Effects of the addition of gum Acacia and probiotics to yogurts with low sugar and fat contents

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ABSTRACT

Hydrocolloids are frequently added to dairy products to modify the texture or to prevent syneresis. The effect of adding different levels of acacia gum and probiotic cultures on the physical, chemical, sensory and microbiological characteristics of low-fat and -sugar yogurts was investigated. Four treatments were used with the following levels of gum inclusion: F1control (0 %); F2 (1 %); F3 (2 %) and F4 (3 %). The treatments were evaluated during a 21-day storage period under refrigeration; in the first 7 days, an increase in acidity and a decrease in pH values was observed, indicating the continued activity of the microbial cultures. There was a significant difference among the treatments (P<0.05) only for the variable "colour". Samples containing acacia gum were darker, with a tendency to a red or yellow colour. The addition of acacia gum did not favour the survival of the evaluated bacteria, although they were at appropriate levels according to the established guidelines. The yogurt with acacia gum was well accepted by the tasters, with satisfactory physico-chemical characteristics and the maintenance of the cellular viability of the final product.

Key words: *Acacia senegal*, milk products, yogurt

Efectos de la adición de goma de Acacia y probióticos a yogures con bajo contenido de azúcar y grasa

RESUMEN

Los hidrocoloides se agregan frecuentemente a los productos lácteos para modificar la textura o prevenir la sinéresis. Se investigó el efecto de la adición de diferentes niveles de goma de acacia y cultivos probióticos sobre las características físicas, químicas, sensoriales y microbiológicas de los yogures bajos en grasa y azúcar. Se utilizaron 4 tratamientos con los siguientes niveles de inclusión de goma: F1control (0 %); F2 (1 %); F3 (2 %) y F4 (3 %). Los tratamientos fueron evaluados durante un período de almacenamiento de 21 días bajo refrigeración; en los primeros 7 días, se observó un aumento en la acidez y una disminución en los valores de pH, lo que indicó la actividad continua de los cultivos microbianos. Hubo una diferencia significativa entre los tratamientos (P<0,05) solo para la variable "color". Las muestras que contenían goma de acacia eran más oscuras, con tendencia a un color rojo o amarillo. La adición de goma de acacia no favoreció la supervivencia de las bacterias evaluadas, aunque se mantuvieron en niveles apropiados de acuerdo con las pautas establecidas. El yogur con goma de acacia fue bien aceptado por los catadores, con características fisicoquímicas satisfactorias y el mantenimiento de la viabilidad celular del producto final.

Palabras clave: *Acacia senegal*, productos lácteos, yogur.

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INTRODUCTION

Yogurt and fermented milk are among the most common fresh dairy products eaten around the world, and their consumption is associated with numerous health benefits (Donovan and Shamir 2014). These days, consumers view food as a way to improve their health and wellbeing, and manufacturers are responding proactively by offering new products that meet these requirements (Gray *et al.* 2003).

Many consumers fail to pay attention to nutritional values and calories when foods are shown as healthy, even if they are not (Chandon and Wansink 2007); this confirms the importance of indicating that the food contributes to consumer health (Küster-Boluda and Vidal-Capilla 2017).

Gum arabic, also referred to as acacia gum, exudes as a viscous fluid from the stems and branches of *Acacia* trees (specifically *Acacia senegal* and *Acacia seyal*), which grow across the Sahelian belt of Africa (Kennedy *et al.* 2012). The term 'gums' refers to a range of natural polymers, mainly polysaccharides, that are widely used in the food industry to control the rheological and organoleptic properties of food products. They are employed to perform a number of functions, including the thickening and gelling of products (Williams 2016). Key food applications include a range of confectionery products, flavoured oil emulsions and capsules and health foods as a source of soluble fibre with prebiotic properties; acacia gum has a fibre content of over 80 % (Thevenet 2012).

The increase in the demand for functional foods, such as fermented milks with probiotic microbial cultures, has sparked research to keep these microorganisms viable in the product (Burkert 2012). The viability of probiotics in yogurt is affected by various factors: the conditions under which the yogurt is stored, the type of the inoculated strain, the product's lactic acid concentration, the starter cultures used and the concentrations of hydrogen peroxide (H_2O_2) and oxygen in the yogurt (Hasani *et al.* 2016). In this sense, the use of acacia gum is promising, as it may also aid in the cellular protection of probiotic bacteria, which has been shown by Souza *et al.* (2009) using *Lactobacillus casei* in ice cream.

The texture of the product and the propensity to syneresis are one of the main characteristics that will define the quality of the yogurt (Lee and Lucey 2010). One of the alternatives is the use of thickeners such as acacia gum, used as a substitute for fat, as it reduces calories with a minimum change in consumer acceptance.

However, a study conducted by Ribeiro *et al.* (2010), evaluating the yogurt market study, found that 39.9 % of the respondents said they did not consume light/diet yogurt because they did not like it. Fermented milk, yogurts and whey beverages are products that are highly appreciated in Brazil by a large population of all ages. They are produced via similar technological processes, but differ in their chemical compositions, ingredients and sensory characteristics. There are many products available on the market, including those containing functional ingredients (probiotic and prebiotic) or for specific nutritional uses (lactose-free as well as light and diet formulations; Pimentel *et al.* 2017).

In view of the above, the objective of this study was to evaluate the effects of the addition of increasing levels of acacia gum on the characteristics of yogurt containing probiotic bacteria and with low levels of fat and sugar.

MATERIAL AND METHODS

Around 40 litres of yogurt, obtained from fermentation in an oven at 43ºC, were prepared using UHT milk (Santa Clara® skimmed). After fermentation, the gel was cooled to 37ºC, and the mass was broken together with 0.6 mL/L passion fruit flavouring (Mix Saborforte®; 0.02 g/L colorant, Arcolor®; 0.07 % aspartame, Zero cal®). The use of aspartame and the concentrations employed are in agreement with Reis (2007).

For the formulations, four concentrations (0 to 3 %) of acacia gum (Fibregum®Nexira) were used, and the samples were packed in 1-L flasks for further microbiological and sensory analyses. A Bio-Rich® Probiotic culture, containing *Lactobacillus acidophilus* LA-5, *Bifidobacterium* BB-12 and *Streptococcus thermophilus*, was added according to the manufacturer's recommendations. The experiment was repeated three times.

- *pH value evaluation*

The pH was determined using a digital pH meter (IAL2008).

- *Titratable acidity evaluation*

Acidity, in terms of degrees Dornic, was determined by titration (Mapa 2007).

- *Bacterial viability evaluation*

The samples were stored for 21 days at 4°C and evaluated on days 1, 7, 14 and 21 for pH value, acidity and viable cells of *Streptococcus salivarius ssp. thermophilus, Lactobacillus acidophilus and Bifidobacterium* spp. The M17 agar media were incubated at 37°C for 48 h for streptococci, the MRS agar at 37ºC for 72 h for lactobacilli and Agar Bifidobacterium for bifidobacteria, both with incubation in anaerobic conditions (Silva *et al*. 1997).

- *Syneresis, texture and colour evaluation*

Syneresis was analysed via centrifugation (Guzmán-González 2000). For this, natural yogurt (25 g) was prepared in 50 mL conical falcon tubes and centrifuged at 500 g for 10 minutes at 4°C. Syneresis was expressed as a percentage of serum released in the centrifugation.

The texture profile was evaluated according to Rawson and Marshall (1997) in a universal texturometer with a 35 mm diameter flat-bottom cylindrical probe (A/BE 35).

The results were obtained with the aid of the Texture Expert version 1.11 for TPA (Texture Profile Analysis). The parameters of interest were firmness, adhesiveness, cohesiveness, gumminess and elasticity. The 50 mL samples fermented in plastic cups with 9.2 cm in height and an inner diameter superior and inferior of 6.6 and 5.0 cm, respectively; were analysed immediately after being removed from the refrigerator. The measurement conditions were standardised in a probe gauge of 60 mm, with a penetration force of 15 g and a compression speed of 3 mms^{-1}.

The colour was determined via the CIELAB system in Minolta® CR400 equipment, using the colour parameters L* (brightness), a* and b* (chromaticity coordinates).

- *Sensory analysis*

Samples were submitted to sensory evaluation by a team of 200 untrained testers (50 people per time); process was approved by the Ethics Committee, number 43116114.1.0000.0104, for each experimental period (1, 7, 14 and 21 days).

The analysed parameters were overall appearance, colour and aroma; in addition, the judges were asked to indicate the frequency of consumption and the intention to buy the products if they were available for sale on the market.

For the evaluation, a token was used with a hedonic scale of four points, with the extremes 1 (I disagree greatly) and 4 (I liked it very much).

For the frequency of consumption, a four-point scale was used, in which 4 represented the maximum grade (always consumed) and 1 the minimum grade (never consumed).

For the calculation of the product acceptability index, the expression:

$$IA\,(\%) = \frac{A \times 100}{B}$$

was adopted, where *A* = average grade obtained for the product and *B* = maximum grade given to the product. The IA with good acceptability was considered greater than or equal to 70 %.

- *Statistical analysis*

The data were submitted to analysis of variance (ANOVA), with averages being compared by Tukey´s test when the differences were verified. The results were significant at $P<0.05$. For all analyses, we used the software package SAS (version 9.1.3, 2003).

RESULTS AND DISCUSSION

- *pH values and bacterial viability*

There was no difference among the treatments in relation to the pH values (Table 1). Maestri *et al.* (2014) and Robinson *et al.* (2006) have obtained similar pH values during evaluating yogurt samples. Thus, the mean pH values between 4.22

Table 1. pH values and microbiological counts of fermented milk with different levels of acacia gum

Parameters	Levels of inclusion of acacia gum (%)				SE	P
	0	1	2	3		
pH	4.27	4.31	4.28	4.30	0.016	0.2154
	Microbiological count (CFU/mL)					
Lactobacillus sp.	8.00	7.54	7.17	8.55	0.440	0.2084
Estreptococcus sp.	8.19	7.70	8.17	8.19	0.294	0.5828
Bífidobacterium	7.76	7.55	7.49	7.84	0.254	0.7375

SE= standard error

and 4.36 are in accordance with the levels considered to be ideal, which range from 4.0 to 4.4 (MAA 2000). At lower pH levels, rejection by consumers and clot contraction may occur; the latter is due to the reduced protein hydration, causing desorption (Bortolozo and Quadros 2007).

During the evaluation period, the product was post-acidified at 1 week after manufacturing (Table 2). The reduction of the pH and the accumulation of organic acids during refrigerated storage of fermented milk are defined as "post acidification", which is mainly attributed to the ongoing metabolic activity of *L. delbrueckii subsp. bulgaricus*. However, Settachaimongkon *et al.* (2016) have observed significantly lower post-acidification in yoghurts, which might be associated with the decrease in viable counts of *L. delbrueckii subsp. bulgaricus,* responsible for the higher production of lactic acid.

Probiotic bacteria are sensitive to several factors, mainly pH values, with no acidic growth (4.0 to 4.5) or alkaline growth (8.0 to 8.5) being present, and the Bifidobacteria may not remain viable throughout the shelf-life of the product and during the passage through the gastrointestinal tract (Silva 2016).

This fact has been proven through microbiological counts, and a significant reduction occurred during the shelf-life of the product (Table 2). A reduced viability has also been observed in studies such as that of Mathias (2011) when assessing the viability of traditional yogurt bacteria, which showed an increase in the microbial population from the 1st to the 15th day, with a decrease in the total number of viable bacteria in the last 15 days of storage, probably related to *Streptococcus thermophilus*. In the present study, for this microbial group, the counts increased at 7 days and were subsequently reduced. For lactobacilli, this increase occurred at 14 days, with reduced levels at 21 days. Such oscillations may have contributed to the reduction of the pH (7 days) and the subsequent increase due to the lower production of lactic acid by microorganisms.

Contradictory effects, related to the presence of acacia gum in yogurt-related microbiological counts,

Table 2. pH values and microbiological counts of fermented milk containing acacia gum as a function of storage times

Parameters	Shelf life (days)				SE	P
	1	7	14	21		
pH	4.35	4.23	4.29	4.29	0.016	0.006[1]
	microbiological count (CFU/mL)					
Lactobacillus sp.	9.36	7.18	8.08	6.64	0.440	0.009[2]
Estreptococcus sp.	7.91	8.97	7.93	7.38	0.294	0.023[3]
Bífidobacterium	7.44	7.70	8.27	7.24	0.254	0.084

[1] $\hat{Y} = 4.39 - 0.053x + 0.005x^2 - 0.0002x^3$ ($r^2 = 64.29$); [2] $\hat{Y} = 10.296 - 1.037x + 0.106x^2 - 0.003x^3$ ($r^2 = 60.16$); [3] $\hat{Y} = 7.993 + 0.139x - 0.008x^2$ ($r^2 = 42.52$). SE= standard error

have been reported by Oliveira (2008), who observed an increase in probiotic bacteria and a better maintenance of viability during the storage period. Similarly, Rokka and Rantamaki (2010) reported that the incorporation of soluble fibre from Arabic gum in a milk-based medium during storage, increased the viability of *Lactobacillus paracasei*. In addition, the Bifidobacteria population encapsulated in Arabic gum survived better when compared to free cells.

The Brazil legislation (MAA 2000) determines the minimum viable quantity for probiotics between 10^8 and 10^9colony-forming units (CFU) in the daily recommendation of the ready-to-eat product; in terms of the use of Bifidobacteria, the count will be 10^6 CFU/mL (MAA 2000). Considering these values, in the present study, the yoghurt samples met the recommended standards, although the bacterial numbers were reduced in the evaluated period (Table 2).

- *Titratable acidity*

There was no significant difference (P>0.05) among treatments in relation to the titratable acidity. The use of acacia gum did not promote greater acidification, most likely because of its prebiotic effect through the stimulation of bacterial fermentation. Linear regression models were adjusted for the behaviour of the variable acidity as a function of time. There was an increase in the acid values with increasing storage time (P<0.05), which is in line with the initial pH values. The mean values were 9.30; 10.44; 10.56 and 10.74 ºD for 1; 7; 14 and 21 days respectively, equivalent to 0.93; 1.044; 1.056 and 1.074 g lactic acid/mL.

In relation to acidity, some studies have found yogurts with a final acidity of 0.6 to 1.5 %, expressed as lactic acid (Cunha *et al.* 2008). Despite the post-acidification observed, the percentages of lactic acid present in the yogurt after the total storage period are in accordance with the current legislation, which establishes concentrations between 0.6 and 2.0 % (MAA 2000).

- *Syneresis*

There was no significant difference among treatments in relation to this variable. Spontaneous whey separation is related to an unstable network, which can be due to an increase in the rearrangement of the gel matrix and negatively affects consumer perception, as the consumer thinks that the product is deteriorated (Lobato Calleros *et al.* 2014). Other type of additives such as starches have been used to achieve fat mimetic properties by retaining substantial quantities of water into weak gel structures (Luo and Gao 2011). Likewise, Bravo-Núnez *et al.* (2019) tested modified and pre gelatinised starches in low-fat yogurts and found, for some samples, higher syneresis values when compared to the control samples.

- *Colour*

The addition of acacia gum influenced the colour of the product (P<0.05). Samples containing 3 % acacia gum were darker, while those containing levels of 2 and 3% tended to be redder and yellow (Table 3).

In the perception and assessment of the overall appearance of food, colour is the most important quality attribute and influences consumer's acceptability. An undesirable colour may therefore lead to a poor acceptance and a lower market value (Wang *et al.*2018). Talib *et al.* (2018) have observed no significant difference among tasters in the sensory perception of colour in samples of yogurt containing acacia gum.

- *Texture*

The addition of acacia gum to the formulations did not influence the parameters (P>0.05) firmness, adhesiveness, cohesiveness, gumminess and elasticity (Table 4 and 5).

Pimentel (2009) found that the addition of inulin decreased the firmness in yogurt. The authors attributed these lower values due to an incompatibility between the polysaccharides and the milk proteins, causing the repulsive forces to generate exclusion of the protein molecules from the domain occupied by the polysaccharides, resulting in a decrease in protein concentration for gel formation and, consequently, a lower firmness of the gel.

In general, probiotic strains are selected on the basis of their safety, nutritive value and health-promoting properties, besides other valuable properties that

Table 3. Color parameters L*, a* and b* of fermented milks with different inclusion levels of Acacia Gum, days of storage and interaction between both

Acacia Gum (%)	Shelf life (days)				Average	SE	P
	1	7	14	21			
				L*			
0	84.89	84.77	84.79	84.79	84.81[a]	0.685	<0.0001[1]
1	84.36	84.16	84.34	84.02	84.22[ab]		0.5855[2]
2	84.19	83.73	83.59	81.85	83.34[b]		0.4557[3]
3	81.13	81.76	80.01	81.78	81.17[c]		
Average	83.64	83.61	83.18	83.11			
				a*			
0	0.58	1.81	1.71	1.35	1.36[b]	0.084	0.0014[1]
1	1.02	1.03	1.77	1.71	1.38[b]		<0.0001[2]*
2	0.73	1.91	1.36	2.41	1.60[a]		<0.0001[3]
3	0.98	1.61	1.69	1.95	1.56[a]		
Average	0.83	1.59	1.63	1.86			
				b*			
0	7.86	11.13	10.96	9.57	9.88[b]	0.320	<0.0001[1]
1	9.54	8.96	10.96	10.93	10.10[b]		<0.0001[2]**
2	8.22	11.44	10.18	13.36	10.80[a]		<0.0001[3]
3	9.43	11.53	11.65	12.70	11.33[a]		
Average	8.76	10.77	10.94	11.64			

[1]Probabilities in relation to percentage inclusion of gum acacia; [2]Probabilities in relation to storage times; [3]Probability of interaction between gum acacia percentage and storage times; *$\hat{Y}=0.776 + 0.117x - 0.0032x^2$ ($r^2 = 57.50$); **$\hat{Y} = 9.139 + 0.129x$ ($r^2 = 41.11$)

Table 4. Texture and acidity of fermented milk with different inclusion levels of gum acacia

Parameters	Levels of inclusion of acacia gum (%)				SE	P
	0	1	2	3		
Acidity (D)	10.37	10.03	10.10	10.54	0.472	0.8561
Firmness (N)	0.32	0.30	0.31	0.30	0.010	0.5471
Adhesiveness (g)	11.87	15.00	22.50	10.12	3.233	0.0696
Cohesiveness	0.92	0.89	0.96	0.96	0.082	0.9074
Gummy (g)	31.00	27.25	31.00	29.75	3.268	0.8301
Elasticity (mm)	2.37	2.53	2.35	2.52	0.257	0.9338

Table 5. Texture and acidity parameters of fermented milk during shelf life

Parameters	shelf life (days)				SE	P
	1	7	14	21		
Acidity (D)	9.30	10.44	10.56	10.74	0.472	0.1698
Firmness (N)	0.31	0.31	0.32	0.29	0.010	0.2465
Adhesiveness (g)	15.62	16.37	13.75	13.75	3.233	0.9148
Cohesiveness	0.89	0.95	0.97	0.92	0.082	0.9090
Gummy (g)	28.37	30.75	32.12	27.75	3.268	0.7610
Elasticity (mm)	2.22	2.33	2.87	2.36	0.257	0.3166

may influence the shelf-life, texture and appearance of the probiotic yogurt.

Some probiotic bacteria grow slowly in milk due to the lack of proteolytic activity, and their acidifying characteristic may affect the texture of the final product. A poor acidification performance in milk reduces the acid accumulation during storage and produces exopolysaccharides, which may provide a better texture. Microbial exopolysaccharides may improve the texture of fermented products as they serve as emulsifying or gelling agents, thickening and stabilising the product (Fazilah *et al.* 2018).

According to Oliveira (2008), the presence of acacia gum and inulin did not modify the elasticity and firmness of yogurts for 64 days, obtaining lower values when compared to those of our study (0.09 ± 0.04N/mm vs 0.17 ± 0.03N/mm for elasticity and 0.20 ± 0.05N vs 0.27 ± 0.04N for firmness).

Acacia gum readily dissolves in hot water and results in low-viscosity solutions; it can be used in concentrations of up to 10 % as soluble fibre without modifying the texture (Phillips *et al.* 2007). Due to its low viscosity in solution, acacia gum does not influence the texture parameters, which was observed in our study, using a maximum concentration of 3 %.

- *Sensorial analyses*

Most tasters preferred the formulation with 3 % acacia gum; 63 % of the tasters on day 1, 56 % on day 7, 72 % on day 14 and 70 % on day 20. However, when asked about the frequency of consumption, only 44 % of the tasters, on average, said they always consumed yogurt (Table 6).

The products had a characteristic colour, without lumps and with a homogeneous appearance, with a mildly acidic flavour. The variable "global appearance" differed significantly among the samples, with the highest scores for the formulation with 3 % gum on the first day and with 2 % and 3 % gum at 7 and 14 days, respectively. There was no significant difference among the samples for aroma and colour attributes (P>0.05).

Table 6. Sensory evaluation of fermented milk formulations containing acacia gum during the shelf life of the product

Days	0 %	1 %	2 %	3 %
1	1,84[a]	2,02[a]	2,38[a]	2,86[b]
7	2,70[a]	2,68[a]	3,40[b]	3,40[b]
14	2,56[a]	2,76[a]	3,24[b]	3,56[b]
21	3,08[a]	3,32[a]	3,48[a]	3,46[a]

Means followed by the same letter, in the same line, do not differ among themselves by the Tukey test at 5% probability.

The overall appearance is translated by the "set", relating to the first impression caused by the product, without representing the average of the scores of the other characteristics evaluated.

Previous studies have shown variations in the acceptance of yogurt samples with probiotic and prebiotic ingredients. An experiment to determine the effects of different acacia gum (AG) concentrations (0.5; 1.0 and 1.5 %) on organoleptic and physicochemical properties of yogurt was conducted using fresh cow's milk with 3 % starter culture. The results showed that the organoleptic properties of the yogurt increased as the percentage of AG increased in all tested samples. Increasing the amount of AG concentrations in the milk significantly decreased the clotting time in all treatments. Yogurt produced with low concentrations of gum had longer coagulation times (100 - 110 min), while curd obtained with high concentrations of AG was firmer. An AG concentration of 1.5 % was ideal in terms of the previous characteristics in the manufacturing of yogurt, scoring highly for 85.3 % of the respondents (Talib 2018).

Oliveira and Jurkiewicz (2009) verified that probiotic yogurts made with the prebiotics inulin and acacia gum obtained an acceptance between 7.4 and 7.6 on a hedonic 9-point scale. For Capitani *et al.* (2014), evaluating yogurt containing probiotics and polydextrose, the scores attributed by the tasters in evaluating the overall impression of the yogurts ranged from 4.86 to 6.71 and from 5.57 to 6.91 on the 1st and 14th day of the manufacturing process, respectively.

When preparing fat-reduced or fat-free yogurts, the lack of fat globules results in textural changes that adversely affect mouthfeel and are not desired by consumers, as mouthfeel is an important parameter when it comes to yogurt product quality (Bravo-Núnez *et al.* 2019).

The consumers preferred the formulations with higher acacia gum contents, indicating the viability of this substance in the production and commercialisation of yogurt.

CONCLUSIONS

Yogurt with acacia gum was well accepted by the tasters, resulting in satisfactory physico-chemical characteristics and the maintenance of cellular viability, indicating its potential use in yogurt production.

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