ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
Food Science and Technology

DOI: https://doi.org/10.1590/fst.61520

Increase in conjugated linoleic acid content and improvement in microbial and physicochemical properties of a novel kefir stored at refrigerated temperature using complementary probiotics and prebiotic

Hamid POURBABA[1], Amir Ali ANVAR[1], Rezvan Pourahmad[2*], Hamed AHARI[3]

Abstract

The present study was aimed to determine the effects of *Lactobacillus acidophilus* LA-5, *L. paracasei* 431, and *Bifidobacterium lactis* BB-12 with lactulose on values of conjugated linoleic acid (CLA) and microbial, physicochemical, and sensory properties of a novel kefir. Thirteen groups were evaluated on days 1, 7, and 14 at 4 °C. The interaction between probiotics and lactulose reduced pH to 4.5 in the first week and slightly decreased on day 14 (4.35). The syneresis value was decreased by increasing the lactulose dose. The interaction could not remarkably increase probiotic survival; the greatest and lowest values were 7.18 and 7.81 log CFU/mL, respectively. The greatest and the lowest lactic acid value was 2.77 and 1.47 g/100 mL, respectively, in kefirs supplemented with *L. acidophilus* LA-5 and *L. acidophilus* LA-5+ *L. paracasei* 431+ *B. lactis* BB-12. A 4-fold increase in the acetic acid value (0.592 g/100 mL) was observed in kefirs supplemented with *B. lactis* BB-12 along with *L. acidophilus* LA-5 and *L. paracasei* 431 (G12). It is concluded that adding 5% lactulose along with *L. acidophilus* LA-5+ *L. paracasei* 431 to kefir could valuably increase the CLA value (3.51-8.07 ppm) and give it more acceptability of flavor, odor, and syneresis.

Keywords: kefir; *Lactobacillus acidophilus*; *Lactobacillus paracasei*; *Bifidobacterium lactis*; lactulose; conjugated linoleic acid.

Practical Application: The interaction between 5% lactulose and the consortium of *L. acidophilus LA-5+ L. paracasei* 431 in the kefirs could valuably: 1: incredibly increase the CLA value (3.51-8.07 ppm). 2: showed low syneresis and appropriate taste, odor, and texture along with a great overall Acceptability. 3: Decrease acetic acid, which gives a bitter taste to kefir. 4: Stabilize the limit of probiotic survival of the kefirs at the standard level (6-7 log CFU/mL).

1 Introduction

Probiotics are living cells and usually modify food contents for health benefit of humans (Roobab et al., 2020). Kefir is a complex-probiotic produced from corresponding grains (Demirci et al., 2019; Kivanc & Yapici, 2019; Tomar et al., 2020) that encompass a consortium of microorganisms (Lim et al., 2019; Mitra & Ghosh, 2020), such as lactic acid bacteria (LAB) containing *Lactococcus*, *Lactobacillus*, occasionally acetic-acid producing bacteria, and non-lactose fermenting yeast, with a long-endured association with a natural substance matrix of proteins and kefiran as polysaccharide (Bengoa et al., 2019b; Rosa et al., 2017; Tomar et al., 2020). *Lactobacillus acidophilus*, *L. paracasei*, and *Bifidobacterium animalis* subsp. *lactis* are due special attention because of their health- and immunity-stimulating properties (Bengoa et al., 2019a). *L. acidophilus* is one of the homofermentative bacteria which directly produce two lactic acid (LA) molecules from one molecule of glucose (Fazio et al., 2020), but heterofermentative ones, including *B. lactis,* convert glucose to lactic acid and acetic acid (AA) or other volatile compounds (Zareba et al., 2012). In consumption of foods with these probiotics, the GITs of consumers are protected against inappropriate situations including extreme pH alterations, GIT enzymes and excretions, and bacterial accumulation (Živković et al., 2016).

The microorganisms existing in kefir produce some metabolites (Costa et al., 2020) such as fatty acids and bacteriocins to inhibit the growth of closely pathogenic bacteria from attaching to intestinal mucosa (Kim et al., 2019). During fermentation, lactose converts to LA and other volatile compounds, which gives the kefir a slightly sour taste (Kök-Taş et al., 2013) and some physiological, preventative, and remedial attributes which make the consumer modify digestibility (Demir, 2020). The biological and physico-chemical criteria of fermented milk beverages are essential for the ultimate properties of the product. These properties are chiefly associated with the milk content, starter type and volume, complementary probiotics, fermentation temperature, and acidity (Wang et al., 2017). Titratable acidity (TA) and pH are two key indicators measured to determine the quality of milk during kefir production. Lactic acid and acetic acid also indicate the quality of the kefir, measuring them is costly and requires more technique (Magalhães et al., 2011b).

Received 06 Nov., 2020
Accepted 09 Nov., 2020
[1]*Department of Food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Department of Food Science and Technology, Varamin- Pishva Branch, Islamic Azad University, Varamin, Iran*
[3]*Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
*Corresponding author: rjpourahmad@yahoo.com; rezvanpourahmad@iauvaramin.ac.ir

A panel of scientists in food and microbiology branches were arranged by the International Scientific Association (ISA) in 2016 for Probiotics and Prebiotics to review the definition of prebiotics. They believed that prebiotic is a substrate that is selectively used by host microorganisms conferring a health advantage (Gibson et al., 2017). On the other hands, Zendeboodi et al. (2020) proposed three chief classes of probiotic containing 'true probiotic' (TP) denoting to live and dynamic probiotic organism, 'pseudo-probiotic' (PP) denoting to live and inactive microorganism, and finally the forms of vegetative or spore (PPV or PPS) and 'ghost probiotic' (GP) referring to dead/nonviable probiotic, in the forms of intact or ruptured (GPI or GPR). Each of these classes are classified into two groups based on their site of action/impact: internal (in vivo) or in vitro. Lactulose, as prebiotic, is a synthetic disaccharide produced with galactose and fructose consumed by *Bifidobacterium* and *Lactobacillus* spp, and it promotes probiotic growth (Delgado-Fernández et al., 2019). Accordingly, nearly 30% of the lactose present in milk is decomposed to acid throughout the fermentation process (Rosa et al., 2017), resulting in a drop in pH and increase in stability. Moreover, the glucose is turned into LA by microbiota existing in kefir (Hikmetoglu et al., 2020).

Subsequently, linoleic acid (LA) is converted to CLA as its isomers, which usually has a relatively low level in dairy products (Gamba et al., 2019). The CLA is a polyunsaturated fatty acid (PUFA) which can be produced by a few strains of LAB and *Bifidobacteria* spp (Linares et al., 2017). The CLA has a few valuable health properties; it reduces the carcinogenic compound effect and the risk of atherosclerosis. However, the nutritional CLA content of food, even in milk, is comparatively too low to enhance the appropriate physiological effect (Vieira et al., 2017). Prebiotics are substrates specifically employed by host probiotics resulting in a well-being advantage (Gibson et al., 2017). Prebiotics are non-digestible components that stimulate the propagation and efficiency of probiotics in the colon and have an advantageous effect on the host (Thongaram et al., 2017). The gut-bone axis can be modulated with live Lactobacillus spp or by milk fermented by that (Eor et al., 2020).

Lactulose (galactopyranosyl-D-fructose) is a synthetic disaccharide prebiotic which may reach the colon and promote the propagation of *Bifidobacterium* and *Lactobacillus* spp (Kailasapathy & Chin, 2000). The interaction effects of probiotics and prebiotics existing in kefir may expand the survivability of the probiotic bacteria and may promote their growth in the colon and upper parts of the intestinal tract. Prebiotics incorporated in kefir, such as lactulose (Delgado-Fernández et al., 2019), fructose (Larosa et al., 2020), oligofructose and fructooligosaccharide (Glibowski & Zielińska, 2015; Shafi et al., 2019), inulin (Santos et al., 2019; Ribeiro et al., 2019), or isomalto-oligosaccharides, as well as pine honey (Coskun & Karabulut Dirican, 2019) have been studied in recent years, but the findings are less focused on CLA value or the survivability of complementary probiotics during storage at refrigerated temperatures.

Thus, the current study assessed the effects of different levels of lactulose, complementary probiotics containing *L. acidophilus* LA-5 and *L. paracasei* 431, individually or in consortium form, and finally along with *B. lactis* BB-12 on the value of conjugated linoleic acid *(*CLA) as well as the physiochemical and sensory properties and bacterial survival of the produced kefir. Samples of cow-milk-based kefir, which were initially incorporated within two commercial starter cultures (CHN22 and LAF4; CHR HANSEN, Denmark), were preserved at refrigerated temperature and analyzed on days 1, 7, and 14 after storage.

2 Materials and methods

2.1 Materials

Approximate 12 L of cow milk containing 2.5% fat and 8.6% solids not fat (Pak Dairy Co., Tehran, Iran) was used. Starter cultures, CHN22 including mesophilic bacteria (*Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *lactis* biovar *diacetylactis, Leuconostoc mesenteroides subsp. cremoris*) and LAF4 containing *Kluyveromyces marxianus* subsp. *marxianus* were purchased from CHR-Hansen (Denmark). They were freeze-dried lactic acid bacteria starter cultures which were added directly to the milk samples as direct vat set (DVS) starters based on the manufacturer instruction (approximate 10^7 CFU/mL). The complementary probiotics including *L. acidophilus* LA-5, *L. paracasei* 431 and *B. lactis* BB-12 were also purchased from CHR-Hansen (Denmark) in 25-g packages for DVS use. The 25-g lactulose powder pack with 98% purity added to the milk samples as a prebiotic was purchased from Sigma-Aldrich (Germany).

2.2 Study design

In this study, 0.1 g of CHN-22 and 0.002 g of LAF4 per liter was added to pasteurized cow milk. The *L. acidophilus* LA-5 and *L. paracasei* 431, individually (0.001 g/L of milk) or in consortium form, were included in the study, while a 3-probiotic mixture group (*L. acidophilus* LA-5 and *L. paracasei* 431 along with *B. lactis* BB-12) was designed as the last treatment (Figure 1). As such, the treatments as well as the control group, which had neither probiotics nor prebiotic (Table 1), were designed as follows: 100 mL of milk (totally 10,700 mL pre-heated at 90 °C for 5 min) and the determined volume of lactulose were poured into 150-mL-126 test tubes and stirred using a plate shaker (RSLAB-7PRO, Rogo-Sampaic, Spain) for 30 minutes. They were incubated at 30 °C until reaching the pH of 4.7 (about 6 h). Then the kefir samples were cooled down until 4 °C and stored at this temperature for 14 days (Figure 1). Accordingly, the kefir was sampled on days 1, 7 and 14 to assess the biological and physico-chemical attributes.

2.3 Microbiological analysis

From each kefir sample containing viable cells, 1.0 mL was mixed with 9.0 mL 0.1% peptone water (Merck, Darmstadt, Germany) in a Stomacher bag and homogenized. The serial dilution was performed with values of 10^{-2}-10^{-6}. Each final dilution of each kefir sample was cultured in triplicate on de Man, Rogosa, and Sharpe (MRS) bile agar (0.15 bile salts; Merck, Darmstadt, Germany) and incubated at 37 °C under anaerobic conditions (10% CO2) for 3d to grow; the lactobacilli bacteria, including *L. acidiphilus* and *L. paracasei* as well as *Bifidobacterium lactis*,

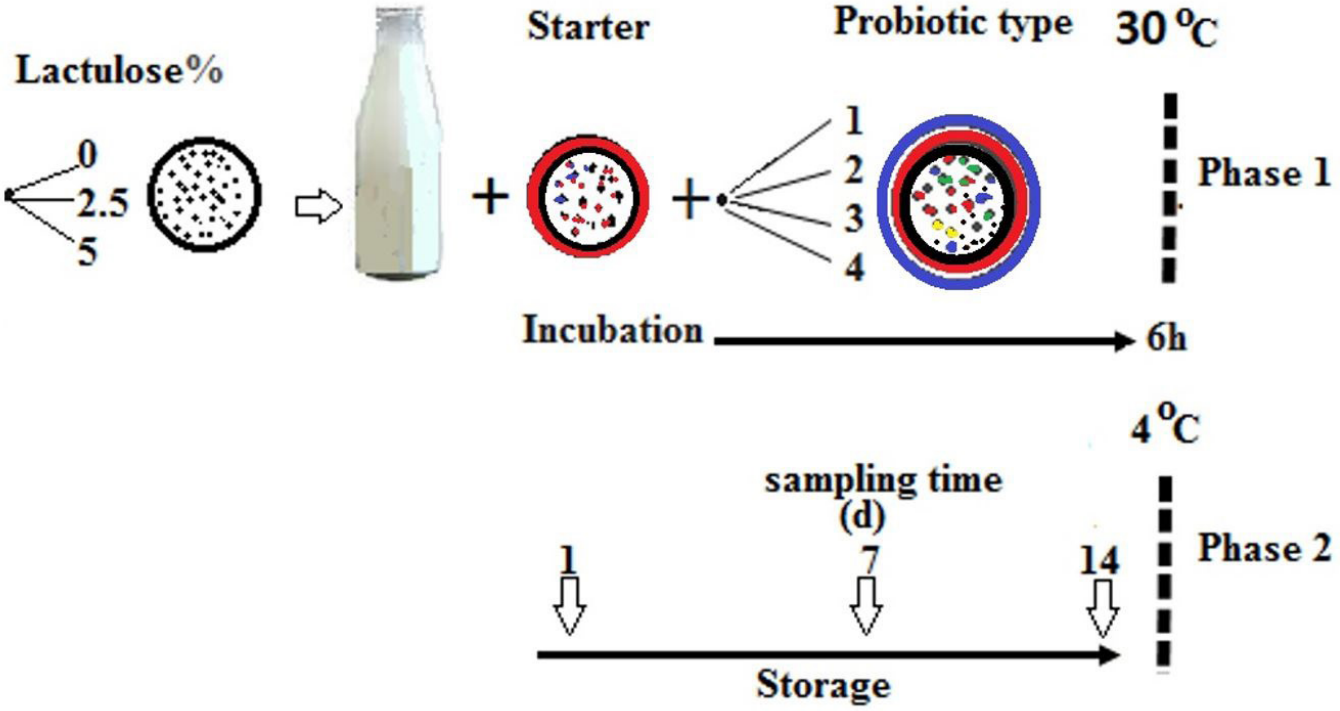

Figure 1. Outline of the experimentations—In addition to the control group, there were 12 treatments including *L. acidophilus*, *L. paracasei, L. acidophilus+L. paracasei*, *L. acidophilus+L. paracasei + B. lactis.* Different concentrations of lactulose were inoculated to cow milk and heated at 90 °C for 5 min. The treated milk (in triplicate) were then supplemented with the determined probiotics, incubated at 30 °C for 6h and ultimately stored at 4 °C. The sampling was carried out on days 1, 7 and 14.

Table 1. The experiment containing constant volume of starter and 100 mL of milk per each group was assigned as G1-G13.

Group N0.	Supplementary Probiotics	Lactulose (%)
G1	*L. acidophilus*	0
G2		2.5
G3		5
G4	*L. paracasei*	0
G5		2.5
G6		5
G7	*L. acidophilus+ L. paracasei*	0
G8		2.5
G9		5
G10	*L. acidophilus+ L. paracasei+ B. lactis*	0
G11		2.5
G12		5
G13	*Control*	0

were then counted. Non-probiotic lactococcus bacteria, which were applied as starters in this study, could not grow in MRS-bile agar (Sabooni et al., 2018; Sohrabvandi et al., 2012).

2.4 Total titratable acidity and pH measurement

Titratable acidity (TA) was defined by the titration of 10 mL of kefir sample with 0.1-N NaOH solution to get pH 8.2, expressed in g of lactic acid 100 g^{-1} (%). The pH was measured by a digital lab-scale pH meter (AZ, 86502, Taiwan). All analyses were conducted in triplicate (Bondia-Pons et al., 2007).

2.5 Syneresis evaluation

Syneresis was determined based on the procedure given by Wang et al. (2017) with minor modifications. At the sampling times, 20 g of each kefir sample was centrifuged at 450 rpm and 4 °C for 30 min (Sigma 3-18KHS, Germany). The centrifuged supernatant was weighed (*s*); the weight was recorded and divided by the initial weight of the kefir sample (20 g), and the result was expressed as a percentage. Equation 1 was used to compute the syneresis:

$$Syneresis\ (\%) = (s/20g) \times 100\% \quad (1)$$

2.6 Determination of organic acid concentration

To determine the concentrations of LA and AA, 5 mL of each kefir sample was mixed with 25 mL of H_2SO_4 (45 mmol/L) and homogenized for 1h. The combination was centrifuged at 5,000 × *g*, and the supernatant fluid was then filtered through 0.45-µm cellulose acetate filters. This method was based on the C18 column (250 × 4.6 mm, 5 µm particle size) following the method of Gaze et al. (2015) with minor modification. The mobile phase was programmed using an isocratic system: A: acetonitrile (5%), B: 0.1% orthophosphoric acid (95%), which was set for 1 mL/min flow rate for 10 min at room temperature. The final centrifuged liquid (100 µL) vortexed with 900 µL of mixture of A+B was re-centrifuged at 5,000 × *g*. Then, 50 µL of supernatant was ultimately injected into an HPLC apparatus (Shimadzu Corp., Tokyo, Japan) in triplicate (Gaze et al., 2015; Leite et al., 2013), and the absorbance at 210 nm was assayed.

2.7 Conjugated linoleic acid measurement

The CLA (*cis*-9,*trans*-11) in the samples of the kefir samples was determined using a gas chromatography HP-6890 series (Hewlett-Packard, Waldbronn, Germany) equipped with a flame ionization detector (FID). The chromatographic separation of CLA was achieved using a RTX-2330 (USA) capillary column (40 m × 0.18 mm × 0.1 μm) containing 10% cyanopropylphenyl and 90% biscyanopropyl polysiloxane in the non-bonded stationary phase. The injector and detector temperatures were adjusted to 240 °C and 260 °C, respectively, following the method of Bondia-Pons et al. (2007) with minor modification. The CLA value was expressed as ppm kefir.

2.8 Sensory analysis

Sensory analysis of the kefir samples was performed with 9 highly-trained panelists who regularly consumed kefir (Gulati et al., 2018). The assessment was done using a 9-point scale hedonic procedure. The attributes were ranked with an increasing format from 1 (extremely disliked) to 9 (extremely liked). Thirteen aliquots of kefir (10 mL each) sampled from different groups were served in transparent pots to each panelist at three sessions. Mean scores of sensory criteria were used as responses of the panelists. The sensory properties evaluated included taste, odor, texture, and overall acceptability.

2.9 Statistical analyses

Data analyses were accomplished using SPSS statistical software, version 26 (SPSS Inc., Chicago, IL). Bacterial and physicochemical data was assessed using a mixed model and repeated-measurement ANOVA. A factorial arrangement was set up to study the impact of 13 groups and 3 sampling times. The Bonferoni test was executed to compare the differences between groups two by two. To determine the differences in scores for the sensory properties, no non-parametric alternative to mixed model and repeated-measurement ANOVA was known to evaluate the qualitative variables of sensory properties; however, two independent variables, the prebiotic (lactulose) concentration and time of sampling, were merged into one variable (lactulose-time) using the compute approach in SPPS software, and subsequently, the Kruskal-Wallis H test was applied followed by the Mann-Whitney U test.

3 Results and discussion

Repeated-measures ANOVA exhibited significant effects of multivariate interactions (η^2=0.956, η^2=0.855, η^2=0.947, and η^2=0.780, respectively) of the independent variables on changing the log cell/mL of the probiotic count (PC), pH, TA, syneresis, LA, AA, and CLA values of the final kefir product.

3.1 Probiotic survival

The results of supplemented probiotic count (PC) in the produced kefirs are listed in Table 2. The PC of each group was linearly decreased by the increased time from the first to the second week, which could be due to the increase in acidity and decrease in pH. Generally, the lowest PC was observed in groups excluding lactulose; the PC value in G1, G4, G7, and G10 was about 7.6, 7.4, and 7.2 log CFU/mL on days 1, 7, and 14, respectively, of cold storage with a negligible exception. The greatest PC was observed in the groups containing 2.5% and 5% lactulose, with values of 7.8, 7.6, and 7.4 log CFU/mL on days 1, 7, and 14, respectively (p>0.05). The application of coating materials and prebiotic in probiotic microencapsulation results in high survivability of probiotics in gastrointestinal situations, which can be further join in food products (García et al., 2019; Siang et al., 2019; Yildiran et al., 2019).

The total probiotic count in kefir should be greater than 7 log CFU/mL (Rosa et al., 2017). Similarly, the PC range was 7.25-7.82 log CFU/mL on day 14 of preservation of the kefir at 4 °C (Table 2). However, this result (Table 2) was not in concordance with that obtained by Delgado-Fernández et al. (2019), who reported that the number of *Lactobacillus* spp reached 9.1 and 9.3 log CFU/mL on days 7 and 14 at refrigerated temperature in kefir supplemented with 2-4% lactulose. Similarly, other researchers (Nacheva, 2019) exhibited that the effect of 3% lactulose-supplemented kefir resulted in the propagation of the *Lactobacillus* spp which reached approximately 7.5 log CFU/mL for both days 7 and 14 during cold storage. They believed that it is commercially non-profitable to deploy higher concentrations of prebiotics. These differences among the researchers might be due to the bacteriological method that carried out with all the *Lactobacillus* spp in the starter culture in the above-mentioned studies (Delgado-Fernández et al., 2019; Nacheva, 2019). In the current study, however, the enumeration of the definite complementary probiotics (not the starter) was investigated under anaerobic conditions at 30 °C. The PC of *L. acidophilus* LA-5 ranged between 5.8 and 6.6 log CFU/mL, respectively, at the fourteenth and first day of storage at 4 °C (Kök-Taş et al., 2013), lower than those of the current study reporting 7.82 ± 0.0 and 7.44 ± 0.0 log CFU/mL in G3 (with 5% lactulose), respectively. Even n G2 (2.5% lactulose-supplemented kefir) exhibited 7.80 ± 0.0 and 7.41 ± 0.0 log CFU/mL, respectively. Unlikely, the PC of *L. acidophilus* presented in cow-milk kefir along with polymerized whey protein showed a great value (10.5 log CFU/mL) with no significant difference (p>0.05) through day 14 of cold storage (Wang et al., 2017). The definite manufactured starter as well as the supplementary probiotics used in the current study probably made a difference in the other previously discussed findings. Other researchers (Leite et al., 2013) found that the growth or survival of each probiotic in kefir is associated with the presence of each other, due to the bacterial quorum-sensing relationship present between kefir probiotics. Similarly, the current study showed that the consortium probiotic administration promoted the growth of probiotics (Table 2). The lower acidification (higher pH values) of the fermented milk can increase the shelf-life of beverages as well as the survival rate of the added probiotics (Nejati et al., 2020).

3.2 pH and titratable acidity

The values of pH were obtained from the kefir samples throughout the cold storage is presented in Table 2. The pH of the initial milk was 6.6. In the control group, the pH of the kefir samples reached 4.5 in the first week, which was significantly

Table 2. Estimated Marginal Means of probiotic count (log CFU/mL), pH, titratable acidity as g of lactic acid.100 g^{-1}(%) kefir, and syneresis (g.100 g^{-1}, %) were affected through the interaction of Species of probiotic bacteria× Lactulose× Time in the kefir samples (n=3).

Species of probiotic	Lactulose (%)	Day	Mean ± SE			
			Probiotic count	pH	Acidity	Syneresis
L. acidophilus	0	1	7.64 ± 0.0^{aA}	4.34 ± 0.02^{aA}	0.80 ± 0.01^{aA}	36.97 ± 0.30^{aA}
	0	7	7.40 ± 0.0^{abA}	4.31 ± 0.02^{aA}	0.81 ± 0.00^{aA}	37.07 ± 0.32^{aA}
	0	14	7.25 ± 0.0^{bA}	4.27 ± 0.01^{aA}	0.83 ± 0.00^{aA}	42.97 ± 0.40^{bA}
	2.5	1	7.80 ± 0.0^{aB}	4.57 ± 0.02^{aB}	0.78 ± 0.01^{aA}	30.85 ± 0.30^{aB}
	2.5	7	7.65 ± 0.0^{abB}	4.57 ± 0.02^{aB}	0.80 ± 0.00^{aA}	32.67 ± 0.32^{aB}
	2.5	14	7.41 ± 0.0^{bA}	4.49 ± 0.01^{aB}	0.82 ± 0.00^{aA}	32.93 ± 0.40^{aB}
	5	1	7.82 ± 0.0^{aB}	4.45 ± 0.02^{aAB}	0.82 ± 0.01^{aA}	32.93 ± 0.30^{aB}
	5	7	7.67 ± 0.0^{aB}	4.35 ± 0.02^{aA}	0.82 ± 0.00^{aA}	33.67 ± 0.32^{aB}
	5	14	7.44 ± 0.0^{bA}	4.39 ± 0.01^{aAB}	0.84 ± 0.00^{aA}	34.90 ± 0.40^{aB}
L. paracasei	0	1	7.63 ± 0.0^{aA}	4.36 ± 0.02^{aA}	0.72 ± 0.01^{aA}	35.93 ± 0.30^{aA}
	0	7	7.36 ± 0.0^{abA}	4.35 ± 0.02^{aA}	0.79 ± 0.00^{aA}	37.27 ± 0.32^{abA}
	0	14	7.22 ± 0.0^{bA}	4.31 ± 0.02^{aA}	0.84 ± 0.00^{aA}	39.13 ± 0.40^{bA}
	2.5	1	7.79 ± 0.0^{aB}	4.47 ± 0.02^{aAB}	0.67 ± 0.01^{aB}	34.25 ± 0.30^{aB}
	2.5	7	7.66 ± 0.0^{aB}	4.45 ± 0.02^{aB}	0.78 ± 0.00^{aA}	33.93 ± 0.32^{aB}
	2.5	14	7.36 ± 0.0^{bA}	4.39 ± 0.01^{aAB}	0.84 ± 0.00^{aA}	36.20 ± 0.40^{bC}
	5	1	7.81 ± 0.0^{aB}	4.46 ± 0.02^{aAB}	0.80 ± 0.01^{aA}	30.60 ± 0.30^{aB}
	5	7	7.68 ± 0.0^{aB}	4.39 ± 0.02^{aA}	0.82 ± 0.00^{aA}	31.80 ± 0.32^{aB}
	5	14	7.39 ± 0.0^{bA}	4.39 ± 0.01^{aAB}	0.89 ± 0.00^{aA}	32.90 ± 0.40^{aB}
L. acidophilus+L. paracasei	0	1	7.63 ± 0.0^{aA}	4.36 ± 0.02^{aA}	0.80 ± 0.01^{aA}	37.25 ± 0.30^{aA}
	0	7	7.40 ± 0.0^{bA}	4.30 ± 0.02^{aA}	0.83 ± 0.00^{aA}	38.07 ± 0.32^{aA}
	0	14	7.37 ± 0.0^{aB}	4.24 ± 0.02^{aA}	0.85 ± 0.00^{aA}	40.80 ± 0.40^{bA}
	2.5	1	7.79 ± 0.0^{aB}	4.36 ± 0.02^{aA}	0.71 ± 0.01^{aA}	34.27 ± 0.30^{aB}
	2.5	7	7.68 ± 0.0^{aB}	4.34 ± 0.02^{aA}	0.73 ± 0.00^{aA}	35.97 ± 0.32^{aA}
	2.5	14	7.38 ± 0.0^{bA}	4.31 ± 0.01^{aA}	0.76 ± 0.00^{aA}	38.70 ± 0.40^{bB}
	5	1	7.81 ± 0.0^{aB}	4.32 ± 0.02^{aA}	0.67 ± 0.01^{aB}	34.60 ± 0.30^{aB}
	5	7	7.68 ± 0.0^{aB}	4.31 ± 0.02^{aA}	0.72 ± 0.00^{aA}	35.23 ± 0.32^{aA}
	5	14	7.39 ± 0.0^{bA}	4.30 ± 0.01^{aA}	0.79 ± 0.00^{aA}	37.23 ± 0.40^{bC}
L. acidophilus+L. paracasei+B. lactis	0	1	7.63 ± 0.0^{aA}	4.45 ± 0.02^{aAB}	0.74 ± 0.01^{aA}	35.03 ± 0.30^{aA}
	0	7	7.39 ± 0.0^{bA}	4.37 ± 0.02^{abA}	0.72 ± 0.00^{aA}	37.20 ± 0.32^{bA}
	0	14	7.18 ± 0.0^{bA}	4.30 ± 0.02^{bA}	0.74 ± 0.00^{aA}	40.10 ± 0.40^{cA}
	2.5	1	7.78 ± 0.0^{aB}	4.48 ± 0.02^{aAB}	0.80 ± 0.01^{aA}	33.10 ± 0.30^{aB}
	2.5	7	7.67 ± 0.0^{aB}	4.41 ± 0.02^{aA}	0.76 ± 0.00^{aA}	37.40 ± 0.32^{bA}
	2.5	14	7.37 ± 0.0^{bA}	4.40 ± 0.01^{aAB}	0.77 ± 0.00^{aA}	38.80 ± 0.40^{bA}
	5	1	7.78 ± 0.0^{aB}	4.36 ± 0.02^{aA}	0.65 ± 0.01^{aB}	31.20 ± 0.30^{aB}
	5	7	7.69 ± 0.0^{aB}	4.32 ± 0.02^{abA}	0.81 ± 0.00^{bA}	31.83 ± 0.32^{aB}
	5	14	7.40 ± 0.0^{bA}	4.27 ± 0.01^{bA}	0.84 ± 0.00^{bA}	37.53 ± 0.40^{bC}
Control	0	1	-	4.57 ± 0.02^{aB}	0.65 ± 0.01^{aB}	35.97 ± 0.30^{aA}
	0	7	-	4.55 ± 0.02^{aB}	0.81 ± 0.00^{bA}	36.50 ± 0.32^{abA}
	0	14	-	4.35 ± 0.01^{bAB}	0.85 ± 0.00^{bA}	38.43 ± 0.40^{bA}

Different small superscripts in each row and same lactulose concentrations indicate a significant difference at a p value of 0.05. Capital superscripts in each column and same days among the probiotic groups indicate a significant difference at a p value of 0.05. SE is the abbreviation for standard error.

different (p<0.05) compared to that of day 14 (4.35). Similar to this study (Table 2), Magalhães et al. (2011a) reported that the pH value of the kefir was 4.42 on 24 h at 25°C. The increase in acidity or decrease in pH of the kefir can be explained by the production of organic acid following the fermentation process performed by the probiotics (Magalhães et al., 2011b). In the kefir samples supplemented with *L. acidophilus* and *L. paracasei* (G1-G3 and G4-G6), the pH showed quadratic curves so that the values neared 4.3, 4.5, and 4.4, respectively, while 0.0, 2.5%, and 5% lactulose was added. It seems that the addition of lactulose to the kefir supplemented with the individual probiotic could slightly increase the pH from the first to the seventh day, but in the consortium groups (G7-G9 and G10-G12), a constant pH (p>0.05) with a slightly lower level was shown. For all three days of sampling, the lowest pH was observed in the *L. acidophilus* LA-5+ *L. paracasei* 431 sample (G9), reaching 4.3 throughout the cold storage with no significant difference (p>0.5) compared to those of G1, G4, G7, G8, and G12. The pHs of the kefir supplemented with consortium probiotics and 2.5-5% prebiotic, particularly in G7-G12 (4.2-4.4), were significantly

lower (p<0.05) than that of the control sample (Table 2) on all days of cold storage, showing more acidifying activities that resulted in reduced pH levels which occurred with the addition of lactulose (2-5%) to the kefir. This is contrary to other results (Delgado-Fernández et al., 2019) that represented that lactulose (2-4%) had no effect on the pH of kefirs.

The *L. paracasei* count reached 9.45 ± 0.24 log CFU/mL in the kefir with pH 3.89 ± 0.07 preserved at 30 °C after the first day (Bengoa et al., 2019a), which is lower than the suggested pH of kefir that should be in the range of 4.4-4.6 (Nambou et al., 2014). The greatest PC of *L. paracasei* 431 (Table 2) was 7.81 ± 0.0 log CFU/mL with a pH value of 4.46 ± 0.07 at 4 °C after 24h. During the storage period, the slight decrease in pH level (close to 4.3) detected in all kefir samples mainly throughout the second week of cold storage could be associated with the acidifying activities of the probiotics (starter and complementary ones) which increased in refrigerated temperatures (Glibowski & Zielińska, 2015). This difference could be due to the fermentative metabolic process (hetero or homo) deployed by the probiotic added to the kefir and the temperature at which the kefir samples were preserved. However, some probiotic species adjust the acid production formed by LAB, increasing the pH and enhancing bacterial growth. As such, the greater the pH is, the higher the survival rate of probiotics in the kefir environment will be (Leite et al., 2013).

On each day of sampling (Table 2), TA values showed no significant difference (p>0.5) between the treatments ranging from 0.71-0.82%, 0.72-0.82%, and 0.74-0.89% for days 1, 7, and 14, respectively, with the exception of G12, which exhibited a significantly lower TA (p<0.05) on the first day (0.65%). The TA of G12 had no significant difference (p>0.05) from those of G9 and G13 (control) on the first day, but it increased 54%, 72%, and 80% (Cui et al., 2013) with increases in sucrose (6, 8, and 10 g/100 mL, respectively). Conversely, the current study showed that different concentrations of lactulose insignificantly (p>0.05) impacted TA in cold storage throughout the study. Some researchers (Yoo et al., 2013) reported that the TA levels were 0.77-0.82% in various kefirs produced through new or conventional methods on the first day of storage; this is in agreement with the current study that showed the TA of the kefirs ranged 0.65-0.82% and increased slightly in a time-dependent manner (Table 2). An exception was shown in G9. Among the groups, the lowest TA was observed in G9, which reached 0.67%, 0.72%, and 0.79%, respectively, for days 1, 7, and 14. Similarly, the LA of G9 was also one of the lowest among the groups, showing that low levels of lactic acid were produced in the kefir supplemented with *L. acidophilus* LA-5 and *L. paracasei* 431 (G9). In agreement with the current study (Table 2), the TA values of the kefir ranged from 0.7% to 0.8% on the second day of cold storage (Tomar et al., 2020) and increased up to the 14th day. In another study (Hong et al., 2019), the TA of kefir inoculated with 6 log CFU/mL *Saccharomyces cerevisiae* KU200284 was 1.1%, representing a slightly higher increase than those of the current study. The differences in TA and pH between the kefir beverages may be due to the differences in microorganism populations as well as the symbiosis between the microorganisms added to the kefir cultures (Nejati et al., 2020). The symbiosis between LA-produced bacteria, including *L. acidophilus* LA-5 and *L. paracasei* 431, led to a moderate volume of organic acids in the current study (Table 3).

3.3 Syneresis analysis

Data on the changes in syneresis of the kefir samples is presented in Table 2. Generally, the percentage of syneresis for each kefir sample increased in a storage time-dependent manner and decreased when the lactulose concentration was increased, indicating more fermentative activity of the kefir resulted in a reduction in the syneresis of the kefir. Syneresis showed a significant decrease (P<0.05) with an incubation time-dependent manner from 40.76% at 18 h to 37.47% at 30 h (Bensmira & Jiang, 2012). Low syneresis mirrors an appropriate kefir fermentation, so that the great values of syneresis show that the water-holding capacity and the gel firmness of the kefir were weak, leading to the detachment of more nutrients from the gel (Setyawardani et al., 2020). The least values of syneresis were found in G6 which was inoculated with 5% lactulose (30.60%, 31.80%, and 32.90%, respectively, for days 1, 7, and 14 of cold storage), but the acid production of this treatment was much greater than those of other treatments, which in turn could be responsible for the bitter and undesirable taste of the kefir. In agreement with G6, the syneresis of other samples inoculated with 5% lactulose, i.e. G9 (34.60% and 35.23%, respectively, for days 1 and 7) and G12 (31.20% and 31.83%, respectively, for days 1 and 7) exhibited lower syneresis compared to those of the groups with less lactulose (0 and 2.5%) at refrigerated temperatures (Table 2). The acid content of G12 was more than that of G9, which is explained in detail in the following sections.

The syneresis values in all treatments were significantly lower (p<0.05) than that of the control sample. In another research (Montanuci et al., 2012), the effect of inulin added to kefir resulted in a decrease in the syneresis value (from 24% to 26.45% and 23% to 22.7% for days 1 and 14, respectively) in contrast to the current study, which demonstrated that the addition of 2.5% lactulose to *L. acidophilus* LA-5-supplemented kefir led to a significant decrease (P<0.05) in syneresis values, from 36.9% to 30.85% and 42.9% to 32.9% on days 1 and 14, respectively. This result was in line with the findings of another study (Wang et al., 2017) reported that the syneresis value was decreased in fermented goat milk with increases in complementary polymerized whey protein. These findings indicate that the addition of prebiotics such as lactulose promotes the fermentative activity of kefir, resulting in an increase in acidity and reduction in syneresis.

3.4 Lactic acid and acetic acid

Table 3 shows that LA reached 1.87 g/100 mL on the day 1 and increased significantly (p<0.05) increased on days 7 and 14 (2.20 and 2.58 g/100 mL, respectively) in the control. Conversely, *L. acidophilus* LA-5-supplemented kefirs (G1-G3, Table 3) showed that LA production was performed with a delay (1.5 and 1.7-1.9 g/100 mL; p>0.05) on the first and seventh days and increased dramatically (p<0.05) on day 14 (2.4-2.7 g/100 mL), irrespective of lactulose concentration. This indicated the role of preservation time in cold storage on day 14 plays for increases in LA and the independency of lactulose in the kefirs supplemented with *L. acidophilus* LA-5,

Table 3. Estimated Marginal Means of conjugated linoleic acid (ppm), lactic acid (g/100mL), and acetic acid (g/100 mL) through the interaction of Species of bacteria× Lactulose × Time (n=3).

Species of probiotic	Lactulose (%)	Day	Mean ± SE		
			CLA (ppm)	Lactic acid (%)	Acetic acid (%)
Lactobacillus acidophilus	0	1	2.49 ± 0.03^{aA}	1.57 ± 0.41^{aA}	0.113 ± 0.30^{aA}
	0	7	4.29 ± 0.02^{bA}	1.76 ± 0.20^{aA}	0.131 ± 0.33^{bA}
	0	14	7.00 ± 0.04^{cA}	2.40 ± 0.18^{bA}	0.137 ± 0.27^{cA}
	2.5	1	2.60 ± 0.03^{aA}	1.51 ± 0.41^{aA}	0.094 ± 0.30^{aA}
	2.5	7	5.20 ± 0.02^{bB}	1.70 ± 0.20^{aA}	0.120 ± 0.33^{bA}
	2.5	14	7.03 ± 0.04^{cA}	2.44 ± 0.18^{bA}	0.146 ± 0.27^{cA}
	5	1	2.71 ± 0.03^{aA}	1.59 ± 0.41^{aA}	0.098 ± 0.30^{aA}
	5	7	5.43 ± 0.02^{bB}	1.92 ± 0.20^{aA}	0.120 ± 0.33^{bA}
	5	14	7.03 ± 0.04^{cA}	2.77 ± 0.18^{bB}	0.124 ± 0.27^{bA}
Lactobacillus paracasei	0	1	2.48 ± 0.03^{aA}	2.17 ± 0.41^{aB}	0.108 ± 0.30^{aA}
	0	7	3.48 ± 0.02^{bC}	2.18 ± 0.20^{aB}	0.142 ± 0.33^{bA}
	0	14	5.00 ± 0.04^{cB}	2.41 ± 0.18^{bA}	0.231 ± 0.27^{cB}
	2.5	1	2.80 ± 0.03^{aAB}	1.91 ± 0.41^{aB}	0.101 ± 0.30^{aA}
	2.5	7	3.65 ± 0.02^{bC}	2.10 ± 0.20^{aB}	0.153 ± 0.33^{bA}
	2.5	14	5.88 ± 0.04^{cC}	2.42 ± 0.18^{bA}	0.177 ± 0.27^{cA}
	5	1	3.03 ± 0.03^{aB}	2.12 ± 0.41^{aB}	0.124 ± 0.30^{aA}
	5	7	3.87 ± 0.02^{bAC}	2.19 ± 0.20^{aB}	0.152 ± 0.33^{bA}
	5	14	5.90 ± 0.04^{cC}	2.75 ± 0.18^{bB}	0.159 ± 0.27^{cA}
L. acidophilus+L. paracasei	0	1	2.42 ± 0.03^{aA}	1.63 ± 0.41^{aA}	0.097 ± 0.30^{aA}
	0	7	3.20 ± 0.02^{bC}	1.88 ± 0.20^{aA}	0.113 ± 0.33^{aA}
	0	14	6.07 ± 0.04^{cC}	2.58 ± 0.18^{bAB}	0.153 ± 0.27^{aA}
	2.5	1	2.81 ± 0.03^{aAB}	1.63 ± 0.41^{aA}	0.106 ± 0.30^{aA}
	2.5	7	3.51 ± 0.02^{bC}	1.84 ± 0.20^{aA}	0.109 ± 0.33^{aA}
	2.5	14	7.07 ± 0.04^{cA}	2.35 ± 0.18^{bA}	0.119 ± 0.27^{aA}
	5	1	3.51 ± 0.03^{aC}	1.61 ± 0.41^{aA}	0.112 ± 0.30^{aA}
	5	7	3.91 ± 0.02^{aA}	1.84 ± 0.20^{aA}	0.121 ± 0.33^{aA}
	5	14	8.07 ± 0.0^{cD}	2.48 ± 0.18^{bA}	0.131 ± 0.27^{aA}
L. acidophilus+L. paracasei+ B. lactis	0	1	2.20 ± 0.03^{aA}	1.47 ± 0.41^{aA}	0.401 ± 0.30^{aB}
	0	7	3.62 ± 0.02^{bC}	1.49 ± 0.20^{aA}	0.466 ± 0.33^{aB}
	0	14	5.07 ± 0.04^{cB}	1.95 ± 0.18^{bC}	0.501 ± 0.27^{bC}
	2.5	1	2.81 ± 0.03^{aAB}	1.51 ± 0.41^{aA}	0.441 ± 0.30^{aB}
	2.5	7	4.14 ± 0.02^{bA}	2.02 ± 0.20^{bA}	0.490 ± 0.33^{aB}
	2.5	14	6.02 ± 0.04^{cC}	2.57 ± 0.18^{cA}	0.511 ± 0.27^{bC}
	5	1	3.27 ± 0.03^{aB}	1.72 ± 0.41^{aA}	0.464 ± 0.30^{aB}
	5	7	4.55 ± 0.02^{bA}	2.07 ± 0.20^{bA}	0.554 ± 0.33^{bC}
	5	14	7.08 ± 0.04^{cA}	2.51 ± 0.18^{cA}	0.592 ± 0.27^{bD}
Control	0	1	0.72 ± 0.03^{aD}	1.87 ± 0.41^{aAB}	0.220 ± 0.30^{aC}
	0	7	0.98 ± 0.02^{aD}	2.20 ± 0.20^{Bb}	0.270 ± 0.33^{aC}
	0	14	3.08 ± 0.04^{bE}	2.58 ± 0.18^{Bab}	0.285 ± 0.27^{aB}

Different small superscripts in each row and same lactulose concentrations indicate a significant difference at a p value of 0.05. Capital superscripts in each column and same days among the probiotic groups indicate a significant difference at a p value of 0.05.

which might be due to the weak consumption of lactulose by *L. acidophilus* (Watson et al., 2013). Similarly, another research revealed that *L. acidophilus* consumed lactulose but in lesser amounts than lactose (Watson et al., 2013). On the other hand, the AA value of the control (close to 0.2) was greater than those of G1-G3 and even G4-G9 (approximate 0.1), indicating that AA decreased the effect of *L. acidophilus* LA-5 in producing AA became weak in the kefir, which could be due to the fact that it is a homofermentative bacterium (Fazio et al., 2020) and can't produce AA. The addition of 1% lactulose to a fermented milk increased LA to 1.1 g/100 mL on the second day of cold storage (Kliks et al., 2019). Based on the above-mentioned discussion, the LA and AA values of the *L. acidophilus* LA-5 samples (G1-G3) could not be greater than those of other samples in which the probiotics could coincidingly ferment lactulose and lactose such as G4-G6. *L. paracasei* proficiently consumed lactulose as well as lactose (Watson et al., 2013). Therefore, an increase in LA coinciding with AA would be expected in G4-G6. As such, the least value of LA among G4-G6 kefirs supplemented with *L. paracasei* 431 was 1.91 g/100 mL on day 1 in G5, being significantly greater (p<0.05) than the greatest values of the other treatments at the same time (1.72 g/100 mL, G12). The LA,

a species-dependent organic compound (Table 3), was 2.17 and 2.18 g/100 mL on the first and seventh days, respectively, in the sample without lactulose (G4), while the values for the *L. acidophilus* LA-5-supplemented kefir were 1.57 and 1.56 g/100 mL, respectively on the same days. Regarding the LAB, LA was claimed to be produced low by *L. paracasei* (Kök-Taş et al., 2013). Conversely, the values of LA typically found in groups G4-G6 inoculated with *L. paracasei* 431 surprisingly showed greater values ($p<0.05$), reaching 1.91-2.19 and 2.10-2.19 g/100 mL on days 1 and 7, respectively, compared to those of G1-G3 (1.5 and 1.7 g/100 mL), G7-G9 (1.6 and 1.8 g/100 mL), and G10-G12 (1.47-1.72 and 1.49-2.7 g/100 mL) at the same time. On day 14, the LA significantly ($p<0.05$) increased, irrespective of probiotic type (individual or consortium) or the prebiotic percentage (Table 3). In agreement with the consortium samples containing 3 bacteria (G10-G12) which included *B. lactis* as a strong heterofermentative AA-producer, this value surprisingly showed a greater value ($p>0.05$) in G4-G6 compared to those of other treatments. This result could be due to the fact that *L. paracasei* 431 is a facultative heterofermentative species of LAB (Fazio et al., 2020) and can produce either lactic acid or acetic acid (Yamamoto et al., 2019). Based on the current results (Table 3), it seems that complementary *L. paracasei* 431 used the heterofermentative pathway less than the homofermentative one (G4-G6), resulting in a relatively higher AA value, but the proportion one is still lower than the LA value.

Due to the smaller proportion (50%) of *L. paracasei* 431 in the consortium of G7-G9 than in those of the individual situation in G4-G6 (100%), the value of LA produced in G7-G9 ranged from 1.61 to 1.88 g/100 mL at first week, which is significantly ($p<0.05$) less than the 1.91 to 2.19 g/100 mL obtained in G4-G6, at the same time. This pattern re-occurred for AA values, but the difference between the groups was not significant ($p>0.05$). The results of this study (Table 3) demonstrated that the AA and LA values of G7-G9 were independent of lactulose concentration. However, the LA in G7-G9 (1.6 and 1.8 g/100 mL; $p>0.05$) was produced with a delay on the first and 7th days, respectively, and increased significantly ($p>0.05$) on day 14 (2.3-2.5 g/100 mL), similar to what took place in G1-G3. Dissimilar to the current study, Delgado-Fernández et al. (2019) also showed that LA was constant through the first week (0.63 g/100 mL). They showed that AA was constantly 0.038 within the 14 days of cold storage, less than the 5% lactulose-supplemented kefirs in this study that ranged from 0.1 to 0.5 g/100 mL (Table 3). However, both criteria in G7-G9 as well as G1-G3 were remarkably less than those of the control group on days 1 and 7 during cold storage in this study, indicating that LA and AA were dependent on complementary probiotics adding to kefir.

Bifidobacterium animalis subsp. *lactis* is classified as a heterofermentative bacterium, but its heterofermentative metabolic pathway differs from that of the LAB due to the conversion of glucose to LA and AA in the ratio of 3:2 (Szajnar et al., 2020). It is confirmed that the major composition of the volatile profile of fermented milk is AA, and bifidobacteria are more responsible for this situation than LAB (Zareba et al., 2012), particularly for the bitter taste of the kefir (Szajnar et al., 2020). Therefore, the production of more AA and consequently bitter taste were expected in the bacterial consortium (G10-G12) than in the other groups (Table 3). In this context, the value of AA was significantly ($p<0.05$) 2-4 times greater ($p<0.05$) in consortium along with *B. lactis* BB-12 (0.40-0.59 g/100 mL) than those of other treatments (Table 3) excluding *B. lactis* BB-12 or the control (0.1-0.2 g/100 mL). In contrast to the weak ability of bifidobacteria to ferment lactose (Watson et al., 2013), the acetic acid content in this study was higher in the kefir samples of G10-G12, which could be due to the heterofermentative activity taking place on lactulose consumption (Thongaram et al., 2017). According to these results (G10-G12), AA was dependent on lactulose concentration, which was significantly increased ($p<0.05$) in G12 supplemented with 5% lactulose compared to G10 and G11 ($p>0.05$).

3.5 Conjugated linoleic acid

The CLA contents of the kefir samples produced during the fermentation process and storage time are presented in Table 3. The CLA content was increased in a time- and lactulose-dose-dependent manner; the CLA values of the control sample at different sampling times were significantly less ($p<0.05$) than those of the other samples. These results (Table 3) show that the lowest CLA value was 2.20 ppm on day 1 in G10 (incorporated with 0% lactulose) more than those of the control on days 1 and 7 (0.72 and 0.98 ppm, respectively), indicating the role of complementary probiotics in increasing CLA produced in the kefirs. The CLA of G10 was 2.42, 3.20, and 6.07 ppm significantly less ($p<0.05$) than those of G11 (2.81, 4.14, and 6.02 ppm) and G12 (3.27, 4.55, and 7.08 ppm, respectively), indicating that CLA was increased by increases in lactulose, particularly in samples of probiotic bacterial consortiums (G7-G12). The linoleate isomerase gene (*lai*), which induces the conversion of linoleic acid to CLA, has significant homology with myosin-cross-reactive antigen (MCRA) proteins (Salsinha et al., 2018) produced in response to stress in bacteria. MCRA proteins in probiotics may cooperate in the first phase of CLA fabrication (Rosberg-Cody et al., 2011). Moreover, it has been proposed that CLA is produced as a result of a stress response induced by more than one gene in a multiple-phase response (Salsinha et al., 2018). Decreases in pH value have been claimed as a sub-lethal stress factor for *Lactobacillus spp.* (Vieira et al., 2015). During microbial activities, the pH drops considerably to below 4.6 during milk fermentation, which ultimately decreases CLA production by the bacteria (Kim & Liu, 2002). This finding was not in line with this study (Table 3), which showed that a decrease in pH and increase in CLA values coincided in a time-dependent manner in cold storage. As such, the greatest value of CLA (8.07 ppm) was observed in the consortium of *L. acidophilus* LA-5+ *L. paracasei* 431 on day 14 (G9), while the pH reached 4.30 (Table 2). The greatest CLA values for days 1 and 7 were found in G9 (3.51 ppm) and G12 (8.07 ppm), respectively. The CLAs of all treatments were significantly greater ($p<0.05$) than those of the control sample (Table 3). This value reached about 2.0 ppm in the kefir supplemented with *Streptococcus thermophilus* and *B. lactis* BB-12 (Florence et al., 2009), which is less than those of kefir samples G1-G12 of this study. The CLA of milk fermented by a consortium of starter and complementary probiotics containing the *Lactococcus lactis* subsp. *cremoris* MRS47 was 0.08 ppm kefir at 40 °C and pH=4.5 after 8 h (Vieira et al., 2017). These

low CLA values might be due to the type of complementary probiotics, sampling time, and temperature for preservation. Contrary to this study (Table 3), CLA reached 1.2 ppm, while it was 0.9-0.95 ppm in the yogurt supplemented with *L. lactis* and *L. reuteri* after 6 days of cold storage (Colakoglu & Gursoy, 2011).

3.6 Sensory properties

The results of sensory evaluation on the kefir samples during storage at a refrigerated temperature are given in Table 4. A score below 5, i.e. "indifferent", was not observed in any samples for the properties of taste, odor, texture, or overall acceptance. The overall acceptability scores for the bacterial consortium samples were increased by increasing the time up to 7 days and by increasing the lactulose concentration. Conversely, the overall acceptability was decreased with increases in a time-dependent manner up to day 14, which might be due to the formation of surface mold (Irigoyen et al., 2005). The transglutaminase yoghurt was firmer and less creamy than Control yoghurt. and consumers did not exhibit a high refusal against that (García-Gómez et al., 2019). The kefir samples containing monocultures *L. plantarum* O20 and *B. Lactis* BB-12 were more acceptable from other goat's kefir (Mituniewicz-Małek et al., 2019).

Table 4. Mean score of sensory attributes, including flavor, odor, texture and general acceptability of the kefir samples through the effect of independent variable (n=3).

Species of probiotic	Lactulose (%)	Days	Mean score			
			flavor	odor	Texture	O. acceptability
Lactobacillus acidophilus	0	1	5.33^{a}	5.66^{a}	5.66^{a}	5.66^{a}
	0	7	6.33^{b}	6.66^{b}	7.00^{b}	7.00^{b}
	0	14	5.66^{a}	5.66^{a}	5.66^{a}	7.00^{b}
	2.5	1	6.66^{bd}	6.33^{b}	6.66^{c}	6.66^{b}
	2.5	7	7.66^{c}	7.00^{b}	8.66^{d}	7.00^{b}
	2.5	14	7.00^{d}	7.00^{b}	7.00^{b}	5.66^{a}
	5	1	7.66^{c}	7.66^{c}	6.66^{c}	7.00^{b}
	5	7	8.33^{e}	8.33^{d}	8.33^{d}	8.66^{c}
	5	14	7.00^{d}	7.00^{b}	7.00^{b}	7.00^{b}
L. paracasei	0	1	5.66^{a}	6.66^{b}	6.66^{c}	6.66^{b}
	0	7	6.66^{bd}	8.00^{d}	7.66^{eg}	8.00^{d}
	0	14	5.66^{a}	6.00^{a}	6.66^{c}	6.66^{b}
	2.5	1	6.66^{bd}	7.00^{b}	6.66^{c}	6.66^{b}
	2.5	7	7.00^{d}	7.00^{b}	7.33^{b}	7.00^{b}
	2.5	14	6.66^{bd}	6.33^{b}	6.66^{c}	5.66^{a}
	5	1	7.66^{c}	6.66^{b}	6.00^{a}	7.66^{d}
	5	7	8.33^{e}	7.66^{c}	7.00^{b}	7.66^{d}
	5	14	7.33^{c}	6.33^{b}	6.00^{a}	7.00^{b}
L. acidophilus+L. paracasei	0	1	7.66^{c}	7.00^{b}	7.00^{b}	6.66^{b}
	0	7	8.00^{ce}	8.33^{d}	8.00^{e}	8.33^{cd}
	0	14	6.66^{e}	5.66^{a}	6.00^{a}	7.00^{b}
	2.5	1	8.00^{ce}	7.00^{b}	7.00^{b}	8.00^{d}
	2.5	7	8.66^{e}	7.66^{c}	7.66^{e}	7.66^{d}
	2.5	14	7.66^{c}	7.00^{b}	6.66^{c}	7.66^{d}
	5	1	8.66^{e}	7.66^{c}	7.66^{e}	8.66^{c}
	5	7	9.00^{f}	8.00^{d}	8.33^{de}	8.66^{c}
	5	14	8.66^{c}	8.00^{d}	8.33^{de}	8.33^{cd}
L. acidophilus+L. paracasei+ B. lactis	0	1	5.66^{a}	5.66^{a}	7.66^{c}	5.66^{a}
	0	7	6.66^{bd}	6.66^{b}	8.00^{e}	6.66^{b}
	0	14	5.33^{a}	5.33^{a}	6.66^{c}	5.33^{a}
	2.5	1	7.66^{c}	7.66^{c}	7.66^{e}	7.66^{d}
	2.5	7	7.00^{d}	7.00^{b}	8.33^{d}	7.00^{b}
	2.5	14	7.00^{d}	6.00^{a}	7.00^{b}	6.00^{a}
	5	1	8.66^{e}	8.66^{e}	8.66^{d}	8.66^{c}
	5	7	8.33^{e}	8.33^{d}	8.33^{e}	8.33^{cd}
	5	14	7.00^{c}	7.33^{bc}	8.00^{e}	7.66^{d}
Control	0	1	5.66^{a}	7.66^{c}	7.00^{b}	5.66^{a}
	0	7	6.00^{b}	8.33^{d}	7.66^{e}	7.66^{d}
	0	14	5.66^{b}	8.00^{d}	6.66^{c}	7.66^{d}

Different small superscripts in each column indicate a significant difference ($p< 0.05$). O: overall.

In another study, the high temperature (35°C) of fermentation was shown as a reason for higher viscosity (Barukčić et al., 2017). The greatest scores on days 1, 7, and 14 of kefir sampling in the current study were 8.66 (G9 and G12), 8.66 (G9 with no significance compared to G3, $p<0.05$), and 8.33 (G9 with no significance compared to G12, $p>0.05$), respectively. The scores for flavor in G9 were 8.66, 9.00, and 8.66, but for odor were 8.66, 8.33, and 8.00, respectively, in G12, G3, and G12, and G9. Dissimilarly, some researchers (Kiliç et al., 1999) believed that kefirs made 3 days after manufacturing did not have an appropriate odor for consumers. Greater concentrations of kefir grains (5%) showed less odor, but on day 14, the intensity of the odor was not acceptable, in contrast to the current study (Irigoyen et al., 2005). The greatest scores for texture (8.66) were found in G12 and G2 with no significance ($p>0.05$) compared to G3 and G9 (8.33). As such, the greatest scores were observed in G9 for overall acceptability (8.66, 8.66, and 8.33 after 1, 7, and 14 days of storage, respectively), when 5% lactulose was added to the kefir (Table 4), indicating that the increase in lactulose affected the palatability of the kefir according to the panelists, which was in line with the findings regarding CLA production (Table 3) in the kefir samples supplemented with *L. acidophilus* LA-5+ *L. paracasei* 431(G9). Similarly, other researchers showed that a more appropriate taste was obtained by increasing the kefir grain concentration, so that the addition of 6% grain produced a more acidic kefir with a better taste compared to the kefir samples supplemented with 2% kefir grains (Sulmiyati et al., 2019).

4 Conclusion

The results indicated that LA and AA measured from the test samples and even the control were significantly greater in the current study than in other studies, which may be explained by the types and quantities of starter probiotics. Furthermore, the interactions between lactulose in different doses and complementary probiotics caused lower syneresis. This situation resulted in more acceptability among the panelists who gave greater scores to the kefir samples with greater lactulose concentrations, particularly the sample with 5% lactulose. Obviously, the scores for the 3-bacterial consortium-supplemented kefirs were slightly lower compared to the *L. acidophilus* LA-5+ *L. paracasei* 431-supplemented kefir samples, which may be due to the higher production of AA, ranging from 0.4 to 0.6 g/ mL, and the bitter taste of this type of kefir. The survival rate of the complementary probiotics added to the kefir samples was higher than the standard level (7 log CFU/ mL), while the pH was decreased by 4.3 during storage at the refrigerated temperature after 14 days. The highest values of produced CLA were measured in G9 and G12, but acceptability for different sensory criteria was greatest for G9. It is concluded that the addition of probiotics with prebiotic improves the characteristics of kefir. In this context, the addition of 5% lactulose along with *L. acidophilus* LA-5+ *L. paracasei* 431 could valuably increase the CLA value (3.51-8.07 ppm) and give it more acceptability of flavor, odor, and syneresis.

Acknowledgements

Technical support for the thesis accomplishment by the Islamic Azad University is gratefully acknowledged.

References

Barukčić, I., Gracin, L., Režek Jambrak, A., & Božanić, R. (2017). Comparison of chemical, rheological and sensory properties of kefir produced by kefir grains and commercial kefir starter. *Mljekarstvo*, 67, 169-176. http://dx.doi.org/10.15567/mljekarstvo.2017.0301.

Bengoa, A. A., Iraporda, C., Acurcio, L. B., Cicco Sandes, S. H., Costa, K., Moreira Guimarães, G., Esteves Arantes, R. M., Neumann, E., Cantini Nunes, Á., Nicoli, J. R., Garrote, G. L., & Abraham, A. G. (2019a). Physicochemical, immunomodulatory and safety aspects of milks fermented with *Lactobacillus paracasei* isolated from kefir. *Food Research International*, 123, 48-55. http://dx.doi.org/10.1016/j.foodres.2019.04.041. PMid:31284997.

Bengoa, A. A., Iraporda, C., Garrote, G. L., & Abraham, A. G. (2019b). Kefir micro-organisms: their role in grain assembly and health properties of fermented milk. *Journal of Applied Microbiology*, 126(3), 686-700. http://dx.doi.org/10.1111/jam.14107. PMid:30218595.

Bensmira, M., & Jiang, B. (2012). Effect of some operating variables on the microstructure and physical properties of a novel Kefir formulation. *Journal of Food Engineering*, 108(4), 579-584. http://dx.doi.org/10.1016/j.jfoodeng.2011.07.025.

Bondia-Pons, I., Molto-Puigmarti, C., Castellote, A., & Lopez-Sabater, M. (2007). Determination of conjugated linoleic acid in human plasma by fast gas chromatography. *Journal of Chromatography. A*, 1157(1-2), 422-429. http://dx.doi.org/10.1016/j.chroma.2007.05.020. PMid:17532324.

Colakoglu, H., & Gursoy, O. (2011). Effect of lactic adjunct cultures on conjugated linoleic acid (CLA) concentration of yogurt drink. *Journal of Food Agriculture and Environment*, 9, 60-64. http://dx.doi.org/10.1234/4.2011.1908.

Coskun, F., & Karabulut Dirican, L. (2019). Effects of pine honey on the physicochemical, microbiological and sensory properties of probiotic yoghurt. *Food Science and Technology*, 39(2, Suppl. 2), 616-625. http://dx.doi.org/10.1590/fst.24818.

Costa, G. M., Paula, M. M., Costa, G. N., Esmerino, E. A., Silva, R., Freitas, M. Q., Barão, C. E., Cruz, A. G., & Pimentel, T. C. (2020). Preferred attribute elicitation methodology compared to conventional descriptive analysis: A study using probiotic yogurt sweetened with xylitol and added with prebiotic components. *Journal of Sensory Studies*, 35, e12602. http://dx.doi.org/10.1111/joss.12602.

Cui, X.-H., Chen, S.-J., Wang, Y., & Han, J.-R. (2013). Fermentation conditions of walnut milk beverage inoculated with kefir grains. *Lebensmittel-Wissenschaft + Technologie*, 50(1), 349-352. http://dx.doi.org/10.1016/j.lwt.2012.07.043.

Delgado-Fernández, P., Corzo, N., Lizasoain, S., Olano, A., & Moreno, F. J. (2019). Fermentative properties of starter culture during manufacture of kefir with new prebiotics derived from lactulose. *International Dairy Journal*, 93, 22-29. http://dx.doi.org/10.1016/j.idairyj.2019.01.014.

Demir, H. (2020). Comparison of traditional and commercial kefir microorganism compositions and inhibitory effects on certain pathogens. *International Journal of Food Properties*, 23(1), 375-386. http://dx.doi.org/10.1080/10942912.2020.1733599.

Demirci, A. S., Palabiyik, I., Ozalp, S., & Tirpanci Sivri, G. (2019). Effect of using kefir in the formulation of traditional Tarhana. *Food Science and Technology*, 39(2), 358-364. http://dx.doi.org/10.1590/fst.29817.

Eor, J. Y., Tan, P. L., Son, Y. J., Lee, C. S., & Kim, S. H. (2020). Milk products fermented by *Lactobacillus* strains modulate the gut–bone axis in an ovariectomised murine model. *International Journal of Dairy Technology*, 73(4), 743-756. http://dx.doi.org/10.1111/1471-0307.12708.

Fazio, A., La Torre, C., Caroleo, M. C., Caputo, P., Cannataro, R., Plastina, P., & Cione, E. (2020). Effect of addition of pectins from jujubes (*Ziziphus jujuba* Mill.) on vitamin C production during heterolactic fermentation. *Molecules*, 25(11), 2706. http://dx.doi.org/10.3390/molecules25112706. PMid:32545249.

Florence, A. C. R., Silva, R. C., Espírito Santo, A. P., Gioielli, L. A., Tamime, A. Y., & de Oliveira, M. N. (2009). Increased CLA content in organic milk fermented by bifidobacteria or yoghurt cultures. *Dairy Science & Technology*, 89(6), 541-553. http://dx.doi.org/10.1051/dst/2009030.

Gamba, R. R., Yamamoto, S., Sasaki, T., Michihata, T., Mahmoud, A.-H., Koyanagi, T., & Enomoto, T. (2019). Microbiological and functional characterization of kefir grown in different sugar solutions. *Food Science and Technology Research*, 25(2), 303-312. http://dx.doi.org/10.3136/fstr.25.303.

García, C., Bautista, L., Rendueles, M., & Díaz, M. (2019). A new synbiotic dairy food containing lactobionic acid and *Lactobacillus casei*. *International Journal of Dairy Technology*, 72(1), 47-56. http://dx.doi.org/10.1111/1471-0307.12558.

García-Gómez, B., Romero-Rodríguez, Á., Vázquez-Odériz, L., Muñoz-Ferreiro, N., & Vázquez, M. (2019). Sensory quality and consumer acceptance of skim yoghurt produced with transglutaminase at pilot plant scale. *International Journal of Dairy Technology*, 72, 388-394. http://dx.doi.org/10.1111/1471-0307.12595.

Gaze, L. V., Costa, M. P., Monteiro, M. L., Lavorato, J. A., Conte Júnior, C. A., Raices, R. S., Cruz, A. G., & Freitas, M. Q. (2015). Dulce de Leche, a typical product of Latin America: characterisation by physicochemical, optical and instrumental methods. *Food Chemistry*, 169, 471-477. http://dx.doi.org/10.1016/j.foodchem.2014.08.017. PMid:25236253.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & Reid, G. (2017). Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews. Gastroenterology & Hepatology*, 14(8), 491-502. http://dx.doi.org/10.1038/nrgastro.2017.75. PMid:28611480.

Glibowski, P., & Zielińska, E. (2015). Physicochemical and sensory properties of kefir containing inulin and oligofructose. *International Journal of Dairy Technology*, 68(4), 602-607. http://dx.doi.org/10.1111/1471-0307.12234.

Gulati, A., Galvin, N., Hennessy, D., McAuliffe, S., O'Donovan, M., McManus, J. J., Fenelon, M. A., & Guinee, T. P. (2018). Grazing of dairy cows on pasture versus indoor feeding on total mixed ration: Effects on low-moisture part-skim Mozzarella cheese yield and quality characteristics in mid and late lactation. *Journal of Dairy Science*, 101(10), 8737-8756. http://dx.doi.org/10.3168/jds.2018-14566. PMid:30122409.

Hikmetoglu, M., Sogut, E., Sogut, O., Gokirmakli, C., & Guzel-Seydim, Z. (2020). Changes in carbohydrate profile in kefir fermentation. *Bioactive Carbohydrates and Dietary Fibre*, 23, 100220. http://dx.doi.org/10.1016/j.bcdf.2020.100220.

Hong, J.-Y., Lee, N.-K., Yi, S.-H., Hong, S.-P., & Paik, H.-D. (2019). Short communication: Physicochemical features and microbial community of milk kefir using a potential probiotic *Saccharomyces cerevisiae* KU200284. *Journal of Dairy Science*, 102(12), 10845-10849. http://dx.doi.org/10.3168/jds.2019-16384. PMid:31629522.

Irigoyen, A., Arana, I., Castiella, M., Torre, P., & Ibáñez, F. C. (2005). Microbiological, physicochemical, and sensory characteristics of kefir during storage. *Food Chemistry*, 90(4), 613-620. http://dx.doi.org/10.1016/j.foodchem.2004.04.021.

Kailasapathy, K., & Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium spp. Immunology and Cell Biology*, 78(1), 80-88. http://dx.doi.org/10.1046/j.1440-1711.2000.00886.x. PMid:10651933.

Kiliç, Ş., Uysal, H., Akbulut, N., Kavas, G., & Kesenkaş, H. (1999). Chemical, microbiological and sensory changes in ripening kefirs produced from starters and grains. *Ege* Üniversitesi *Ziraat Fakültesi Dergisi*, 36, 111-118.

Kim, D.-H., Jeong, D., Kim, H., & Seo, K.-H. (2019). Modern perspectives on the health benefits of kefir in next generation sequencing era: Improvement of the host gut microbiota. *Critical Reviews in Food Science and Nutrition*, 59(11), 1782-1793. http://dx.doi.org/10.1080/10408398.2018.1428168. PMid:29336590.

Kim, Y., & Liu, R. (2002). Increase of conjugated linoleic acid content in milk by fermentation with lactic acid bacteria. *Journal of Food Science*, 67(5), 1731-1737. http://dx.doi.org/10.1111/j.1365-2621.2002.tb08714.x.

Kivanc, M., & Yapici, E. (2019). Survival of Escherichia coli O157: H7 and *Staphylococcus aureus* during the fermentation and storage of kefir. *Food Science and Technology*, 39(Suppl. 1), 225-230. http://dx.doi.org/10.1590/fst.39517.

Kliks, J., Leśniak, A., Spruch, M., Szołdra, S., & Czabaj, S. (2019). Quality characteristics of natural yoghurts produced with lactulose supplementation. *Integrative Food, Nutrition and Metabolism*, 6, 1-4. http://dx.doi.org/10.15761/IFNM.1000253.

Kök-Taş, T., Seydim, A. C., Özer, B., & Guzel-Seydim, Z. B. (2013). Effects of different fermentation parameters on quality characteristics of kefir. *Journal of Dairy Science*, 96(2), 780-789. http://dx.doi.org/10.3168/jds.2012-5753. PMid:23245957.

Larosa, C. P., Balthazar, C. F., Guimarães, J. T., Rocha, R. S., Silva, R., Pimentel, T. C., Granato, D., Duarte, M. C. K. H., Silva, M. C., Freitas, M. Q., Cruz, A. G., & Esmerino, E. A. (2020). Sheep milk kefir sweetened with different sugars: Sensory acceptance and consumer emotion profiling. *Journal of Dairy Science*. In press. http://dx.doi.org/10.3168/jds.2020-18702. PMid:33162085.

Leite, A. M. O., Miguel, M. A. L., Peixoto, R. S., Rosado, A. S., Silva, J. T., & Paschoalin, V. M. F. (2013). Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage. *Brazilian Journal of Microbiology*, 44(2), 341-349. http://dx.doi.org/10.1590/S1517-83822013000200001. PMid:24294220.

Lim, H. W., Kim, D. H., Jeong, D., Kang, I. B., Kim, H., & Seo, K. H. (2019). Biochemical characteristics, virulence traits and antifungal resistance of two major yeast species isolated from kefir: Kluyveromyces marxianus and Saccharomyces unisporus. *International Journal of Dairy Technology*, 72(2), 275-281. http://dx.doi.org/10.1111/1471-0307.12582.

Linares, D. M., Gomez, C., Renes, E., Fresno, J. M., Tornadijo, M. E., Ross, R. P., & Stanton, C. (2017). Lactic acid bacteria and bifidobacteria with potential to design natural biofunctional health-promoting dairy foods. *Frontiers in Microbiology*, 8, 846. http://dx.doi.org/10.3389/fmicb.2017.00846. PMid:28572792.

Magalhães, K. T., Dragone, G., Melo Pereira, G. V., Oliveira, J. M., Domingues, L., Teixeira, J. A., Silva, J. B. A., & Schwan, R. F. (2011a). Comparative study of the biochemical changes and volatile compound formations during the production of novel whey-based kefir beverages and traditional milk kefir. *Food Chemistry*, 126(1), 249-253. http://dx.doi.org/10.1016/j.foodchem.2010.11.012.

Magalhães, K. T., Pereira, G. V. M., Campos, C. R., Dragone, G., & Schwan, R. F. (2011b). Brazilian kefir: structure, microbial communities and chemical composition. *Brazilian Journal of Microbiology*, 42(2), 693-702. http://dx.doi.org/10.1590/S1517-83822011000200034. PMid:24031681.

Mitra, S., & Ghosh, B. C. (2020). Quality characteristics of kefir as a carrier for probiotic *Lactobacillus rhamnosus* GG. *International Journal of Dairy Technology*, 73(2), 384-391. http://dx.doi.org/10.1111/1471-0307.12664.

Mituniewicz-Małek, A., Zielińska, D., & Ziarno, M. (2019). Probiotic monocultures in fermented goat milk beverages–sensory quality of final product. *International Journal of Dairy Technology*, 72(2), 240-247. http://dx.doi.org/10.1111/1471-0307.12576.

Montanuci, F. D., Pimentel, T. C., Garcia, S., & Prudencio, S. H. (2012). Effect of starter culture and inulin addition on microbial viability, texture, and chemical characteristics of whole or skim milk Kefir. *Food Science and Technology*, 32(4), 580-865. http://dx.doi.org/10.1590/S0101-20612012005000119.

Nacheva, I. (2019). Kinetic and microbiological dependencies in the process of fermentation of goat milk, enriched with lactulose. *Bulgarian Journal of Agricultural Science*, 25, 187-190.

Nambou, K., Gao, C., Zhou, F., Guo, B., Ai, L., & Wu, Z.-J. (2014). A novel approach of direct formulation of defined starter cultures for different kefir-like beverage production. *International Dairy Journal*, 34(2), 237-246. http://dx.doi.org/10.1016/j.idairyj.2013.03.012.

Nejati, F., Junne, S., & Neubauer, P. (2020). A big world in small grain: a review of natural milk kefir starters. *Microorganisms*, 8(2), 192-201. http://dx.doi.org/10.3390/microorganisms8020192. PMid:32019167.

Ribeiro, A. S., Silva, M. N., Tagliapietra, B. L., Brum Jr., B. S., Ugalde, M. L., & Richards, N. S. P. S. (2019). Development of symbiotic yoghurt and biological evaluation (New Zealand White Rabbits) of its functional properties. *Food Science and Technology*, 39(2, Suppl. 2), 418-425. http://dx.doi.org/10.1590/fst.20618.

Roobab, U., Batool, Z., Manzoor, M. F., Shabbir, M. A., Khan, M. R., & Aadil, R. M. (2020). Sources, formulations, advanced delivery and health benefits of probiotics. *Current Opinion in Food Science*, 32, 17-28. http://dx.doi.org/10.1016/j.cofs.2020.01.003.

Rosa, D. D., Dias, M. M. S., Grześkowiak, Ł. M., Reis, S. A., Conceição, L. L., & Peluzio, M. C. G. (2017). Milk kefir: nutritional, microbiological and health benefits. *Nutrition Research Reviews*, 30(1), 82-96. http://dx.doi.org/10.1017/S0954422416000275. PMid:28222814.

Rosberg-Cody, E., Liavonchanka, A., Göbel, C., Ross, R. P., O'Sullivan, O., Fitzgerald, G. F., Feussner, I., & Stanton, C. (2011). Myosin-cross-reactive antigen (MCRA) protein from Bifidobacterium breve is a FAD-dependent fatty acid hydratase which has a function in stress protection. *BMC Biochemistry*, 12(1), 9. http://dx.doi.org/10.1186/1471-2091-12-9. PMid:21329502.

Sabooni, P., Pourahmad, R., & Adeli, H. R. M. (2018). Improvement of viability of probiotic bacteria, organoleptic qualities and physical characteristics in kefir using transglutaminase and xanthan. *Acta Scientiarum Polonorum. Technologia Alimentaria*, 17(2), 141-148. http://dx.doi.org/10.17306/J.AFS.0556. PMid:29803216.

Salsinha, A. S., Pimentel, L. L., Fontes, A. L., Gomes, A. M., & Rodríguez-Alcalá, L. M. (2018). Microbial production of conjugated linoleic acid and conjugated linolenic acid relies on a multienzymatic system. *Microbiology and Molecular Biology Reviews*, 82(4), e00019-18. http://dx.doi.org/10.1128/MMBR.00019-18. PMid:30158254.

Santos, D. C., Oliveira, J. G. Fo., Santana, A. C. A., Freitas, B. S. M., Silva, F. G., Takeuchi, K. P., & Egea, M. B. (2019). Optimization of soymilk fermentation with kefir and the addition of inulin: Physicochemical, sensory and technological characteristics. *LWT*, 104, 30-37. http://dx.doi.org/10.1016/j.lwt.2019.01.030.

Setyawardani, T., Sumarmono, J., & Widayaka, K. (2020). Physical and microstructural characteristics of kefir made of milk and colostrum. *Buletin Peternakan*, 44(1), 43-49. http://dx.doi.org/10.21059/buletinpeternak.v44i1.49130.

Shafi, A., Naeem Raja, H., Farooq, U., Akram, K., Hayat, Z., Naz, A., & Nadeem, H. R. (2019). Antimicrobial and antidiabetic potential of synbiotic fermented milk: a functional dairy product. *International Journal of Dairy Technology*, 72(1), 15-22. http://dx.doi.org/10.1111/1471-0307.12555.

Siang, S. C., Wai, L. K., Lin, N. K., & Phing, P. L. (2019). Effect of added prebiotic (Isomalto-oligosaccharide) and coating of beads on the survival of microencapsulated *Lactobacillus rhamnosus* GG. *Food Science and Technology*, 39(Suppl. 2), 601-609. http://dx.doi.org/10.1590/fst.27518.

Sohrabvandi, S., Mortazavian, A.-M., Dolatkhahnejad, M.-R., & Monfared, A. B. (2012). Suitability of MRS-bile agar for the selective enumeration of mixed probiotic bacteria in presence of mesophilic lactic acid cultures and yoghurt bacteria. *Iranian Journal of Biotechnology*, 10, 16-21.

Sulmiyati, S., Said, N. S., Fahrodi, D. U., Malaka, R., & Maruddin, F. (2019). The physicochemical, microbiology, and sensory characteristics of kefir goat milk with different levels of kefir grain. *Tropical Animal Science Journal*, 42(2), 152-158. http://dx.doi.org/10.5398/tasj.2019.42.2.152.

Szajnar, K., Znamirowska, A., & Kuźniar, P. (2020). Sensory and textural properties of fermented milk with viability of *Lactobacillus rhamnosus* and *Bifidobacterium animalis* ssp. *lactis* Bb-12 and increased calcium concentration. *International Journal of Food Properties*, 23(1), 582-598. http://dx.doi.org/10.1080/10942912.2020.1748050.

Thongaram, T., Hoeflinger, J. L., Chow, J., & Miller, M. J. (2017). Prebiotic galactooligosaccharide metabolism by probiotic lactobacilli and bifidobacteria. *Journal of Agricultural and Food Chemistry*, 65(20), 4184-4192. http://dx.doi.org/10.1021/acs.jafc.7b00851. PMid:28466641.

Tomar, O., Akarca, G., Çağlar, A., Beykaya, M., & Gök, V. (2020). The effects of kefir grain and starter culture on kefir produced from cow and buffalo milk during storage periods. *Food Science and Technology*, 40(1), 238-244. http://dx.doi.org/10.1590/fst.39418.

Vieira, C. P., Cabral, C. C., Costa Lima, B. R. C., Paschoalin, V. M. F., Leandro, K. C., & Conte-Junior, C. A. (2017). *Lactococcus lactis* ssp. *cremoris* MRS47, a potential probiotic strain isolated from kefir grains, increases cis-9, trans-11-CLA and PUFA contents in fermented milk. *Journal of Functional Foods*, 31, 172-178. http://dx.doi.org/10.1016/j.jff.2017.01.047.

Vieira, C., Álvares, T., Gomes, L., Torres, A., Paschoalin, V., & Conte-Junior, C. (2015). Kefir grains change fatty acid profile of milk during fermentation and storage. *PLoS One*, 10(10), e0139910. http://dx.doi.org/10.1371/journal.pone.0139910. PMid:26444286.

Wang, H., Wang, C., Wang, M., & Guo, M. (2017). Chemical, physiochemical, and microstructural properties, and probiotic survivability of fermented goat milk using polymerized whey protein and starter culture Kefir Mild 01. *Journal of Food Science*, 82(11), 2650-2658. http://dx.doi.org/10.1111/1750-3841.13935. PMid:29125639.

Watson, D., O'Connell Motherway, M., Schoterman, M., van Neerven, R. J., Nauta, A., & Van Sinderen, D. (2013). Selective carbohydrate utilization by lactobacilli and bifidobacteria. *Journal of Applied Microbiology*, 114(4), 1132-1146. http://dx.doi.org/10.1111/jam.12105. PMid:23240984.

Yamamoto, N., Shoji, M., Hoshigami, H., Watanabe, K., Watanabe, K., Takatsuzu, T., Yasuda, S., Igoshi, K., & Kinoshita, H. (2019). Antioxidant capacity of soymilk yogurt and exopolysaccharides produced by lactic acid bacteria. *Bioscience of Microbiota, Food and Health*, 38(3), 97-104. http://dx.doi.org/10.12938/bmfh.18-017. PMid:31384521.

Yildiran, H., Başyiğit Kiliç, G., & Karahan Çakmakçi, A. G. (2019). Characterization and comparison of yeasts from different sources for some probiotic properties and exopolysaccharide production. *Food Science and Technology*, 39(Suppl. 2), 646-653. http://dx.doi.org/10.1590/fst.29818.

Yoo, S.-H., Seong, K.-S., & Yoon, S.-S. (2013). Physicochemical properties of kefir manufactured by a two-step fermentation. *Han-gug Chugsan Sigpum Hag-hoeji*, 33(6), 744-751. http://dx.doi.org/10.5851/kosfa.2013.33.6.744.

Zareba, D., Ziarno, M., & Obiedzinski, M. (2012). Volatile profile of non-fermented milk and milk fermented by *Bifidobacterium animalis* subsp. *lactis*. *International Journal of Food Properties*, 15(5), 1010-1021. http://dx.doi.org/10.1080/10942912.2010.513024.

Zendeboodi, F., Khorshidian, N., Mortazavian, A. M., & Cruz, A. G. (2020). Probiotic: conceptualization from a new approach. *Current Opinion in Food Science*, 32, 103-123. http://dx.doi.org/10.1016/j.cofs.2020.03.009.

Živković, M., Miljković, M. S., Ruas-Madiedo, P., Markelić, M. B., Veljović, K., Tolinački, M., Soković, S., Korać, A., & Golić, N. (2016). EPS-SJ exopolisaccharide produced by the strain *Lactobacillus paracasei* subsp. *paracasei* BGSJ2-8 is involved in adhesion to epithelial intestinal cells and decrease on *E. coli* association to Caco-2 cells. *Frontiers in Microbiology*, 7, 286. http://dx.doi.org/10.3389/fmicb.2016.00286. PMid:27014210.