An Acad Bras Cienc (2022) 94(3): e20210875 DOI 10.1590/0001-3765202220210875
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

ENGINEERING SCIENCES

The Effect of Fermentation Time and Yogurt Bacteria on the Physicochemical, Microbiological and Antioxidant Properties of Probiotic Goat Yogurts

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Abstract: In this study, monocultures of *L. casei*, *L. acidophilus, B. lactis* and their combination with yogurt starter culture were used with goat yogurt. Yogurts containing only probiotic bacteria were observed for 12 hours of fermentation, and yogurts containing both probiotic bacteria and yogurt bacteria were followed for 8 hours of fermentation. The use of yogurt culture increased the lactic acid contents, hardness values and antioxidant activities – using ABTS (2,2-azino-di-(3-ethylbenzothialozine sulfonic acid) and DPPH (2,2-Diphenyl-1-picrylhydrazyl) methods – and exhibited a shortened fermentation time. DPPH radical scavenging activity of all probiotic yogurt samples without yogurt culture decreased significantly at the end of fermentation (after 8 hours) compared to the beginning of fermentation (p<0.05). Across all the samples, *L. acidophilus* and *B. lactis*-containing yogurts exhibited the maximum viability at the end of fermentation. *L. casei* could not maintain viability at the end of the 8 hour fermentation. A high positive correlation was determined between antioxidant activity (ABTS) and the free amino acid results of probiotic yogurts containing yogurt culture. In this study, it was concluded that antioxidant activity, probiotic viability and amino acid content of probiotic goat yogurts changed with fermentation time.

Key words: goat milk, probiotic yogurt, fermentation, antioxidant activity, free amino acid content.

INTRODUCTION

Yearly global non-bovine milk production has reached 133 million tons, corresponding to more than 17% of worldwide milk production (Ranadheera et al. 2019). Goat milk production makes up 13.5% of non-bovine milk, and it is regarded as being among the biggest contributors to non-bovine milk production (Nunez & de Renobales 2016, Ranadheera et al. 2018).

One of the most important factors affecting the increase in consumption of goat milk and its products is the beneficial effect of goat milk on human health (Akan & Kinik 2015). Goat milk produces less allergic reactions (Park et al. 2007) and exhibits a higher digestibility (Jandal 1996) compared to milk from cows. Goat milk contains a lower proportion of trans C18:1 fatty acid than cow's milk, which is an important advantage in terms of lowering the risk of heart disease (Haenlein 2004). Despite these advantages, many consumers may avoid consumption of goat milk and its products because of the characteristic unpleasant goat odor and taste. However, it is possible to mask the unpleasant odor and taste of goat milk and its products, and to improve its rheological properties by enriching it with probiotic microorganisms (Slacanac et al. 2010).

Probiotics are live microorganisms that benefit the health of the host when taken in adequate amounts (García-Burgos et al. 2020). In order for probiotics to have a beneficial effect on health, they must remain alive in the passages of the gastrointestinal tract. However, in recent years, it has been reported that non-viable probiotic cells (paraprobiotics) and their metabolites (postbiotics) also contribute to a positive effect on health (Zendeboodi et al. 2020). It has been reported in many studies that fermented milk products have a health-promoting effect (Jia et al. 2016, Yang et al. 2008, Grom et al. 2020, Nyanzi et al. 2021). It has been reported in published literature that goat milk plays an important role as a probiotic carrier due to its high buffering capacity and nutrients, and that probiotics increase the functionality of goat milk (Ranadheera et al. 2012, Slacanac et al. 2010). In addition, it has been reported that probiotic goat milk products have higher antioxidant activity, cholesterol-lowering activity, and antimicrobial activity than cow milk products (Zhang et al. 2015, Balakrishnan & Agrawal 2014, Slacanac et al. 2004). Yogurt is consumed frequently around the world and is a good carrier of probiotics and bioactive substances. In recent years, new studies have been carried out on the use of goat milk in yogurt production. According to Hadjimbei et al. (2020) produced probiotic yogurt containing *Pistacia atlantica* resin extracts using S*acchoromyces boulardii* as a starter. El-Shafei et al. (2020) enriched goat milk yogurt with quinoa extract. Pradeep Prasanna & Charalampopoulos (2019) Bifidobacterium animalis subsp. encapsulated lactis BB-12 and used it in probiotic goat yogurt. Mituniewicz-Małek et al. (2019) and Lucatto et al. (2020) evaluated 86 probiotic goat yogurt and beverages in terms of sensory quality.

Lactic acid bacteria (LAB) are widely used in the fermentation process in the food industry, especially in the dairy industry (Peighambardoust et al. 2011). During fermentation, as a result of the proteolytic activity of LAB, bioactive peptides with many health effects, including antioxidant activity, may be released. Free radicals and reactive oxygen species affect the development of many diseases, including cardiovascular diseases.

Studies on the biological properties of fermented milk products, including probiotic viability and antioxidant activity during fermentation, are very limited. Only Ozcan et al. (2019) investigated the changes in antioxidant activity during the fermentation of kefir. Therefore, this present study aimed to produce probiotic yogurts using goat milk – a research subject studied less because of a focus on cow's milk – and some properties of these yogurts were determined during fermentation. *L. casei*, *L. acidophilus* and *B. lactis* are widely used in the dairy industry in the production of probiotic yogurts. In this study, six types of probiotic yogurt were produced using monoculture probiotics (*L. casei*, *L. acidophilus* and *B. lactis*) and yogurt culture (*S. thermophilus* and *L. bulgaricus*). Acidification kinetics, hardness values, viability levels of probiotic and yogurt bacteria, antioxidant activities (measured using DPPH and ABTS methods) and the total free amino acid content parameters were investigated during 12 hours of fermentation of yogurts containing only probiotic bacteria, and 8 hours of fermentation of yogurts containing both probiotic bacteria and yogurt culture. The effects of the yogurt culture and fermentation time on these parameters were then examined.

MATERIALS AND METHODS

Material

Raw goat's milk was purchased from Sütüm Keçiden Farm in Manisa, Turkey. Probiotic

bacteria (*Lactobacillus casei* 431, *L. acidophilus* LA-5, *Bifidobacterium* subsp. *lactis* BB-12) and yogurt culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) were obtained from Chr. Hansen (Copenhagen, Denmark).

Method

Yogurt production

Raw goat's milk (pH: 6.90, protein: 3.40% and fat: 3.70%) was heated to 85 °C for 15 minutes. Then the resulting pasteurized milk was divided into 6 groups and inoculated with probiotic bacteria (10 mg per liter milk to reach 10^7-10^8 CFU/g viability) and yogurt culture according to the manufacturer's instructions. Probiotic yogurts were incubated at 37 °C for 12 hours and the probiotic yogurts containing yogurt bacteria were incubated at 42 °C for 8 hours. During the fermentation process, samples were periodically taken. Sample codes are given below:

LC: Monoculture of *Lactobacillus casei* 431 with yogurt

LA: Monoculture of *Lactobacillus acidophilus* LA-5 with yogurt

BL: Monoculture of *Bifidobacterium animalis* subsp. *lactis* BB-12 with yogurt

LCY: *Lactobacillus casei* 431 and yogurt bacteria with yogurt

LAY: *Lactobacillus acidophilus* LA-5 and yogurt bacteria with yogurt

BLY: *Bifidobacterium animalis* subsp. *lactis* BB-12 and yogurt bacteria with yogurt

The pH, titratable acidity and acidification kinetics

The pH values of yogurt samples were determined using a digital pH meter (Hanna HI 83141, Woonsocket, Rhode Island, USA) and the titratable acidity values were determined using the alkali titration method with 0.1 mol/L NaOH and expressed as a lactic acid percentage (AOAC 2003). The acidification rate (V_{max}) was calculated as the time change of pH (dpH/dt) and expressed as pH units/min (Oliveira et al. 2009). At the end of the incubation, the following kinetic parameters were also calculated: t_f : time to reach pH 4.6; t_{max}: time at which V_{max} was reached.

Hardness

Hardness values of the yogurts were determined using a Brookfield CT3 Texture Analyzer (Middleboro, USA) device and a TA4/1000 acrylic probe (38.1 mm in diameter and 20 mm in height). The device parameters were set as follows: a load cell of 4500 x *g*, a trigger load of 6.8 g and a test speed of 1.00 mm/s. Hardness values were calculated using Brookfield Texture Pro CT software and expressed in grams (Yerlikaya et al. 2020).

Antioxidant activity

The preparation of pH 4.6 soluble nitrogen extracts

A 20 g sample of yogurt was mixed with 40 mL of water, and the pH of the mixture was adjusted to 4.6 with 1 mol/L HCl. The mixture was kept in a water bath at 40 °C for 1 hour and then centrifuged at 3000 × *g* at 4 °C for 30 minutes. After centrifugation, the upper fat layer was removed, and the supernatant was filtered through Whatman No. 42 filter paper. The filtrate obtained was used to determine the antioxidant activity and the total amount of free amino acids.

DPPH radical scavenging activity

A determination of the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging

activity was carried out according to Pavithra & Vadivukkarasi (2015), with modifications. Accordingly, 100 µL of DPPH solution (0.2 mmol/L prepared in methanol) was added to a 100 µL sample, and the mixture was incubated at room temperature for 30 minutes in the dark. The absorbance was measured at a wavelength of 517 nm using a 96-well Microplate Reader (Thermo Scientific, Multiskan Sky, Waltham, Massachusetts, USA). For the control sample, 100 µL of methanol was used instead of a 100 µL sample. Blank solutions were used for both the control and sample. The results were expressed as a percentage of DPPH radical scavenging activity (RSA).

$$\%RSA = [(Abscontrol - Abssample)/Abscontrol] \times 100$$

ABTS radical scavenging activity

The ABTS [(2,2-azino-di-(3-ethylbenzothialozine sulfonic acid)] radical scavenging activity was determined by the method described in Re et al. (1999). A 10 µL sample and 240 µL of ABTS solution were mixed and incubated for 10 minutes at room temperature. Then, absorbance was measured at a wavelength of 734 nm using a 96-well Microplate Reader. The results were expressed as µmol/L of Trolox equivalent antioxidant capacity or TEAC.

Total free amino acid content

The total free amino acid (TFAA) levels of the pH 4.6 soluble extracts of the yogurt samples were determined by the Cadmium (Cd)–ninhydrin spectrophotometric method described in Hayaloglu (2007). A 200 µL amount of the Cd-Ninhydrin reagent (prepared daily) was added to 10 µL of sample. The mixture was heated at 84°C for 15 minutes and cooled rapidly afterwards. The mixture absorbances were measured at a wavelength of 507 nm using a 96-well Microplate Reader (Thermo Scientific, Multiskan Sky, Waltham, Massachusetts, USA). The results were expressed as µg of leucine/mL.

Microbiological analyses

Under aseptic conditions, 10 g of sample was homogenized with 90 mL of 0.1% peptone water. Then, serial dilutions were prepared in peptone water. MRS agar was used for *L. bulgaricus* counts, and samples were incubated at 37 °C for 48 to 72 hours in an anaerobic environment. M17 agar was used for *S. thermophilus* counts, and samples were incubated at 37 °C for 24-48 hours in an aerobic environment (Terzaghi & Sandine 1975). MRS-sorbitol agar (Merck, Darmstadt, Germany) was used for counting *L. acidophilus*, and samples were incubated at 37 °C for 48 to 72 hours in an anaerobic environment. MRS agar containing 1 mg/L of vancomycin, and bromcresol green was used for *L. casei* counts. Petri dishes were incubated at 37 °C for 72 hours under anaerobic conditions. TOS-Propionate Agar (Merck, Darmstadt, Germany) was used for *Bifidobacterium animalis* subsp. *lactis* counts. MUP Selective Supplement was added to the medium through a sterile filter with a pore size of 0.22 µm, and incubation was carried out under anaerobic conditions at 37 °C for 72 hours (Thitaram et al. 2005).

Statistical analyses

The trials in the present study were replicated twice. All analyses were performed in triplicate. For the statistical analyses, one-way analysis of variance (ANOVA) was adopted using SPSS software version 25.0 (SPSS Inc. Chicago, Illinois). The significantly different groups were determined using the Duncan test ($p<0.05$). The correlation coefficient was calculated using the Pearson Correlation Test.

RESULTS AND DISCUSSION

Acidification kinetics

In this study, pH values of plain probiotic yogurts and probiotic yogurts containing yogurt culture were determined during the 12 hours and 8 hours of fermentation, respectively (Figure 1a and 1b). At the end of the 12th and 8th hour of fermentation, pH values fell below 4.6 pH. After 12 hours of fermentation, the lowest pH value was observed in the LC sample. In the LCY, LAY and BLY samples, yogurt cultures accelerated lactose degradation and lactic acid production, and thereby shortened fermentation time. V_{max} expresses pH units per minute. The maximum V_{max} value was calculated in the BLY sample (Table I). This shows that BLY is the sample whose acidity progresses the fastest during the incubation period. It was observed that the incubation period of LA and LAY yogurts containing *L. acidophilus* was longer than the other samples. This showed that *L. acidophilus* grew more slowly in goat milk than *B. lactis* and *L. casei*. During the fermentation, lactic acid values of all yogurt samples increased and reached a maximum level at the end of the

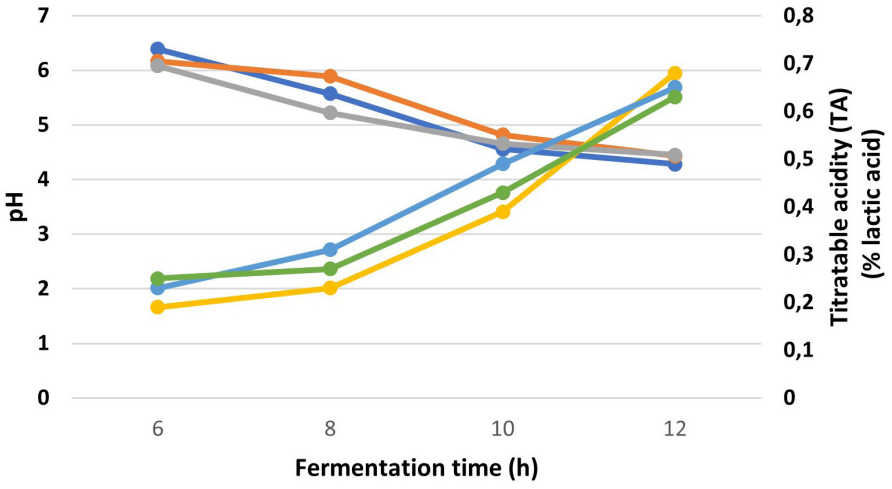


Figure 1a. pH and titratable acidity values of LC, LA and BL samples during the fermentation.

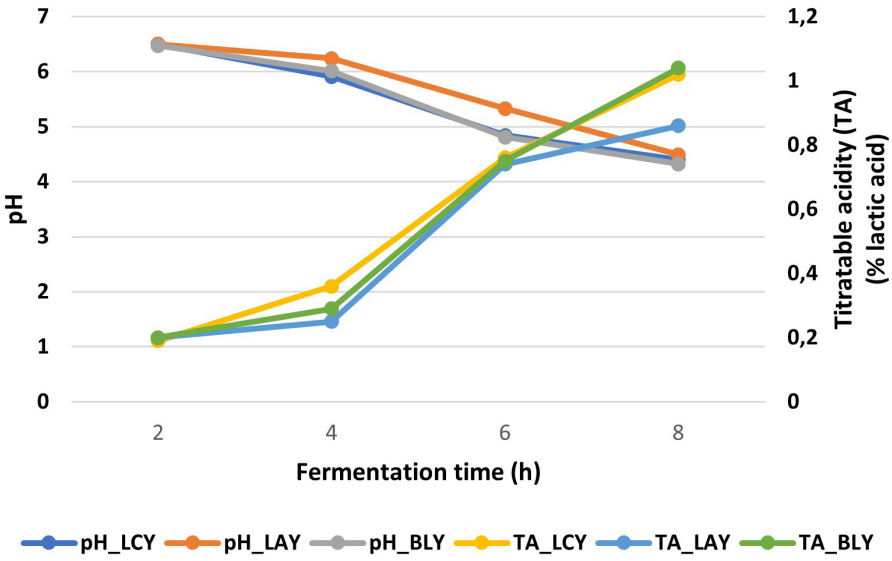


Figure 1b. pH and titratable acidity values of LCY, LAY and BYL samples during the fermentation.

Table I. Acidification kinetics of yogurt samples.

Sample	V_{max} (10^{-3} pH units/min)	t_f (time (h) to pH reached 4.6)	t_{max} (incubation time (h))
LC	3.65	10	12
LA	3.44	11	12
BL	3.47	10	12
LCY	5.21	7	8
LAY	5.02	7,5	8
BLY	5.35	7	8

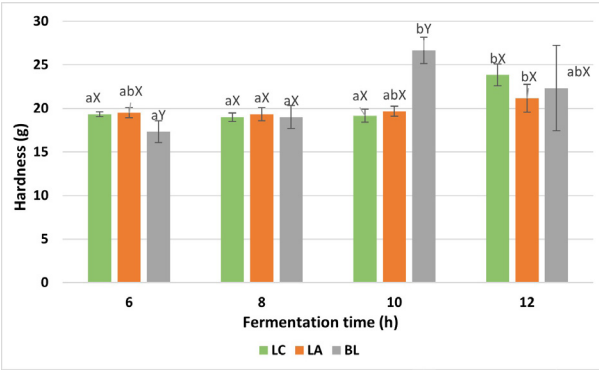
fermentation. The lactic acid content exhibited the highest increase in probiotic yogurts with yogurt bacteria at the 6th hour of fermentation (Figure 1a).

Hardness

The most important parameter in evaluating the textural properties of yogurt is hardness. Yogurt has its maximum water-holding capacity when the pH value of yogurt reaches the isoelectric point of the casein. Later, water released from the yogurt may develop increasing acidity. The strength of the gel structure varies due to the pH drop and casein aggregation resulting from disulfide bonds between the casein and denatured whey proteins (Ozcan et al. 2020). In addition, parameters such as starter culture type/amount, compatibility of bacteria, storage time, fermentation time and temperature, and the food matrix, all affect the textural parameters (Pereira et al. 2003, Pakseresht et al. 2017).

The hardness values of yogurts containing only probiotic bacteria did not change significantly at hour 6 and hour 8 of fermentation (p>0.05) (Figure 2a). At hour 10 of fermentation, only the hardness value of the BL sample (26.66 g) increased significantly (p<0.05). The LC and LA samples had the highest hardness values at hour 12 of fermentation. While the BL sample had a higher hardness at hour 10 of fermentation, compared to the other samples, it was observed that the hardness value decreased at hour 12 of fermentation. The reason for this may be the release of water as a result of the decrease in pH value of the BL sample by the 12th hour. Nevertheless, at the end of the fermentation period, there was no statistically significant difference in the hardness values of the samples (p>0.05).

Hardness values of yogurts containing both probiotic bacteria and yogurt culture are given in Figure 2b. There were no significant differences in the hardness values of the samples at hour 2 and hour 4 of fermentation (p>0.05). Although it was observed that the hardness values of the LCY and BLY samples increased (p<0.05) by the 6th hour of fermentation, there was no change in the hardness value of the LAY sample. It was observed that pH values of the LCY and BLY samples at this hour were at a value of 4.80, while the LAY sample had a value of 5.33. Based on this, it can be said that the curd hardness of probiotic yogurts containing yogurt culture increased significantly at a pH of 4.80. Lee & Lucey (2010), on the other hand, reported that gel formation occurs when the pH value of high



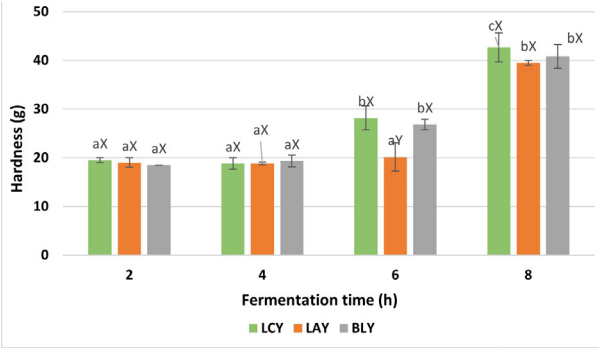


Figure 2a. Hardness values of LC, LA and BL samples during the fermentation.
Figure 2b. Hardness values of LCY, LAY and BLY samples during the fermentation.

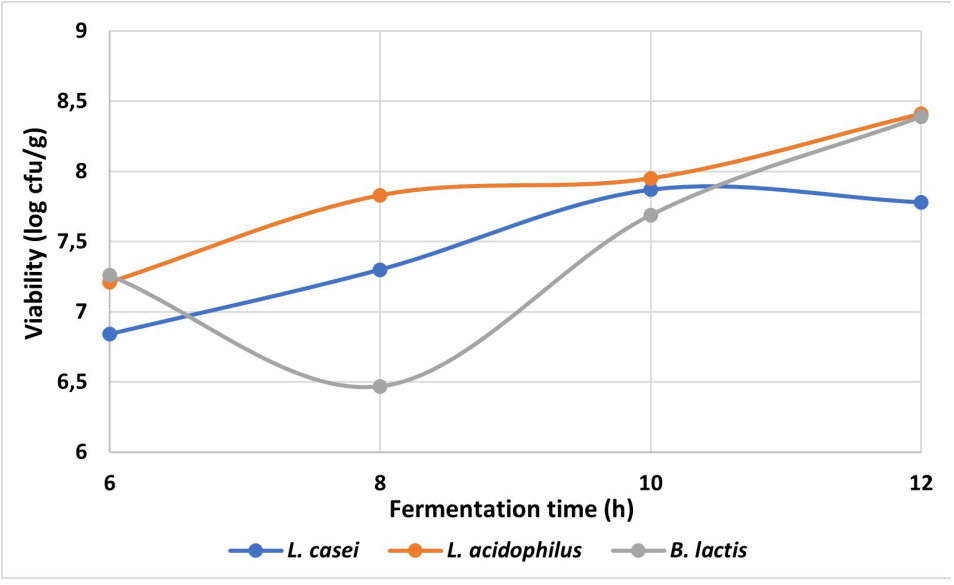


Figure 3. Probiotic viability of LC, LA and BL samples during the fermentation.

heat-treated milk is between 5.2 and 5.4. At hour 8 of fermentation, it was determined that the hardness values of all samples reached a maximum value, and the hardest sample was yogurt containing *L. casei* (42.66 g), followed by *B. lactis* and *L. acidophilus*, respectively.

Goat's milk contains lower α_{s1} casein and higher β casein than cow's milk (Ranadheera et al. 2019). Since the α_{s1} casein level affects the coagulation ability of the milk, this deficiency causes poor coagulation in goat milk and a poor yogurt structure (Hodgkinson et al. 2018). In this study, it was seen that the hardness values of the samples containing only probiotic cultures were considerably lower than the probiotic yogurts produced with the yogurt culture. It can be said that with the use of yogurt culture, the hardness values of yogurts increased significantly ($p<0.05$), and the weak coagulation ability of goat's milk was therefore improved.

Viability of probiotic bacteria and yogurt bacteria

In this study, the viability of probiotic bacteria in yogurts containing only that bacteria over 12 hours of fermentation, and the viability of yogurt bacteria and probiotic bacteria in yogurts containing both over 8 hours of fermentation were investigated.

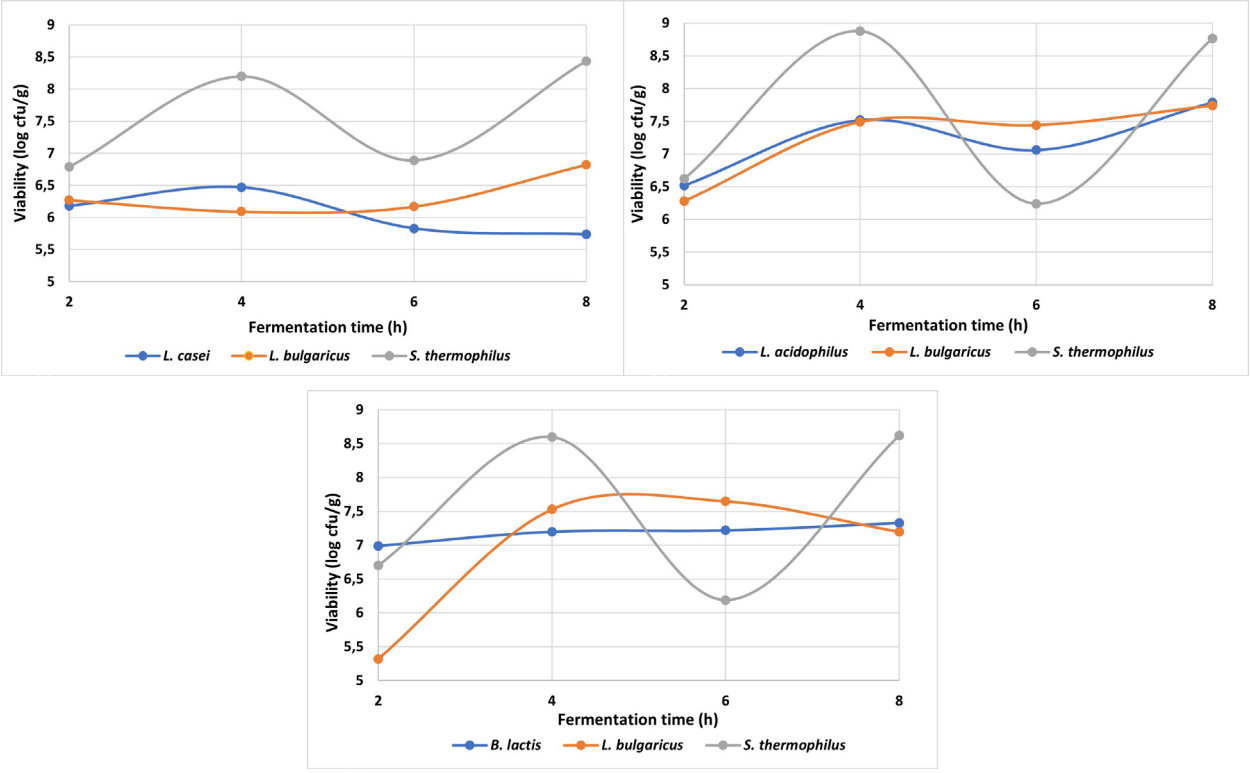


Figure 4a. ***L. casei*****,** ***S. thermophilus*** **and** ***L. bulgaricus*** **counts in LCY sample during the fermentation.**
Figure 4b. ***L. acidophilus*****,** ***S. thermophilus*** **and** ***L. bulgaricus*** **counts in LAY sample during the fermentation.**
Figure 4c. ***B. lactis*****,** ***S. thermophilus*** **and** ***L. bulgaricus*** **counts in BLY sample during the fermentation.**

It was observed that the viability of LC, LA, and BL yogurts at the end of 12 hours of fermentation period increased significantly (p<0.05) (Figure 3). At the end of the 12-hour fermentation period, the LA (8.42 log cfu/g) and BL samples (8.39 log cfu/g) had the highest viability, while the lowest viability was observed in the LC sample (7.78 log cfu/g). Although *Lactobacilli*, with a few exceptions, are mostly reported to be more resistant to acid and oxygen than bifidobacteria (Tripathi & Giri 2014), *B. lactis* is known to tolerate harmful factors such as acid and dissolved oxygen in yogurt compared to other bifidobacteria (Cruz et al. 2012). Therefore, in this study, it was seen that yogurt containing *B. lactis* and *L. acidophilus* showed the best growth potential in goat's milk.

The highest probiotic viability was detected in *L. acidophilus*-containing yogurt (7.79 log cfu/g) after 8 hours of fermentation and was followed by the BLY (7.31 log cfu/g) and LCY (5.83 log cfu/g) samples, respectively. Among these samples, it was determined that the probiotic viability of LCY was very low. By hour 6 of fermentation, the number of *L. casei* decreased significantly (p<0.05) and there were no further significant changes by the 8th hour of fermentation. This may be due to the rapid increase of the lactic acid level at hour 6 of fermentation and the inability of *L. casei* to be resistant to high acidity. However, it was observed that the LC sample containing *L. casei* did survive in an environment with high acidity (Figure 3). Based on this result, it would be more correct to say that *L. casei* is affected by substances such as organic acids produced by yogurt bacteria and that a symbiotic relationship can not be established with yogurt bacteria. Dave & Shah (1997) reported that

S. thermophilus has an antagonistic effect on the growth of bifidobacteria. In this study, it can be said that *S. thermophilus* had a negative effect on the viability of *L. casei*.

L. bulgaricus and *S. thermophilus* counts for LCY, LAY and BLY yogurts are shown in Figure 4a, 4b and 4c. At hour 2 of fermentation, the count for *S. thermophilus* varied from 6.62 to 6.79 log cfu/g, while at hour 8 of fermentation it varied from 8.44 to 8.77 log cfu/g. While there was a regular increase in *L. bulgaricus* counts in yogurts during fermentation, a significant decrease was observed in the *S. thermophilus* count for all samples at hour 6 of fermentation (p <0.05). This may be due to *S. thermophilus* growing better at high pH values and being affected by the rapidly increasing lactic acid level at hour 6 of fermentation. At hour 8 of fermentation, the *S. thermophilus* count increased again. It can be said that the amount of free amino acid increased at this time as a result of the proteolytic activity of *L. bulgaricus*, so *S. thermophilus* increased, growing again by consuming free amino acids.

Both *L. bulgaricus* and *S. thermophilus* counts reached their maximum value in the 8th hour of fermentation for LCY, LAY and BLY yogurts. In addition, *L. acidophilus* in LAY yogurt and *B. lactis* in the BLY sample had the highest viability at the 8th hour of fermentation. During the 8th hour of fermentation of this study, despite there being no statistically significant increase (p>0.05) in the *B. lactis* count, *B. lactis* maintained its viability during the fermentation period in the high acidity medium. It can be said that *L. bulgaricus* and its proteolytic products – like free amino acids – can help *B. lactis* to survive in the fermentation medium.

Antioxidant activity

Since antioxidant activity detection methods determine antioxidant activity through different mechanisms, it is not very accurate to determine that activity using a single method. It is therefore necessary to use several different methods to understand the antioxidant properties of dairy products well (Chen et al. 2003).

In this study, the antioxidant activities of yogurt samples during fermentation were determined in pH 4.6 soluble extracts. The aim was to precipitate casein at a pH of 4.6 and to obtain peptides and amino acids and then determine the antioxidant activities of these substances. Antioxidant activity of yogurt peptides containing only probiotic, and both probiotic and yogurt bacteria, were determined during fermentation by DPPH (RSA%) and ABTS (Trolox equivalent) methods, and the results are given in Table II and Table III.

According to the DPPH method results for the LC, LA and BL samples, antioxidant activity of all samples decreased significantly at the end of the fermentation compared to the beginning of fermentation (p<0.05). Especially in the BL sample, there was a rapid decrease in antioxidant activity at the 8th hour of fermentation, and it was observed that the antioxidant activity did not change significantly at the 10th and 12th hour of fermentation (p>0.05). At the end of 12 hours of fermentation, the LC and LA samples had significantly higher antioxidant activity than the BL sample (p<0.05). The highest RSA activity for LC and LA samples during the whole fermentation period was observed at the 10th hour of fermentation. It was seen that the antioxidant activities of these samples determined by the ABTS method are quite different from the results obtained by the DPPH method. According to the ABTS method, the antioxidant activities of the LC, LA and BL samples increased significantly at the end of the fermentation time compared to the beginning of fermentation (p<0.05). In particular, antioxidant activity increased rapidly in the LA

Table II. Antioxidant activities of LC, LA and BL samples during the fermentation time.

Sample	Fermentation time (h)	DPPH (RSA%)	ABTS (μmol/L Trolox)
LC	6	32.25 ± 2.44^{aX}	70.43 ± 8.18^{aX}
	8	27.23 ± 1.22^{bX}	81.66 ± 13.53^{aX}
	10	37.85 ± 1.54^{cX}	129.76 ± 7.38^{bX}
	12	25.55 ± 2.86^{bX}	223.81 ± 21.52^{cXY}
LA	6	37.14 ± 1.26^{aY}	104.43 ± 10.29^{aY}
	8	33.14 ± 0.77^{abY}	101.43 ± 14.12^{aX}
	10	45.90 ± 2.56^{cY}	266.09 ± 24.56^{bY}
	12	30.05 ± 3.66^{bX}	271.09 ± 44.56^{bY}
BL	6	35.33 ± 1.90^{aXY}	77.00 ± 17.23^{aX}
	8	13.07 ± 1.06^{bZ}	27.23 ± 4.85^{bY}
	10	13.57 ± 1.29^{bZ}	238.95 ± 2.41^{cY}
	12	14.26 ± 0.82^{bY}	210.52 ± 5.87^{dX}

$^{a, b, c, d}$: It expresses changes in samples during the fermentation period. Changes are significant at $p<0.05$ level.
$^{X, Y, Z}$: It expresses changes between samples in the same fermentation time. Changes are significant at $p<0.05$ level.

and BL samples at the 10th hour of fermentation and in the LC sample at the 12th hour. It was observed that DPPH and ABTS method results did not show correlation with the LC, LA and BL samples (r = 0.03) (Table IV). In both methods, it was observed that the sample containing *L. acidophilus* had higher antioxidant activity at the beginning and end of the fermentation.

It was seen that the antioxidant activities of yogurts containing both yogurt culture and probiotic culture, determined by both DPPH and ABTS methods, generally increased during the fermentation period. While the antioxidant activities determined by the DPPH method at the beginning of the fermentation period did not differ from each other ($p>0.05$), at the end of the fermentation period, the LCY (59.04% RSA) sample containing *L. casei* had the highest antioxidant activity followed by the LAY (54.83% RSA) and BLY (50.20% RSA) samples, respectively. Similarly, based on ABTS method results, the LCY sample had the highest antioxidant activity at the end of the fermentation period. It was seen that DPPH and ABTS method results for these samples showed a positive correlation with each other (r = 0.67). In particular, the correlation between DPPH and ABTS results for the LAY sample is quite high (r = 0.85) (Table IV). Based on the DPPH method results at the end of the fermentation period, it was observed that the use of yogurt culture significantly increased the antioxidant activity of probiotic yogurts (Table III). In the ABTS method, it was observed that the antioxidant activities of the samples containing yogurt culture increased significantly at the 6th hour of fermentation ($p<0.05$) and the antioxidant activity of the LC and BL samples did not change significantly at the 8th hour compared to the 6th hour of fermentation ($p>0.05$). In the DPPH method it was not the same, and the antioxidant activity increased significantly at the 8th hour of fermentation

Table III. Antioxidant activities of LCY, LAY and BLY samples during the fermentation time.

Sample	Fermentation time (h)	DPPH (RSA%)	ABTS (µMol/L Trolox)
LCY	2	29.76 ± 2.61^{aX}	186.14 ± 12.66^{AY}
	4	40.48 ± 2.01^{bX}	176.76 ± 16.41^{AX}
	6	40.93 ± 2.50^{bX}	246.81 ± 20.27^{bX}
	8	59.04 ± 1.24^{cX}	253.90 ± 4.79^{bX}
LAY	2	31.07 ± 1.61^{aX}	69.23 ± 8.93^{aY}
	4	22.80 ± 0.85^{bY}	121.81 ± 14.58^{bY}
	6	43.34 ± 1.10^{cX}	196.47 ± 12.00^{cY}
	8	54.83 ± 1.29^{dY}	234.62 ± 13.64^{dXY}
BLY	2	31.85 ± 2.62^{aX}	176.42 ± 17.57^{AX}
	4	34.45 ± 0.99^{aZ}	91.19 ± 12.01^{bZ}
	6	33.80 ± 1.61^{aY}	216.42 ± 10.11^{cY}
	8	50.20 ± 1.44^{bZ}	216.90 ± 19.43^{cY}

$^{a, b, c, d}$: It represents changes in samples during the fermentation period. Changes are significant at $p<0.05$ level.
$^{X, Y, Z}$: It represents changes between samples in the same fermentation time. Changes are significant at $p<0.05$ level.

($p<0.05$). Similar to the DPPH method results, the LCY sample had the highest antioxidant activity at the end of the fermentation period.

It has been reported that the unstable changes in ABTS and DPPH radical scavenging activity are due to many factors, such as the activity of the microbiota and the antioxidant abilities of the many compounds formed during the fermentation process, and because phenolic compounds also play an important role in antioxidant activity (Ozcan et al. 2019). Hydrolysis and release of cell wall components through fermentation causes the release of phenolic compounds from food, which in turn affects antioxidant activity (Yoon et al. 2019). In addition, antioxidant activity methods can give different results due to the structural differences in antioxidative compounds such as polarity, ionic conditions, hydrogen bonding abilities, solubility and stereostructure (Ozcan et al. 2019). Virtanen et al. (2007) reported that the antioxidant activity of yogurt increased as a result of the hydrolysis of milk components by lactic acid bacteria, and that hydrolysis products weighing from 4 to 20 kDa in particular, were responsible for the antioxidant activity.

In this study, it was seen that the antioxidant activity (Trolox equivalent) determined by the DPPH method is lower than the antioxidant activity obtained by the ABTS method (data not shown). This situation is thought to be caused by the solubility of the DPPH radical only in organic solvents and inadequate interpretation of hydrophilic peptides. Sanlidere Aloglu & Oner (2011) reported that the ABTS method gave more sensitive and accurate results than the DPPH method in determining the antioxidant activity of yogurt water-soluble extract.

In addition, no significant correlation was found between the DPPH and ABTS method results, especially in yogurts containing only probiotic culture. Similarly, Yılmaz-Ersan et al. (2018) showed that the DPPH radical scavenging activity of goat milk kefir was highest at the 8th hour of fermentation and the highest value for ABTS was at the beginning of fermentation.

Table IV. Correlation coefficents between DPPH, ABTS and TFFA methods.

Sample	Correlation coefficent (r)		
	DPPH-ABTS	DPPH-TFFA	ABTS-TFFA
LC	-0.352	0.046	-0.696*
LA	0.272	-0.354	0.719**
BL	-0.359	0.696*	0.874**
LCY	0.664*	0.597*	0.738**
LAY	0.835**	0.769**	0.628*
BLY	0.410	0.626*	0.797**
PY	0.030	0.562**	0.160
PYY	0.670**	0.611**	0.553**

***Correlation is significant at $p<0.05$ level.**
**** Correlation is significant at $p<0.01$ level.**

Ozcan et al. (2019) determined that although the antioxidant activities of kefir increase and decrease during 24 hours of fermentation, ABTS and DPPH results showed different trends. In addition, it was reported that the antioxidant activity results obtained with the DPPH method were lower than the results obtained with ABTS, similar to our study. Alenisan et al. (2017) attributed the increase in antioxidant activity during the storage period of fermented dairy products to the increased concentration and bioavailability of antioxidant components such as organic acid derivatives and milk protein hydrolysis. In this study, changes in antioxidant activity during the fermentation can be similarly interpreted.

One of the important parameters affecting antioxidant activity in fermented dairy products is the variety and strain of probiotic bacteria. Gjorgievski et al. (2014) reported that the DPPH radical scavenging activity of yogurt containing only *L. acidophilus* was significantly higher than yogurts containing *L. casei*, *S. thermophilus* and *L. bulgaricus*. Similarly, in our study, it was observed that the antioxidant activity of the LA sample containing only *L. acidophilus* at the end of the fermentation period was higher than the LC and BL samples. Najgebauer-Lejko & Sady (2015) reported that protein content, probiotic variety and microflora are the main factors affecting the antioxidant properties of fermented milk. In addition, the researchers reported that the antioxidant activity of yogurt containing *L. casei* with yogurt bacteria was significantly higher than the other probiotic samples. Similarly, in this study, it was observed that the LCY sample had higher antioxidant activity than the LCA and BLA samples.

Total free amino acid content

Although milk is an important source of protein, it does not contain enough free amino acids and peptides to be used by lactic acid bacteria. In order for lactic acid bacteria to continue their development during fermentation, peptides and free amino acids must be present in the environment. It has been determined in many studies that the proteolytic activity of *L. bulgaricus* is higher than *S. thermophilus*. During yogurt fermentation, *L. bulgaricus* breaks down casein to stimulate the development

Table V. Total free amino acid contents of all yogurt samples during the fermentation time.

Sample	Fermentation time (h)	Total free amino acid (µg/mL leucine)	Sample	Fermentation time (h)	Total free amino acid (µg/mL leucine)
LC	6	137.66 ± 6.25^{aX}	**LCY**	2	92.17 ± 7.89^{aX}
	8	86.17 ± 2.77^{bX}		4	60.92 ± 5.19^{bX}
	10	60.47 ± 8.34^{cX}		6	90.84 ± 6.24^{aX}
	12	60.86 ± 5.71^{cX}		8	123.16 ± 468^{cX}
LA	6	112.16 ± 345^{abY}	**LAY**	2	92.26 ± 10.09^{aX}
	8	108.18 ± 20.10^{aX}		4	83.72 ± 412^{aY}
	10	135.73 ± 9.93^{bY}		6	84.99 ± 4.29^{aX}
	12	182.98 ± 15.99^{cY}		8	143.81 ± 6.34^{bY}
BL	6	64.05 ± 4.61^{aZ}	**BLY**	2	80.21 ± 3.94^{aX}
	8	56.43 ± 0.64^{bY}		4	37.89 ± 8.43^{bZ}
	10	35.00 ± 1.41^{cZ}		6	74.63 ± 14.47^{aX}
	12	37.67 ± 6.35^{cZ}		8	109.08 ± 4.28^{cZ}

$^{a, b, c, d}$: It represents changes in samples during the fermentation period. Changes are significant at $p<0.05$ level.
$^{X, Y, Z}$: It represents changes between samples in the same fermentation time. Changes are significant at $p<0.05$ level.

of *S. thermophilus* and releases peptides and free amino acids. *S. thermophilus* continues to develop by using these nitrogen sources. Since the proteolytic activity of probiotic bacteria is rather limited, their capacity to break down casein and release peptides and amino acids is not sufficient. For this reason, they try to use the free nitrogen resources in the environment and so their fermentation periods are long. The proteolytic activity of microorganisms, assimilation of peptides, and the release of amino acids during fermentation cause differences in the amount of free amino acids (Ozcan et al. 2019).

In this study, the Cd-Ninhydrin method was used to determine the total amount of free amino acids (TFAA) in yogurts. It was observed that the total amount of free amino acids in the LC, LA and BL samples progressed differently with fermentation time. The highest amount of TFAA of the LC sample was detected at the 2nd hour of fermentation, and a decrease in the amount of TFAA was observed in the later hours (Table V). A similar trend was observed in the BL sample, and the lowest amount of TFAA was determined at the end of the fermentation period. Similarly, the BL sample had the lowest antioxidant activity at the 12th hour of fermentation. In contrast to the LC and BL sample, an increase in TFAA was observed during the fermentation period of the LA sample. The maximum amount of TFAA was determined in the LA sample at the 12th hour of fermentation. This situation may suggest that the proteolytic activity of *L. acidophilus* is higher than *L. casei* and *B. lactis*.

It was observed that the TFAA amounts in the LCY, LAY and BLY samples increased significantly at the end of 8 hours of fermentation compared to the beginning of fermentation ($p<0.05$). Among these samples, the sample containing *L. acidophilus* had the highest TFAA amount at the end of the fermentation period. There was a statistically significant decrease in the amount of TFAA in the LCY and BLY samples containing

yogurt culture at the 4th hour of fermentation (p<0.05). This situation may be associated with the decrease in pH and the use of increased nitrogen resources in the environment by *S. thermophilus* because of the development and proteolytic activity of *L. bulgaricus*.

It has been reported in many studies that there is a linear relationship between antioxidant activity and the degree of proteolysis (Virtanen et al. 2007, Solieri et al. 2015, Sah et al. 2014). Sah et al. (2014) reported that DPPH, ABTS and FRAP (ferric reducing antioxidant power) radical scavenging activity increased as the degree of hydrolysis of milk proteins increased. The high antioxidant activity of fermented yogurt stored at low temperature is thought to be due to a higher degree of hydrolysis of milk proteins (Yoon et al. 2019). In this present study, when DPPH, ABTS and TFAA analysis results were correlated within each sample separately, it was found that ABTS antioxidant activity results and TFAA results showed a significant positive correlation to all samples except LC and BL (p<0.01). In the LC and BL samples, a negative correlation was found between the TFFA and ABTS results. In addition, the DPPH results for the LC sample were positively correlated with the TFAA results.

CONCLUSIONS

In this study, probiotic yogurts were produced from goat's milk using various probiotic bacteria and yogurt bacteria. It was observed that the use of yogurt bacteria in goat milk with probiotic bacteria was important to obtain higher hardness values and higher antioxidant activity. It was determined that the DPPH and ABTS methods gave different antioxidant activity results, especially in plain probiotic yogurts. In general, it was observed that the total amount of free amino acids was correlated with the antioxidant activity results. It was observed that probiotic viability was higher in plain probiotic yogurts. It has been concluded that the use of probiotic bacteria together with the yogurt starter culture in the production of probiotic yogurt from goat milk is important in terms of both shortening the fermentation time and increasing the functional properties of yogurt.

Acknowledgments

This research was not supported by any funding agencies in the public, commercial, or not-for-profit sectors. I would like to thank to Prof. Dr. Ali Adnan Hayaloglu for helping to plan of study and Prof. Dr. Harun Rasit Uysal for providing laboratory facilities.

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How to cite

AKAN E. 2022. The Effect of Fermentation Time and Yogurt Bacteria on the Physicochemical, Microbiological and Antioxidant Properties of Probiotic Goat Yogurts. An Acad Bras Cienc 94: e20210875. DOI 10.1590/0001-3765202220210875.

Manuscript received on June 11, 2021;
accepted for publication on August 11, 2021

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