An Acad Bras Cienc (2024) 96(1): e20230658 DOI 10.1590/0001-3765202420230658
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

MICROBIOLOGY

A Novel Approach to the Use of Xanthan Gum: Evaluation of Probiotic Promoter, Postbiotic Formation and Techno-Functional Effect

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Abstract: In the present study, the effect of xanthan gum was evaluated on the metabolic activity and survival of two probiotic strains, namely *B. lactis* and *L. casei* using *in vitro* assay and skim milk model system. *In vitro* assay was carried out identifying by pH, optical cell density (OD), and formation of postbiotics (lactic, acetic, propionic, and butyric acids) in different basal media including glucose, inulin, and xanthan gum as carbon source. The highest pH values were recorded for control (without carbon source) and media with xanthan gum, whereas the media with glucose and xanthan gum had the highest OD values. In comparison to strains, *B. lactis* had higher pH and lower OD values than *L. casei*. It was found that xanthan gum supported the formation of postbiotics as a result of bacterial fermentation. In the skim milk model system, xanthan gum did not negatively affect probiotic viability, and the counts of both strains were above the required level for health benefits (8 log cfu g^{-1}) after 28-day storage. The use of xanthan gum in skim milk matrix positively affected techno-functional properties such as syneresis, color, and textural parameters of samples.

Key words: Xanthan gum, *in vitro,* milk, postbiotic, probiotic, promoter.

INTRODUCTION

Xanthan gum is a bio and microbial exo-polysaccharide obtained from various carbon sources by fermentation using *Xanthomonas* species such as *X. campestris, X. pelargonii, X. phaseoli,* and *X. malvacearum*. It is formed by a β-D-glucose backbone with every second glucose unit linked through a trisaccharide consisting of D-mannose, D-glucuronic acid, and D-mannose. D-mannose has carboxyl groups such as acetic acid and pyruvic acid on the side chains, and thereby giving anionic properties to the gum. It is identified as a GRAS food additive (E 415) by the European Union and the United States Food and Drug Administration (FDA). It has demonstrated techno-functional properties such as biodegradability, high viscosity, and solubility at low concentrations in acidic, alkaline and salt solutions, pseudoplastic behavior, excellent thermal stability, good mouthfeel, the ability to extend shelf life, potential antioxidant activity, and good freeze-thaw stability. Therefore, it has been used by the food industry for as thickener, stabilizer, emulsifier, gel former, fat replacer, as well as encapsulation agent. Moreover, since the backbone of the gum is a cellulosic β-(1,4)- D-glucan, it is resistant to variations in temperature, pH, and shear stress and to degradation by various enzymes (GarcõÂa-Ochoa et al. 2000, Rather et al. 2015, Habibi & Khosravi-Darani 2017, Vega-Sagardía et al. 2018, Diantom et al. 2020).

When taking into account the complex heteropolysaccharide structure and enzymatically undegradable property of xanthan gum, it may show a promoter effect

on the metabolism and growth of probiotic microbiota and might be considered as a candidate prebiotic with industrial importance. Even though probiotics are defined as live microorganisms that have beneficial effects on the health of the host when consumed in sufficient quantities, paraprobiotics, also termed inactive, non-living microbial cells and ghost probiotics, have gained importance in recent years due to their therapeutic properties (Hill et al. 2014, Omak & Yilmaz-Ersan 2022). *Lactobacillus* spp. and *Bifidobacterium* spp. are probiotic bacteria generally regarded as safe. Prebiotics are described as indigestible foods and/or food compounds that are selectively metabolized by beneficial microbiota mainly the Lactobacilli and Bifidobacteria in the large intestine and thus they positively affect the well-being and health of hosts (Gibson et al. 2017). Recently, postbiotics, a new probiotic and prebiotic-related term, are gaining importance due to their beneficial effects on human health and their multiple functional impacts on food qulity. As non-viable bacterial products, cell constituents, or metabolic by-products secreted by live bacteria or released after bacterial lysis are classified as postbiotics, they are also named as biogenics, cell-free supernatants, metabolomics, metabolites, metabiotics, and metabolic by-products of probiotic activity. Postbiotics include short-chain fatty acids (SCFAs; acetic, butyric, and propionic acids), organic acids (lactic and 3-phenyllactic acid), carbohydrates (secreted and/or extracellular polysaccharides, galactose-rich polysaccharides and teichoic acid), cell surface proteins, peptides (p40, p75, Lactocepin and peptidoglycan-derived muropeptides), bacteriocins (acidophilin, bifidin, and reuterin), enzymes and vitamins (B-vitamins). Short-chain fatty acids (SCFAs), the most important postbiotics formed during probiotic fermentation, have biological functions such as reducing the risk of intestinal infections, increasing mineral bioavailability, immunomodulation, hypocholesterolemic, antitumorigenic, antiinflammatory, antiproliferative, antiobesogenic, antiallergic, and antioxidant effects (Aguilar-Toalá et al. 2021, Salminen et al. 2021, Omak & Yilmaz-Ersan 2022, Anvar et al. 2023, Asif et al. 2023). However, recent studies have focused on the utilization of postbiotics produced by probiotics as a food additive owing to their health and technological effects (Mogahed Fahim et al. 2021, Hosseini et al. 2022, Darwish et al. 2022, Sharafi et al. 2022, Anvar et al. 2023, Pimentel et al. 2023).

The beneficial health effects attributed to bacterial cell mass, postbiotics (lactic acid, short chain fatty acids), and gases (CH_4, CO_2, H_2,) are formed by fermentation of prebiotics. Acetic, propionic and butyric acids with multiple health effects are the most common short-chain fatty acids (SCFAs) via saccharitic microbial fermentation. Current researchers have focused on producing novel ingredients with potential prebiotic activity from various sources. In order to investigate the prebiotic potential of a substrate or food, firstly an *in vitro* model simulating fermentation processes is used, which provides a good system for small-scale screening of novel substrates. The metabolism and growth of probiotic bacteria in this model are performed by measuring optical density, pH, acidity, counting viable cells, and analyzing the quantity of postbiotics such as lactic acid and SCFAs. Secondly, prebiotic activity is confirmed by controlled animal and human clinical trials, mainly *in vivo* (de Souza Aquino et al. 2017, Khangwal & Shukla 2019).

However, research into the probiotic promoter effect and fermentation characteristics of xanthan gum has not yet been implemented in detail. Previous studies mainly focused on the utilization of dairy products as a fat

replacer (Nateghi et al. 2012, Murad et al. 2016, Murtaza et al. 2017), probiotic encapsulation (Chen et al. 2017, Nguyen et al. 2017, Tantratian et al. 2018) or a stabilizer (Mohsin et al. 2019). The objective of this research was to identify the fermentability of xanthan gum by some probiotic strains. The probiotic promoter effect was studied in both basal media and skim milk matrix with xanthan gum. The metabolism and survival of strains were tested by determining the pH, optical density (OD), and quantity of postbiotics (lactic, acetic, propionic, and butyric acids) in basal media. Furthermore, the present study investigated probiotic-growth promoter and techno-functional effect (e.g. stabilizer, fat replacer) of xanthan gum in the skim milk matrix during storage at 4°C for 28 days.

MATERIALS AND METHODS

In vitro model system

Bacterial basal media preparation and inoculation

Bifidobacterium animalis subsp. *lactis* and *Lactobacillus casei* were purchased from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). Both strains were activated at 37°C using an anaerobic condition (Anaerocult A, Merck, Germany). Xanthan gum was obtained from the As Food Co., (Istanbul, Turkiye). To test the effect of xanthan gum on the growth of *B. lactis* and *L. casei*, the basal carbohydrate-free medium, Tryptone Peptone Yeast Extract (TPY) for *B. lactis* and a De Man Rogosa ve Sharpe (MRS) broth for *L. casei* were prepared (Table I).

Both media were sterilized at 121°C for 15 min. Stock solutions of xanthan gum were prepared in distilled water and 0.45 µm-filter-sterilizer (Millipore-Stericup-GP). In order to evaluate the effect of xanthan gum on metabolic activity and viability of *B. lactis*, based on the TPYcomposition, five different basal media were prepared using TPY without carbon source (negative control), TPY+ glucose (0.50% w/v; Merck, Germany) and TPY + inulin (0.50% w/v Orafti® HSI, Belgium) as positive controls, and lastly TPY + 0.25% and 0.50% w/v xanthan gum (test substrates). Similarly, in order to investigate the effect of xanthan gum on *L. casei*, five different basal media were prepared using MRS broth composed of glucose (0.5% w/v), inulin (0.5% w/v), xanthan gum (0.25 and

Table I. TPY and MRS Broth used as the basal medium.

Carbohydrate-free TPY broth		Carbohydrate-free MRS broth	
Components	**g L^{-1}**	**Components**	**g L^{-1}**
Yeast extract	2.50	Yeast extract	5.00
Peptone from meat	5.00	Tryptone	10.0
Tween 80	1.00	Peptone from meat	10.0
$K_2HPO_4.3H_2O$	2.00	Tween 80	1.00
$MgCl_2$	0.50	$K_2HPO_4.3H_2O$	2.00
$ZnSO_4.7H_2O$	0.20	Na- acetate	5.00
$CaCl_2$	0.15	Ammonium citrate	2.00
$FeCl_3.6H_2O$	0.003	$MgSO_4.7H_2O$	0.20
L-cysteine HCl	0.50	$MnSO_4.4H_2O$	0.05

0.50% w/v) and without carbon source (negative control). 2% (v/v) of overnight probiotic strains were inoculated into the TPY and MRS broth supplemented with glucose, inulin (0.5%), and xanthan gum (0.25 and 0.50%) as the sole carbon source. Bacteria were grown in 50 mL test tubes prepared separately for each sample and each fermentation time in anaerobic conditions (Anaero Gen Gas Packs, Oxoid, Basingstoke, UK). Each substrate concentration for bacterial strains was carried out with at least 4 replicates throughout *in vitro* assays.

pH assay

The pH measurement for each sample was performed at 0, 12, 24, 36, and 48 hours of fermentation using a pH meter (pH 315i / SET; WTW, Germany).

Optical density (OD) assay

The optical cell density of samples was measured via absorbance at a wavelength of 600 nm with a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan) at 0, 12, 24, 24, 36, and 48 hours of fermentation. The sterile TPY and MRS broth without bacteria were used as blanks for OD measurements.

Prebiotic activity score (PAS) assay

In this assay, TPY and MRS broth supplemented with 0.50% xanthan gum and glucose were prepared for *B. lactis and L. casei*, respectively. A tryptic soy broth supplemented with 0.50% of xanthan gum and glucose was prepared for *Escherichia coli* as the representative enteric species. Afterward, 1% of the overnight culture for each strain was added to a TPY, MRS, and TSB medium with 0.50% xanthan gum and glucose. Test cultures were incubated at 37°C for 24 hours in anaerobic conditions. Samples were taken at 0 and 24 hours of fermentation, and the cell counts were determined by using TPY agar for *B. lactis*, MRS agar for *L. casei* and Tryptic Soy Agar for *E. coli*. PAS was calculated according to Huebner et al. (2007).

$$\frac{\left[\left(\text{Probiotic cell count}_{24\text{ h xanthan gum}}\right)-\left(\text{Probiotic cell count}_{0\text{ h xanthan gum}}\right)}{\left(\text{Probiotic cell count}_{24\text{ h glucose}}\right)-\left(\text{Probiotic cell count}_{0\text{ h glucose}}\right)\right]}$$

$$-\frac{\left[\left(\textit{E. coli}\text{ cell count}_{24\text{ h xanthan gum}}\right)-\left(\textit{E. coli}\text{ cell count}_{0\text{ h xanthan gum}}\right)}{\left(\textit{E. coli}\text{ cell count}_{24\text{ h glucose}}\right)-\left(\textit{E. coli}\text{ cell count}_{0\text{ h glucose}}\right)\right]}$$

Analytical methods for postbiotics

Postbiotics such as lactic acid and SCFAs were evaluated in media with glucose (0.50%), inulin (0.50%), xanthan gum (0.25 and 0.50%), and without carbon source (negative control) after 48-hour fermentation. Standards for postbiotics were obtained from Merck, Germany. The samples (50 mL test tube) for postbiotic analysis were filtered through a 0.45 µm syringe filter. High-Performance Liquid Chromatography (HPLC, Shimadzu Prominence, Japan) equipped with a Diode Array Detector (DAD, SPD-M20A) and Shimadzu CTO-10ASvp Column was used for the lactic acid assay. An orthophosphoric acid, adjusted pH 3, was used as an eluent at a flow of 1 mL min^{-1}. The amount of lactic acid was calculated with the assistance of an LC solution computer program. Analysis of short-chain fatty acids (acetic, propionic, and butyric acids) was carried out using Headspace Gas Chromatography (HS-GCMS, Agilent 7697A Headspace, USA). The filtered sample (20 µL) was injected into HS-GCMS. The column temperature was first set at 35°C for 5 min and then at 150°C and maintained for another 5 min. The system was set to the following conditions: detector and injector temperatures, 200° C and 240° C; fluent speed, 25 psi (He); needle, 90° C; transfer line, 120° C; vial oven, 85° C; thermostat time, 5 min.; pressurize time, 0.5 min.; inject time, 0.08 min. and withdraw time, 0.5 min. Relative standard deviation values for the responses of the retention times obtained from standard solution mixtures containing different levels of each acid were calculated for

the verification of the method. Retention time (RT), coefficient of correlation (R2), and LOD for acetic acid were 24.5, 0.999, and 1.46, respectively. RT, R2 and LOD for propionic acid were 26.7, 0.999, and 1.82, respectively. RT, R2 and LOD for butyric acid were 26.8, 0.999, and 0.86, respectively. Lactic acid and SCFAs values calculated as ppm (mg L^{-1}) were converted to g L^{-1}.

Skim milk model system

Production of skim milk model system with xanthan gum

Freeze-dried *Bifidobacterium lactis* (DANISCO, Sassenage, France) and *Lactobacillus casei* (Chr-Hansen, Istanbul, Turkiye) were used in the milk model system. Reconstituted sterile non-fat milk (12 g 100 mL^{-1}) was autoclaved at 121°C for 15 min for culture activation. The strains were inoculated in sterile reconstituted skim milk and incubated at 37±1° C anaerobically (Anaerobic System Anaerogen, Oxoid, Basingstoke, UK). Skim milk powder was reconstituted in distilled water at 10.70%. Four groups of milk model systems were prepared by using probiotic strains and xanthan gum; BL (milk with *B. lactis),* BLG *(*milk with *B. lactis* and 0.25% xanthan gum), LC (milk with *L. casei)* and LCG (milk with *L. casei* and 0.25% xanthan gum). Maximum levels of xanthan gum (E 415) as food additive in unflavoured fermented milk products are defined as quantum satis (Mortensen et al. 2017); various studies have reported this gum may be used in dairy products in a concentration range of 0.05-0.3% (El-Sayed 2002, Bahrami et al. 2013, Karlton-Senaye et al. 2015, Murad et al. 2016, Habibi & Khosravi-Darani 2017, Murtaza et al. 2017, Nguyen et al. 2017). In light of this information, in a preliminary study, several parameters were determined using various concentrations of xanthan gum (0.05; 0.10; 0.15: 0.20; 0.25; 0.30%). Based on the preliminary experimental data, a 0.25% concentration was selected for this study. Reconstituted milk was divided into two experimental batches, one of which was supplemented with xanthan gum to final concentrations of 0.25% w/v. The gum was completely dissolved by continuous stirring and each batch was subjected to pasteurization at 90° C for 10 min and subsequently cooled to 37° C. Samples were transferred into 100-mL plastic containers and were inoculated with the probiotic starter cultures. The inoculum levels of each bacterium were determined according to the standard plate count agar method to give a final concentration of approximately 8–9 log cfu mL^{-1} in milk. The samples inoculated with the probiotic starter were incubated at 37° C until the final pH of 4.7 was achieved. They were cooled and stored for 28 days. They were then analyzed for probiotic viability, and techno-functional properties (titratable acidity, syneresis, color, and textural attributes) at days 1, 7, 14, 21, and 28 of cold storage (4° C.)

Enumeration of probiotic viability in milk model system

For analysis, 10 g of each sample was homogenized with 90 mL of a sterile physiological saline solution for 2 min. in a Stomacher bag (Seward, Theford, Norfolk, UK). Serial decimal dilutions were prepared by adding 1 mL to 9 mL sterile physiological saline solution. *B. lactis* and *L. casei* counts were enumerated according to the method reported by Tharmaraj & Shah (2003) using Man, Rogosa and Sharpe agar (MRS; Merck, Germany). The plates were incubated at 37° C for 72 h under anaerobic conditions (AnaeroGen Gas Packs, Oxoid, Basingstoke, UK). Plates containing 30–300 colonies were counted and converted into Log colony-forming units (cfu g^{-1}). At the end of storage time, the viability proportion index (VPI) of microorganisms was calculated according to the following formula:

$$VPI = \frac{\text{Final cell population (cfu g}^{-1})}{\text{Initial cell population (cfu g}^{-1})}$$

Titratable acidity assay

10 g sample was titrated with 0.1 N NaOH to a phenolphthalein endpoint (to pH 8.2) and expressed as the percentage lactic acid equivalent (AOAC 2005).

Syneresis assay

25 g sample was weighed and then filtered through Whatman filter paper at 4°C for 2 h at syneresis analysis. The syneresis was determined as the collected whey divided by the initial sample weight (mL 25 g^{-1}) (Ozcan et al. 2021)

Instrumental color assay

Color analysis of samples was performed by using a Minolta Chromameter (Konica Minolta Co., Ltd., Osaka, Japan) calibrated with a white calibration plate as specified by the manufacturer. In the CIELab color scale, L* parameter ranges from 0 (black) to 100 (white), a* shows from red (+a*) to green (–a*), and b* varies from yellow (+b*) to blue (–b*).

Instrumental texture assay

Textural characteristics of samples were evaluated instrumentally using a texture analyzer TA-XT Plus (Stable Micro System Ltd, Model TA-XT plus, Surrey, UK). Before textural analysis, the samples were left at room temperature (20°C). Textural attributes of samples in 100 mL plastic containers were determined by the back extrusion method fitted with a 5 kg load cell. A 40-mm-diameter cylinder probe was used to measure the texture profile of yogurt and penetrated the samples to 75% of their original depth. The speed of the probe was fixed at 0.1 mm s^{-1} during the pretest compression and relaxation of the samples. The distance of penetration from the surface of the sample was set at 20 mm. Firmness, consistency, cohesiveness, and viscosity indexes were measured using the Texture Expert Exceed software (v 2.55) extracted from the resulting force-time curves. All measurements were carried out in triplicate (Patrignani et al. 2007).

Statistical analysis

The results obtained from the present study were statistically evaluated using analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSDs) method ($P<0.05$ and $P<0.01$, Minitab 17, USA). Statistically significant differences were indicated using different letters. Hierarchical cluster analysis was performed following an unweighted pair group method with an arithmetic average based on a dissimilarity matrix. In order to analyze the relationship among techno-functional properties of skim milk matrices, the Pearson Correlation Coefficient (r) was calculated on individual data using statistical software (IBM SPSS Statistics, USA).

RESULTS AND DISCUSSION

Acidity and probiotic activity in the *in vitro* system

A decrease in pH occurs as a result of the fermentation of various carbon sources by strains in the *in vitro* system, reflecting the accumulation of postbiotics such as lactic acid and SCFAs. Two probiotic strains, *B. lactis* and *L. casei*, were selected for the fermentation studies. These species probably differ in their capability for growing on xanthan gum-derived components. Figure 1 shows that the pH values of the medium decreased during fermentation. Throughout fermentation, average pH values for *B. lactis* varied between 5.01 and 6.55. pH values for *B. lactis* in the medium with inulin and 0.50% xanthan gum were found statistically similar at the 24-hour fermentation, whereas these values showed a decrease after 24-hour fermentation.

In the present study, it was found that the pH values identified for *B. lactis* were similar to those values revealed in the study of Polari et al. (2012) in which they used galactoglucomannan extracted from spruce (Picea abies) as a carbohydrate source. Average pH values for *L. casei* ranged from 5.09 to 6.47 and showed a significant decrease during fermentation (Figure 2). It was found that the pH values identified in the 0.25% and 0.50% xanthan gum medium fermented with *L. casei* were lower than the negative control sample. It was also found that it had the highest pH value for *L. casei* at the end of fermentation. Of both species compared with media containing xanthan, *L. casei* could develop acidity by activating in the media containing xanthan gum and reached the lowest pH during the 48-hour fermentation due to a higher capacity to produce acids. All of the *in vitro* fermentation systems for both strains showed a significant decrease in their pH values after 48 hours, which is most probably due to fermentation metabolites such as lactic acid and SCFAs. The decrease in pH during fermentation, preventing the growth of harmful microorganisms and increasing the survival of beneficial ones leads to positive results in the colonic ecosystem. It was found that due to the structural properties of xanthan gum, pH values in the present study were above those values previously identified by Yilmaz-Ersan et al. (2018) and Karlton-Senaye & Ibrahim (2013).

Figures 1 and 2 show that the OD values of the medium were correlated with increased

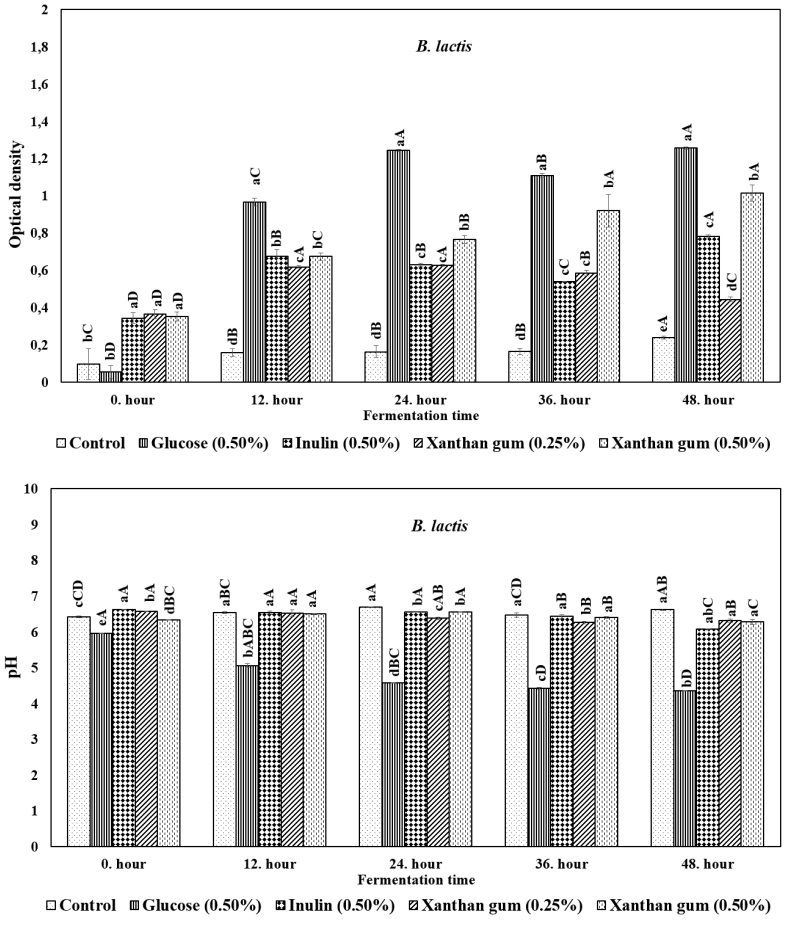

Figure 1. pH and OD values of *B. lactis*.

B. lactis, whereas those of *L. casei* showed an increase during 24-hour fermentation. It was found that *B. lactis* in media containing 0.50 % xanthan gum had the highest cell density value compared to media with glucose, which may give the bifidogenic potential of xanthan gum. *B. lactis* in the media with 0.25 % xanthan gum reached the lag phase after 24-hour fermentation, whereas *B. lactis* in media containing 0.50 % xanthan gum reached the lag phase after 36-hour fermentation (Figure 1). It was found that the cell density values identified for *B. lactis* in this study were similar to the values found in studies by García-Cayuela et al. (2014) and Usta & Yilmaz-Ersan (2017) in which the growth of *B. lactis* was measured in different substrates. When the results were examined, it was found that in the MRS broth containing 0.25 and 0.50% xanthan gum, *L. casei* had a higher cell density than inulin (positive control) (Figure 2). *L. casei* can develop in media containing xanthan gum as well as inulin. The increase in the cell density of *L. casei* may be related to the high amounts of β-D-glucose in xanthan gum. Alarifi et al. (2018) tested the efficacy of gum acacia and fructooligosaccharide in batch cultures inoculated with human feces over 48 hours. They found that gum acacia caused an increase in *Bifidobacterium* spp. and *Lactobacillus* spp. similar to those of fructooligosaccharides. In addition, it was revealed that the 1% concentration of substrates demonstrated more increased selectivity compared to a 2% concentration. The opposite result was observed by Modrackova et al. (2019) in that *Bifidobacterium* species were tested for

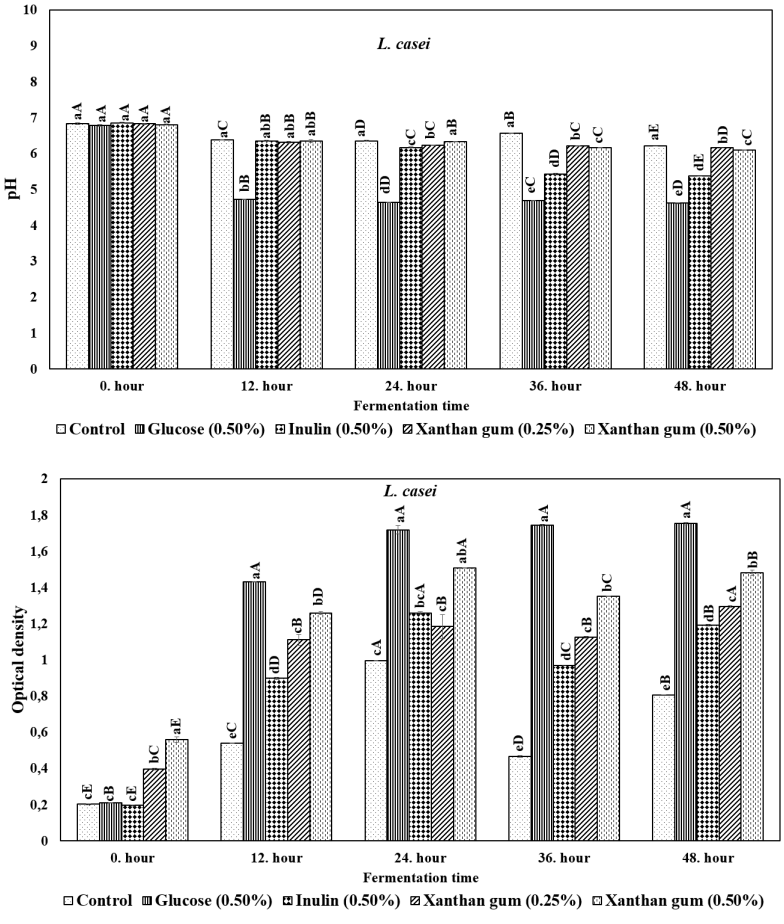

Figure 2. pH and OD values of *L. casei*.

their ability to utilize xanthan, tragacanth, locust bean, guar, arabic, and karaya gums. They found that gum utilization was found to be species and strain-dependent and that xanthan and karaya gums were not suitable as a potential prebiotic source for bifidobacterial strains tested in their study.

Table II shows pH and OD values after the five fermentation times (0, 12, 24, 36, and 48 hours) in anaerobic culture. The results are presented as the mean value of each fermentation time, regardless of the different carbon sources and probiotic strains. There were significant differences (P<0.01) within the level of pH and OD values for different carbon sources, strains, and fermentation times. Significant interactions among parameters were detected (P<0.01). Regarding the carbon source, the highest pH values were recorded for negative control and media with xanthan gum. As pH is a function of the total hydrogen ions (H^+) formed from acid production in the media, the alkaline property of xanthan gum may react with H^+ to neutralize acidity and thereby high pH values might be

Table II. The pH and OD values of *B. lactis* and *L. casei* grown on different carbon sources during 48-hour fermentation.

Carbon sources	N	pH	OD
Negative Control (without carbon source)	30	6.53^{a}	0.311^{d}
Glucose (0.50 %)	30	5.05^{c}	1.075^{a}
Inulin (0.50 %)	30	6.24^{b}	0.675^{c}
Xanthan gum (0.25%)	30	6.38^{ab}	0.702^{c}
Xanthan gum (0.50%)	30	6.39^{a}	0.915^{b}
Strains			
B. lactis	15	6.14^{a}	0.591^{b}
L. casei	15	6.05^{b}	1.025^{a}
Fermentation time (hour)			
0	30	6.53^{a}	0.258^{d}
12	30	6.15^{b}	0.774^{c}
24	30	6.11^{b}	0.855^{b}
36	30	5.95^{bc}	0.860^{ab}
48	30	5.86^{c}	0.931^{a}
ANOVA			
Carbon sources		**	**
Strains		**	**
Fermentation time		**	**
Carbon sources x Strains		**	**
Carbon sources x Fermentation time		**	**
Strains x Fermentation time		**	**
Carbon sources x Strains x Fermentation time		**	**

Significance level, significant at P<0.01(**), different lowercase on the same column indicate significant differences for different carbon sources , strains and fermentation time.

detected in the media with xanthan gum. The highest OD values were detected in media with glucose and xanthan gum. This suggests that xanthan gum is used by strains as a carbon source. When compared with the probiotic strains, *B. lactis* had higher pH and lower OD values than *L. casei*. A significant decrease in the pH *L. casei* was matched by increases in OD values, which means that xanthan gum provides a better growth-promoting effect for *L. casei*. The pH values decreased significantly, while OD values increased during the 48-hour fermentation as a result of the bacterial enzymes. García-Cayuela et al. (2014) found that the OD values of *B. lactis* BB-12, *B. breve* 26M2, and *B. bifidum* HDD541 with the different carbohydrates including lactulose, lactulosucrose, lactosucrose, glucose, kojibiose, and 40-galactosyl-kojibiose used as carbon sources varied between 0 and 1.21 after 48-hour fermentation. The metabolic activity and growth of probiotics are affected by chemical structure, composition of monomer units, degrees of polymerization, and water solubility of carbohydrate sources (Voragen 1998).

PAS measures changes in cell counts and/or cell density values of probiotics to those of opportunistic microorganisms as a quantitative score. A positive PAS obtained in testing different carbon sources indicates that carbon sources are fermented by probiotic bacteria preferably better than glucose, and increases probiotics but not other undesirable bacteria such as *Enterobacterium* spp. (Omak & Yilmaz-Ersan 2022). PAS of xanthan gum and inulin versus glucose for *B. lactis* and *L. casei* were investigated, and the species showing positive PAS are shown in Figure 3. The PAS for both strains was found to be higher than zero, which refers to the fact that growth of the strains is higher for xanthan gum compared to glucose, and higher than the reference bacteria (*E. coli*). Furthermore, it was found that PAS for *L. casei* in media with xanthan gum was higher than that of inulin, whereas for *B. lactis* PAS was lower than inulin.

Postbiotic formation in the *in vitro* system

Postbiotics are secondary metabolomics formed by probiotics, which provide beneficial health effects on the human. Especially, lactic acid and SCFAs formed via the glycolytic pathway during *in vitro* and *in vivo* fermentation offer beneficial health effects such as antitumorigenic, antiobesogenic, antiallergic, reduction of intestinal infections and increasing mineral bioavailability (Omak & Yilmaz-Ersan 2022). In this study, for both strains, lactic acid was the most abundant metabolite produced. Figure 4 illustrates the lactic acid concentrations of *B. lactis* and *L. casei* in media including different carbon sources after 48-hour fermentation. The quantity of lactic acid varied from 1.10 g L^{-1} of *B.*

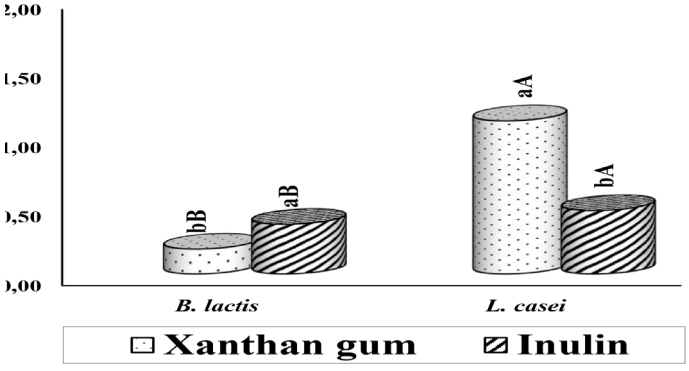

Figure 3. PAS values for *B. lactis* and *L. casei*.

lactis in media with glucose to 15.74 g L^{-1} of *L. casei* in media without carbon source (negative control), depending on the strain and type of substrate. More lactic acid was produced by *L. casei* than *B. lactis*. After 48-hour fermentation, there were significant differences in total SCFA values among strains depending on the substrate type used (Figures 4 and 5). Acetic acid used as an energetic substrate in muscle tissue is the most abundant SCFA produced by human colonic microbiota (Fukuda et al. 2012, Usta-Gorgun & Yilmaz-Ersan 2020). Acetic acid concentrations of strains are shown in Figure 4. Its quantity ranged from 0.03 g L^{-1} of *B. lactis* in media with xanthan gum (0.25%) to 2.55 g L^{-1} of *L. casei* in media with glucose (Figure 4). Propionic acid, formed as a result of colonic fermentation, is metabolized by the liver. Propionic acid values varied from 0.002 g L^{-1} of *B. lactis* in media with inulin and xanthan gum (0.25%) to 0.428 g L^{-1} of the same microorganism in media with glucose (Figure 5). Butyric acid is important for the prevention and treatment of colonic diseases (Pessione et al. 2015). A maximum butyric acid value (0.50 g L^{-1}) of *L. casei* fermented in the negative control (without carbon source) was observed, while *B. lactis* fermented in media with inulin had the lowest butyric acid value (0.03 g L^{-1}) (Figure 5). Generally, higher butyric acid values were present in media with xanthan gum than with inulin (positive control).

The source and chemical structure of substrates, microorganism population, and gut transit time affect SCFA formation which is an important parameter for evaluating the fermentation capacity of microorganisms used with substrates. There are no studies concerning the formation of these postbiotics in media with xanthan gum. There were significant differences ($P<0.01$) within the level of lactic acid and total SCFAs for different carbon sources and strains (Table III). Regarding the substrate type, the highest quantity of lactic acid was recorded in the media without a carbon source (negative control) whereas glucose was the main contributor substrate for SCFAs. It was determined that higher lactic acid and total SCFAs were produced by *L. casei*. *Lactobacilli* can use the glycolytic pathway and the phosphoketolase pathway under the hetero-fermenting conditions, whereas *Bifidobacteria* are unable to use the usual glycolytic pathway or the hexose

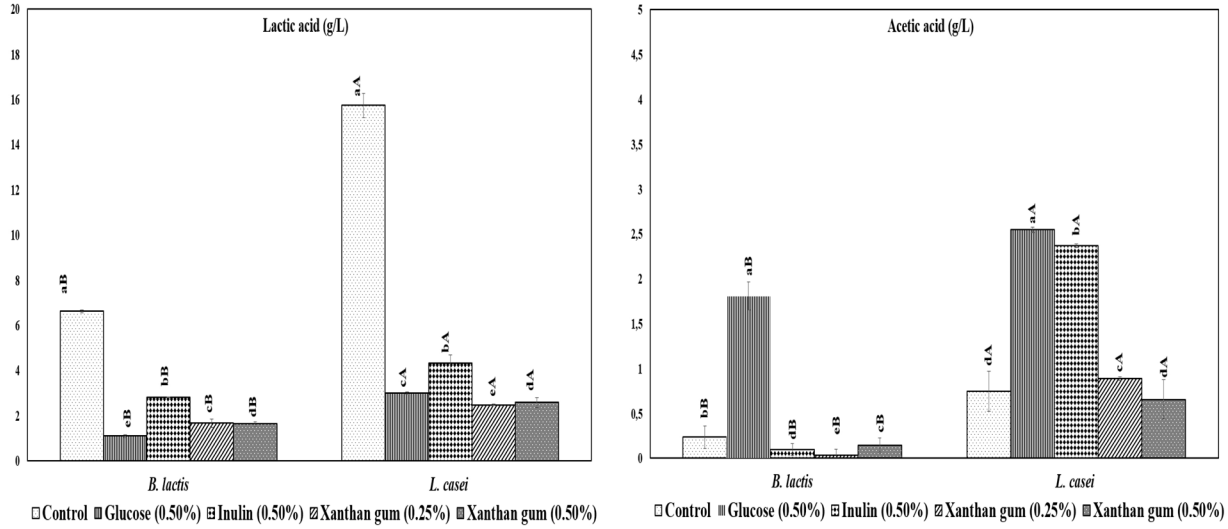

Figure 4. Lactic acid and acetic acid production by *B. lactis* and *L. casei* after 48-hour fermentation.

monophosphate shunt pathway due to a lack of aldolase and glucose-6-phosphate $NADP^+$ oxidoreductase. Differences in the metabolic pathway between strains and substrate types influenced the quantity of postbiotics. As with the lactic acid values, a significant interaction was detected ($P<0.01$) between "carbon sources and strains". Taking into account the above-mentioned results, the media without carbon source had higher total SCFAs because the majority of acids might be consumed by strains in media with xanthan gum. Xu et al. (2021) reported that high amounts of SCFA were produced during *in vitro* fermentation of human feces with xanthan gum oligosaccharides (XGOS) and curdlan oligosaccharides (COS) between 12 and 24 hours. Also, they stated that xanthan gum oligosaccharides exerted the best prebiotic effects in enriching beneficial Bacteroides and butyric acid-producing bacteria proliferation and increasing SCFA levels.

In hierarchical cluster analysis, the mathematical relationship among different carbon sources on the growth of *B. lactis* and *L. casei* is calculated using the results of attributes (pH, OD, lactic acid and SCFAs) analyzed throughout the present study (Figure 6). In respect to the cluster analysis results for *B. lactis*, inulin classified as a prebiotic source and xanthan gum revealed high similarity. As a result of the clustering analysis for *L. casei*, whereas high similarity was found between glucose and inulin, xanthan gum (0.25 and 0.50%) was closer to positive controls than negative.

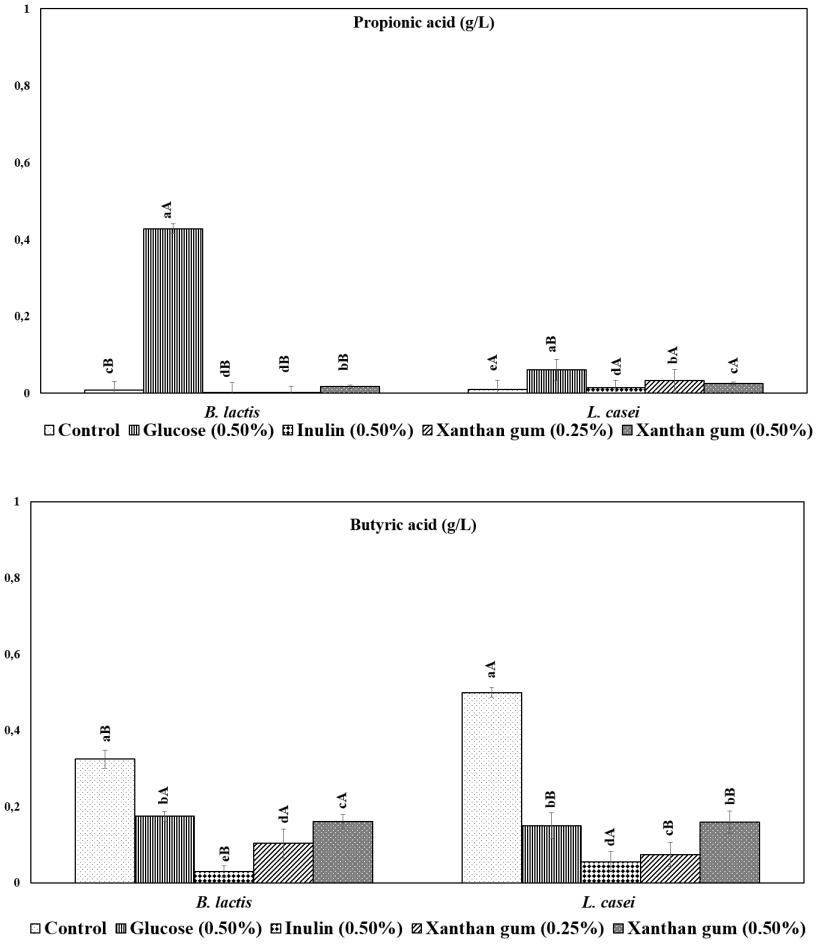

Figure 5. Propionic acid and butyric acid production by *B. lactis* and *L. casei* after 48-hour fermentation.

The classification from the highest to the lowest for both strains is as follows: control > glucose > inulin > xanthan gum (0.25 and 0.50%). These results indicated that xanthan gum could show a stimulating effect on the growth *B. lactis* and *L. casei* and their acidity and SCFAs formation ability during the *in vitro* anaerobic fermentation.

Probiotic viability in skim milk model system with xanthan gum

The utilization of xanthan gum by *B. lactis* and *L. casei* in the skim milk model system was studied during 28-day storage; the results are shown in Table IV. It was found that the viability of *B. lactis* and *L. casei* was influenced by xanthan gum except for the 7, 14, and 21 days of storage ($P<0.01$). On the first day of storage, the log number of *B. lactis* was 9.30 log cfu g^{-1} for the sample with xanthan gum and 9.44 log cfu g^{-1} for the sample without gum. It should be noted here that *B. lactis* count presented a continuous increment for up to 7 days of storage, and the highest *B. lactis* counts were observed after one week in both matrices. A steady decrease occurred in *B. lactis* counts at the end of storage where the final number reached 8.29 log cfu g^{-1} for the sample without gum and 8.05 log cfu g^{-1} for the sample with gum. *L. casei* counts were identified as 9.90 log cfu g^{-1} for the sample without gum and 9.47 log cfu g^{-1} for the sample with gum on the first day of storage. *L. casei* counts were stable during storage and maximum counts were observed at the end of storage. Although there is no universally accepted probiotic microorganism number for beneficial health effects, the recommended number ranges from 6 log cfu g and/or mL^{-1} to 8 log cfu g and/or mL^{-1} (EFSA 2010, Codex Alimentarius 2011, Mousavi et al. 2019) in the fermented product. The results of this study indicated that high levels (7 log cfu mL^{-1}) of both strains could be maintained at the end of storage; the VPI values for both strains ranged between 0.87-1.02. It was found that there was a relationship between the acidity increase and viability of *L. casei*. Generally, it was determined that the highest VPI throughout the

Table III. Lactic acid and total SCFA production by *B. lactis* and *L. casei* in media with different carbon sources after 48-hour fermentation.

Carbon sources	N	Lactic acid	Total SCFA
Negative Control (without carbon source)	4	11.180[a]	0.995[c]
Glucose (0.50%)	4	2.055[e]	2.582[a]
Inulin (0.50%)	4	3.568[b]	1.658[b]
Xanthan gum (0.25%)	4	2.075[d]	0.591[d]
Xanthan gum (0.50%)	4	2.110[c]	0.582[d]
Strains			
B. lactis	10	2.769[b]	0.712[b]
L. casei	10	5.626[a]	1.668[a]
ANOVA			
Carbon sources		**	**
Strains		**	**
Carbon sources x strains		**	**

Significance level, significant at P<0.01(**), different lowercase on the same column indicate significant differences for different carbon sources, strains and fermentation time.

storage was determined for *L. casei*. However, the stimulation effect of gum on the *L. casei* counts may be attributed to its buffering capacity due to acid and enzymatic hydrolysis. It was found that xanthan gum was found to be less helpful in the viability and activity of probiotics in skim milk matrix, presumably due to its highly branched and inherently complex structure. However, there have been only a few studies on the prebiotic potential of xanthan gum in a food matrix. Ziaolhagh & Jalali (2017) investigated the effect of kakuti or wild thyme essence and xanthan gum on the viability of *B. lactis* in dough. They reported that by increasing the amounts of xanthan gum to 0.075%, *B. lactis* count increased as well, but increasing the gum further had no significant effect on microorganism number. Karlton-Senaye et al. (2015) stated that the viability of *L. reuteri* strains in milk drink samples containing gums remained stable during the first 2 weeks of storage and then decreased slightly, whereas the viability of *L. rhamnosus* GG B103 and *L. rhamnosus* GG B101 remained stable during storage. Similar to our findings, zedo gum showed an insignificant effect on the survivability of *L. acidophillus* and *B. bifidum* during 28-day storage (Ghasempour et al. 2012). Bahrami et al. (2013) stated that the statistical analysis showed

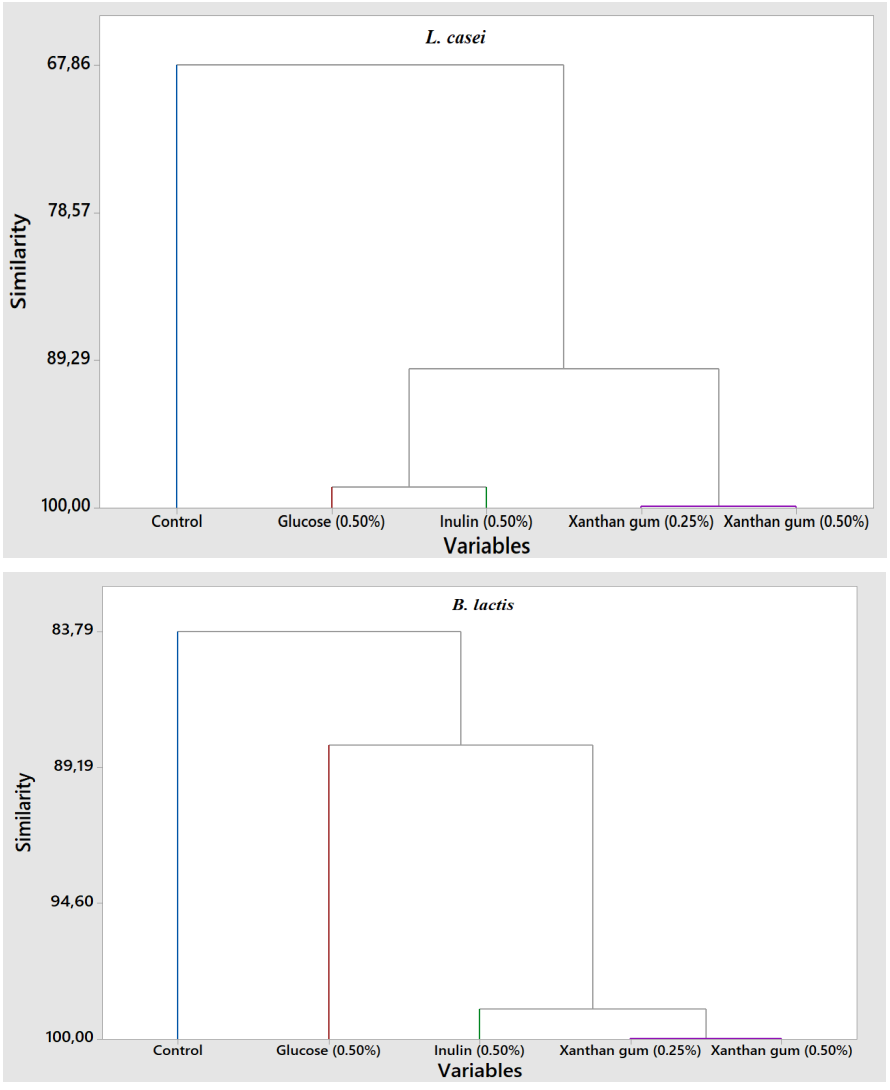

Figure 6. Cluster analysis of *B. lactis* and *L. casei*.

that xanthan gum, guar gum, and barley beta-glucan additions had no marked effect on *L. bulgaricus*, *S. thermophilus* and *L. acidophilus* counts of yogurt samples. El-Sayed et al. (2002) determined that the use of xanthan gum and mixes of guar gum, *carboxymethyl cellulose*, and locust bean gum in yogurt and soy yogurt did not affect the counts of probiotic bacteria. The findings of current study are in accordance with the results of Khalid et al. (2022) who focused on the the prebiotic potential of xanthan gum and its hydrolyzed forms (acid, base and enzyme) in synbiotic yogurt production. They found that all the samples with hydrolyzed xanthan gum had a viable count of *Bifidobacterium longum* BB536 above 6 log cfu g^{-1} after 28 days of storage. The enzyme hydrolyzed xanthan gum demonstrated the maximum positive effect the viable count of probiotics. A notable result of another study (Park et al. 2019) is that for xanthan gum-fortified goat yogurts, the *Bifidobacterium* spp. counts decreased from initial counts of 7.81 log cfu g^{-1} to 5.68 log cfu g^{-1} after four weeks in refrigerated storage.

Techno-functional properties in skim milk model system with xanthan gum

The titratable acidity values of samples displayed significant differences depending on the skim milk matrix and storage time (P<0.01, Table V). The titratable acidity of control and samples with gum varied from 0.67 to 1.29 % throughout the storage time. The acidity of samples except for the BL sample showed a significant decrease during storage. The samples fermented by *L. casei* had the highest acidity at the beginning and end of the storage. Ziaolhagh & Jalali (2017) found that the acidity increased in the yogurt drink including kakuti or thyme essence and xanthan gum during storage and the samples containing xanthan gum had the highest acidity. El-Sayed et al. (2002) reported that the use of xanthan gum or its mixtures had an insignificant effect on the acidity values of yogurt or soy yogurt samples compared to the corresponding controls. Similarly, Khalid et al. (2022) determined that the titratable acidity values ranged from 0.963% to 1.086% in synbiotic yogurts including hydrolyzed xanthan gum during 28-day storage. The type and amount of the produced organic acids in the presence of gums depending on specific probiotic strains resulted in different acidity values in various studies (Ghasempour et al. 2012, Bahrami et al. 2013, Karlton-Senaye et al. 2015).

It was determined that the syneresis defined as the separation of the aqueous phase from the continuous phase or gel network was found

Table IV. Viable cell counts of *B. lactis* and *L. casei* in skim milk model system during storage.

Samples	Storage days					VPI
	1. day	7. day	14. day	21. day	28. day	
BL	9.44 ± 0.000^{bB}	9.65 ± 0.658^{aA}	9.00 ± 0.001^{aD}	9.23 ± 0.002^{aC}	8.29 ± 0.014^{cE}	0.88^{b}
BLG	9.30 ± 0.005^{cA}	9.70 ± 0.785^{aA}	9.40 ± 0.636^{aA}	9.60 ± 0.001^{aA}	8.05 ± 0.001^{dA}	0.87^{b}
LC	9.90 ± 0.007^{aA}	9.80 ± 0.289^{aA}	9.81 ± 0.389^{aA}	9.65 ± 0.353^{aA}	9.98 ± 0.028^{aA}	1.01^{a}
LCG	9.47 ± 0.014^{bA}	10.10 ± 0.572^{aA}	9.80 ± 0.282^{aA}	9.70 ± 0.001^{aA}	9.66 ± 0.028^{bA}	1.02^{a}

[a-d]Different lowercase superscripts in the same column indicate the significant differences among samples for the same storage days (P<0.01).
[A-D]Different uppercase superscripts in the same row indicate the significant differences among storage days for the same sample (P<0.01).
BL: Skim milk with *B. lactis*; BLG: Skim milk with *B. lactis* and 0.25% xanthan gum.
LC: Skim milk with *L. casei*; LCG: Skim milk with *L. casei* and 0.25% xanthan gum.

to be significantly influenced by the sample type and storage time (P<0.01, Table V). The highest syneresis was identified in the sample with fermented *L. casei* at the end of the storage, whereas the lowest value was obtained in the sample with gum. It was noticeable that the BL sample exhibited lesser syneresis values than LC sample after the beginning of the storage. These results may be explained by the fact that the increase of acidity in LC sample during storage altered the development of a more open structure of the sample network and higher syneresis values. The minimum values for syneresis were observed in samples that added xanthan gum. The findings are in harmony with the results of Khalid et al. (2022) who reported the increase in the level of added xanthan gums reduced the syneresis in synbiotic yogurt samples. Bahrami et al. (2013) reported that syneresis decreased with an increased xanthan gum concentration of up to 0.2%, but then the syneresis of the yogurt samples increased. Ghaderi-Ghahfarokhi et al. (2020) and Prasanna et al. (2013) reported that exopolysaccharides

Table V. The titratable acidity, syneresis and color values of skim milk model systems during storage

Properties	Samples	Storage days				
		1. day	7. day	14. day	21. day	28. day
Titratable acidity (%)	BL	0.76 ± 0.009^{bA}	0.73 ± 0.009^{bA}	0.76 ± 0.000^{cA}	0.76 ± 0.016^{bA}	0.67 ± 0.014^{cB}
	BLG	0.81 ± 0.005^{bA}	0.74 ± 0.022^{bA}	0.70 ± 0.000^{dA}	0.78 ± 0.009^{bA}	0.82 ± 0.055^{bA}
	LC	0.91 ± 0.016^{aE}	1.07 ± 0.041^{aD}	1.16 ± 0.009^{aC}	1.20 ± 0.005^{aB}	1.28 ± 0.022^{aA}
	LCG	0.95 ± 0.036^{aD}	1.07 ± 0.013^{aC}	1.07 ± 0.014^{bC}	1.16 ± 0.063^{aB}	1.29 ± 0.059^{aA}
Syneresis	BL	10.00 ± 0.000^{bA}	10.00 ± 0.354^{aA}	10.00 ± 0.000^{bA}	10.75 ± 0.453^{aA}	9.50 ± 0.000^{bA}
	BLG	2.25 ± 0.353^{cA}	2.00 ± 0.000^{bA}	0.00 ± 0.000^{cB}	0.00 ± 0.212^{bB}	0.00 ± 0.000^{cB}
	LC	12.00 ± 0.707^{aA}	9.25 ± 0.000^{aC}	11.50 ± 0.000^{aA}	11.25 ± 0.253^{aA}	10.00 ± 0.000^{aB}
	LCG	0.75 ± 0.353^{dA}	0.00 ± 0.000^{cB}	0.00 ± 0.000^{cB}	0.35 ± 0.000^{bA}	0.00 ± 0.000^{cB}
*L**	BL	95.51 ± 1.104^{aA}	72.69 ± 1.256^{bB}	72.69 ± 0.507^{aB}	72.25 ± 0.918^{bB}	71.06 ± 0.987^{bB}
	BLG	55.11 ± 2.368^{dB}	62.40 ± 1.060^{dA}	61.06 ± 0.307^{cA}	61.64 ± 1.205^{dA}	62.27 ± 0.478^{cA}
	LC	76.78 ± 1.087^{bA}	75.80 ± 0.384^{aB}	74.76 ± 1.839^{aB}	74.59 ± 0.230^{aB}	73.79 ± 1.066^{aB}
	LCG	61.65 ± 0.121^{cC}	66.87 ± 0.281^{cA}	66.43 ± 0.136^{bA}	65.80 ± 0.240^{cAB}	64.71 ± 1.164^{cB}
*a**	BL	-1.02 ± 0.000^{cB}	-2.01 ± 0.510^{bA}	$-1.78\ \pm0.072^{cA}$	-1.70 ± 0.141^{cA}	-2.10 ± 0.529^{bA}
	BLG	-2.47 ± 0.442^{bB}	-3.90 ± 0.042^{aA}	$-3.98\pm\ 0.080^{aA}$	-3.88 ± 0.031^{aA}	-3.97 ± 0.000^{aA}
	LC	-2.16 ± 0.070^{bA}	-1.73 ± 0.046^{bB}	-1.88 ± 0.095^{cAB}	-1.96 ± 0.010^{bAB}	-1.89 ± 0.145^{bAB}
	LCG	-3.08 ± 0.020^{aD}	-3.44 ± 0.044^{aC}	-3.60 ± 0.029^{bC}	-3.89 ± 0.079^{aB}	-4.09 ± 0.114^{aA}
*b**	BL	3.66 ± 0.350^{bC}	8.39 ± 0.345^{aAB}	8.50 ± 0.214^{aAB}	8.00 ± 0.523^{aB}	9.19 ± 0.459^{aA}
	BLG	1.15 ± 0.142^{dB}	4.50 ± 0.286^{cA}	4.10 ± 0.199^{cA}	4.39 ± 0.323^{cA}	4.56 ± 0.185^{dA}
	LC	5.81 ± 0.382^{aB}	6.88 ± 0.295^{bAB}	7.28 ± 0.754^{bA}	7.19 ± 0.417^{aA}	7.15 ± 0.325^{bA}
	LCG	1.94 ± 0.012^{cD}	4.42 ± 0.031^{cC}	4.88 ± 0.085^{cC}	5.53 ± 0.159^{bB}	6.11 ± 0.398^{cA}

[a-d]Different lowercase superscripts in the same column indicate the significant differences among samples for the same storage days (P<0.01).

[A-D]Different uppercase superscripts in the same row indicate the significant differences among storage days for the same sample (P<0.01).

BL: Skim milk with *B. lactis*; BLG: Skim milk with *B. lactis* and 0.25% xanthan gum;LC: Skim milk with *L. casei*; LCG: Skim milk with *L. casei* and 0.25% xanthan gum.

produced by *Bifidobacterium* species could hold free water, reduce the syneresis and create firmer fermented milk gels. The findings showed that the syneresis decreased during cold storage for samples including gum. The addition of gum to milk products can retard syneresis or improve yogurt texture due to the interaction between the gum and milk protein concentration.

Color is an important parameter that affects consumer acceptability and marketing value of a product and is directly influenced by the ingredients and starter culture used in the formulation (Topcuoglu & Yilmaz-Ersan 2020). Statistical analysis illustrated that the addition of xanthan gum and storage time had a significant ($P<0.05$) effect on the color parameters (Table V). Generally, samples without gum (BL and LC) had higher L^* values than others and L^* values of these samples decreased during the first seven days of storage. The a^* values, all with minus measurements, confirmed the greenish was dominating over red in all samples. The samples including gum (BLG and LCG) had higher a^* values than others without gum, whereas b^* values of these samples were the lowest compared to the others. The b^* values, all with measurements above zero, confirmed that the yellow coloration was dominating over blue in all samples. During storage time, generally, the a^* and b^* values of all samples increased, except for a^* values of the LC sample.

It was revealed that there were significant differences ($P<0.01$) between the textural parameter values during storage (Figure 7). Firmness, the force necessary to attain a given deformation, is the higher value means a firmer sample. There were significant differences in the use of xanthan gum and storage time for firmness values ($P<0.05$). Generally, the highest firmness values throughout storage were detected in sample formulated with gum and *L. casei* (LCG). Mohsin et al. (2019) reported that maximum

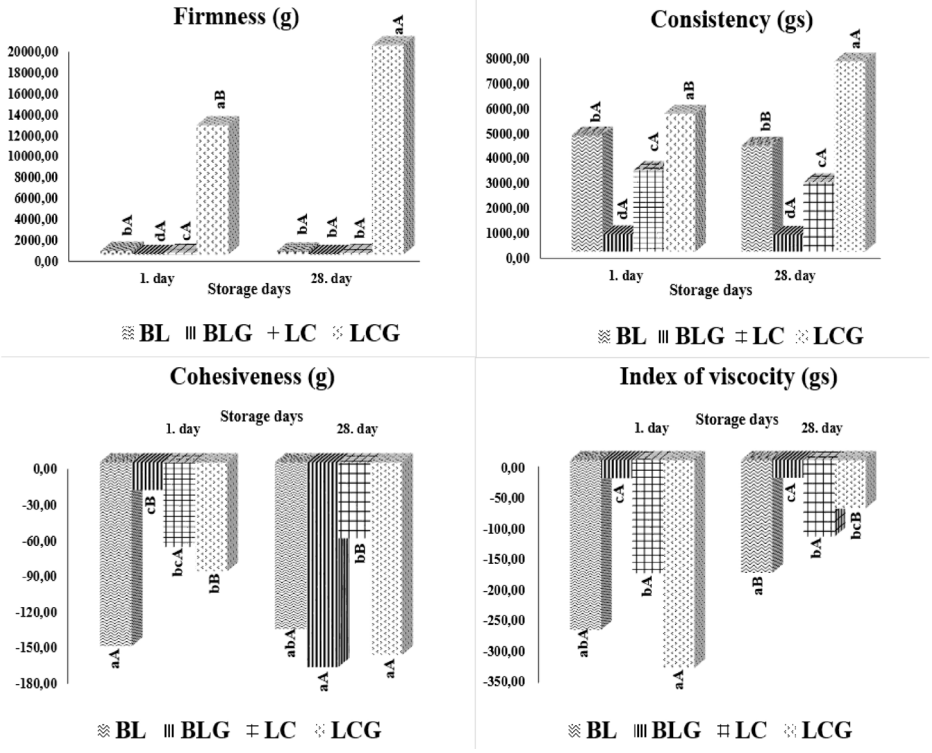

Figure 7. The instrumental texture parameters of the samples in the first and final day of storage.

firmness was found in camel milk date yogurt including 0.5% and 0.75% biosynthesized xanthan gum. Using a similar approach, Park et al. (2019) showed that xanthan and locust bean gums possessed higher textural binding abilities than the other gums (modified food starch with agar pectin, carrageenan, guar and modified food starch with gums) with regard to forming firmer yogurt texture. The higher consistency refers to a highly viscous or thicker sample. It was found that the consistency values were found to be statistically different among the samples during storage (P<0.01). The maximum levels of consistency values were observed at the beginning and end of storage for the LCG sample. Since cohesiveness is related to the strength of the internal bonds in fermented milk structure, the lower the cohesiveness, the smoother the product texture. The cohesiveness values of all samples except for the LC sample during storage showed an increase. Higher cohesiveness values were detected for the BL sample at the beginning of storage, whereas BLG and LCG samples had higher values at the end of storage. The higher values of the index of viscosity refer to being more resistant to gradual deformation of the sample by shear stress, meaning thickness. There were significant differences in the sample type and storage time for viscosity values (P<0.01). During storage, the viscosity values of BL and LCG samples decreased (P<0.05) while those of BLG and LC samples showed statistically insignificant differences (P>0.05). A greater value in the index of viscosity was observed at the beginning of storage for the LCG sample, whereas the BL sample had the highest value at the end of storage. This may be attributed to the combined effect of polysaccharides produced by Bifidobacteria and the low acidity activity of these strains. The findings of the current study are in line with the results of Khalid et al. (2022) who determined that the addition of xanthan gum exhibited a higher viscosity in yogurt samples with *Bifidobacterium longum* BB536. Ziaolhag & Jalali (2017) reported that the viscosity of probiotic yogurt drinks increased as the concentration of xanthan gum increased from 0 to 0.15%. The interaction of the negatively charged xanthan gum with the positively charged surface of

Table VI. Pearson's correlation coefficients among all the techno-functional attributes.

	Probiotic cell counts	Titratable acidity	Syneresis	L*	a*	b*	Firmness	Consistency	Cohesiveness	Index of viscosity
Probiotic cell counts	1									
TA	0.998**	1								
Syneresis	0.011	-0.014	1							
L*	0.069	0.066	0.950*	1						
a*	-0.085	-0.100	0.986*	0.973*	1					
b*	0.011	0.010	0.943	0.998**	0.975*	1				
Firmness	0.494	0.548	-0.604	-0.353	-0.558	-0.363	1			
Consistency	0.399	0.459	-0.031	0.276	0.057	0.276	0.787	1		
Cohesiveness	0.540	0.481	0.145	-0.086	-0.023	-0.139	-0.333	-0.550	1	
Index of viscosity	-0.218	-0.265	-0.414	-0.678	-0.510	-0.685	-0.405	-0.883	0.560	1

***Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.**

the casein micelles in milk contributes to the highly structured network formation, increasing sample viscosity and gel strength (Nguyen et al. 2017). In this study, it was determined that the textural differences were found to be significant among the samples, depending on the probiotic type and xanthan gum, and it was observed that the textural parameters increased for matrix including xanthan gum and *L. casei* (LCG). Thus, further studies are required to characterize the hydrophilic properties, the protein network, physical states of fats and proteins, protein-polysaccharide interactions, and the gelation behavior in xanthan gum which may be responsible for the elevated textural parameters.

Pearson's correlation coefficients were used to explain the use of xanthan gum in skim milk among the techno-functional properties (Table VI). It was found that a strong positive correlation existed between the probiotic cell counts and titratable acidity ($r=0.998$; $P<0.01$). Syneresis was significantly positively correlated with L^* ($r=0.950$; $P<0.05$) and a^* ($r=0.986$; $P<0.05$). L^* was significantly positively correlated with a^* ($r=0.973$; $P<0.05$) and b^* ($r=0.998$; $P<0.05$).

CONCLUSION

Xanthan gum has been used in the food industry due to its multiple techno-functional properties. In this study, an attempt was made to identify the effect of xanthan gum on the growth of probiotic bacteria. The results demonstrated that *B. lactis* and *L. casei* were capable of utilizing xanthan gum as a carbon source of fermentation comparably to the known prebiotic inulin. As a result of xanthan fermentation via bacterial enzymes, postbiotics (lactic, acetic, propionic, and butyric acids) with beneficial health effects were formed. The findings of skim milk model system indicated that *L. casei* could remain viable in the presence of xanthan gum during 28-day storage period and that there was a potential for incorporating this gum in other probiotic dairy products. The utilization of xanthan gum in a skim milk matrix affected important techno-functional parameters like acidity, syneresis, color, and texture. The findings of this study show that xanthan gum is an inexpensive, biodegradable, and sustainable source in order to develop synbiotic products. However, these results only represent the *in vitro* fermentation as well as a skim milk matrix. Further investigations should be carried out as *in vivo* model using animal and human trials and as a gut model using fecal microbiota. In addition, more research is needed concerning synbiotic products containing suitable probiotics and xanthan gum.

This study is a part of Mervenur KANDIL's M.Sc. thesis submitted to the Bursa Uludag University, Graduate School of Natural and Applied Sciences.

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How to cite

KANDIL M & YILMAZ-ERSAN L. 2024. A Novel Approach to the Use of Xanthan Gum: Evaluation of Probiotic Promoter, Postbiotic Formation and Techno-Functional Effect. An Acad Bras Cienc 96: e20230658. DOI 10.1590/0001-3765202420230658.

Manuscript received on June 8, 2023;
accepted for publication on August 14, 2023

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