands. The dashed line shows the minimum total growth pattern of BAL (7 Log_{10} CFU/g) defined by legislation.

Studies carried out by Reis *et al.* (2011) concluded that high concentrations of non-fat solids result in higher levels of casein, an acidic milk protein, which consequently generates an increase in the spent amount of sodium hydroxide (NaOH N/9) during the titration process, causing the increase in titratable acidity.

On the other hand, ingredients used in the composition of Greek yogurt that present chemical characteristics close to pH neutrality are supposed to be an interference factor in the total acidity parameter in Greek yogurts (Bezerra *et al.*, 2019).

Figure 2 – Titratable acidity (g of lactic acid/100g of product ± SD) from Greek yogurt of different brands and flavors



Caption: SD=standard deviation, T=traditional flavor, M=strawberry flavor, different numbers indicate different brands. The dashed line shows the titratable acidity standard defined by legislation.

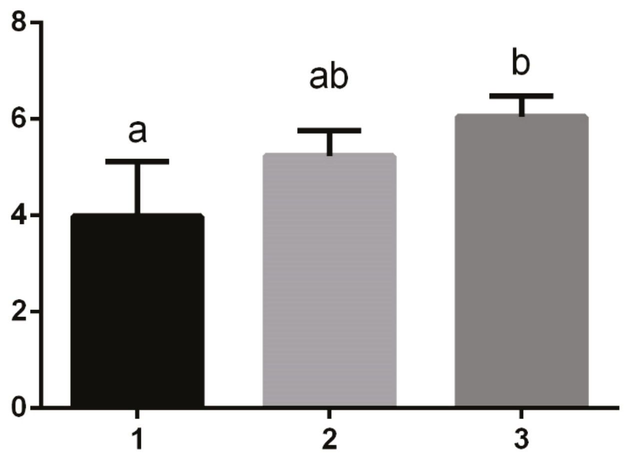
Total growth of BAL and titratable acidity for traditional and strawberry flavor samples with comparison between brands is shown in Fig. 3 and 4. Difference ($p<0.05$) between brands 1 and 3 is shown in both parameters analyzed. Brand 2 showed no difference ($p>0.05$) with brand 3 in both parameters, as well as no difference with brand 1 when comparing total growth of lactic acid bacteria (CFU/g), however, when comparing titratable acidity results, there was a difference ($p<0.05$).

In Fig. 3 we can see that samples from brand 1 (which have higher fat content) presented lowest total count of lactic acid bacteria ($4x10^3$ CFU/g). Samples 2 and 3, on the other hand, did not show any difference ($P>0.05$).

Using whole, reduced-fat, and low-fat cheddar cheeses, Ganesan *et al.* (2014) concluded that products with added lactic acid bacteria *Lactobacillus acidophilus* LA-5 and *Lactobacillus casei* CRL-431 resulted in a count of 10^8 CFU/g of total lactobacilli in low-fat cheeses while non-fat and reduced fat cheeses had a fall of up to eight times in the total count of lactobacilli. This reinforces our results, where milk fat can be related to a reduction in lactic acid bacteria count in a dairy fermented product.

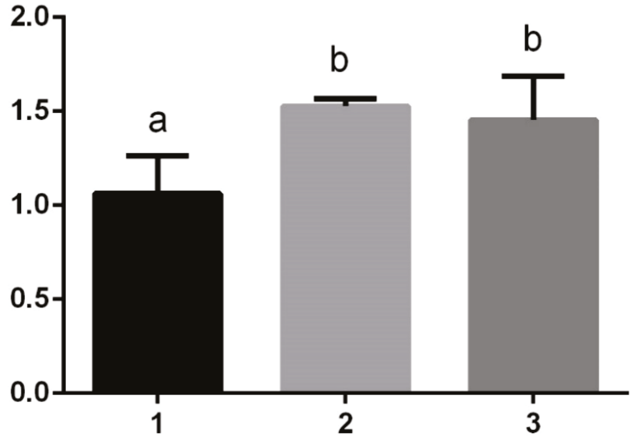
Andrade *et al.* (2015) found different results for titratable acidity ($p<0.05$) using fermented dairy products of different compositions and brands. Costa *et al.* (2019) found similar results ($p<0.05$) when using sweeteners of different compositions in yogurt, when compared to traditional presentations.

Figure 3 – Mean total lactic acid bacteria count (Log_{10} CFU/g $\pm$ SD) in MRS agar of Greek yogurts of each brand



Caption: SD = standard deviation. Different numbers indicate different brands. Different letters represent different results ($p<0.05$) by the One-way ANOVA test with Turkey post-test.

Figure 4 – Mean total titratable acidity (g of lactic acid/100g product $\pm$ SD) for Greek yogurt results of each brand.



Caption: SD = standard deviation. Different numbers indicate different brands. Different letters represent different results ($p<0.05$) by the One-way ANOVA test with Turkey post-test.

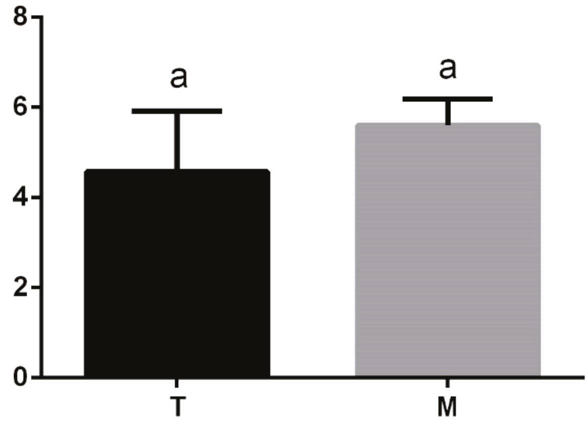
Fig. 5 and 6 show that, based on the total BAL count and titratable acidity, respectively, there was no difference when comparing strawberry and traditional Greek yogurt flavors ($p>0.05$).

Barros *et al.* (2020) obtained different results from this work, where the difference in flavors of Greek yogurt (maize starch and pumpkin jelly) resulted in a significant influence ($p<0.05$) in the results of titratable acidity.

Studies evaluated by Junior *et al.* (2016) also obtained different results from this work when titratable acidity and other parameters were compared between samples containing different formulations of sour flavor (such as citric) addition and samples that did not contain flavor.

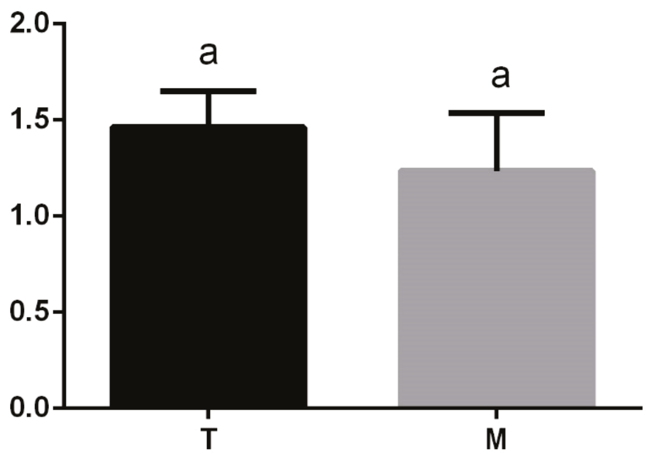
These studies show that each food matrix may have a different effect over final quality of fermented dairy products such as yogurts.

Figure 5 – Mean total lactic acid bacteria count (Log_{10} CFU/g $\pm$ SD) in MRS agar of Greek yogurt of each flavor



Caption: SD=standard deviation, T=traditional flavor, M=strawberry flavor. Different letters represent different results ($p<0.05$) by the Unpaired t test ANOVA test with Turkey post-test.

Figure 6 – Mean total titratable acidity (g of lactic acid/100g of product $\pm$ SD) for Greek yogurt results for each flavor



Caption: SD=standard deviation, T=traditional flavor, M=strawberry flavor. Different letters represent different results ($p<0.05$) by the Unpaired t test NOVA test with Turkey post-test.

Conclusion

The sample with the highest fat content showed lowest count of lactic acid bacteria in Greek yogurt, which confirms the suspicion on which the study was based. Further studies are of paramount importance to investigate the influence of fat content over protossymbiotic cultures. With this work it is possible to emphasize the need to implement a specific legislation for Greek yogurts, since different brands have different compositions and different results regarding legislation requirements.

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